• 国际交流 •

导读：

肾癌是临床常见的肿瘤类型之一。既往研究表明，缺氧在多种癌症的发生和发展过程中均起到了很重要的作用，但在肾癌领域，缺氧及其导致的病理生理反应对疾病转归的意义尚不明了。本文作者利用肿瘤数据库进行研究，识别肾癌中差异表达的缺氧相关基因，开发基于缺氧相关基因的预后指数，提示缺氧可能是肾癌预后的危险因素，缺氧相关环节可能是肾癌的潜在治疗靶点，对临床上肾癌的诊治有着一定指导意义。

张石川
四川省肿瘤医院　放疗中心

Development of a Prognostic Index for Kidney Cancer Based on Hypoxia Landscape

Wang Hesong, Feng Yanyan, Wang Ting, Feng Yuyin, Jiang Haixu, Wang Tingjian, Huang Guangrui
School of Life Sciences, Beijing University of Chinese Medicine, Beijing 100029, *China* (*Wang Hesong, Feng Yanyan, Wang Ting, Feng Yuyin, Jiang Haixu, Huang Guangrui*); *Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA*, 02215, *USA* (*Wang Tingjian*)
Corresponding author: Huang Guangrui, E-mail: hgr@ bucm. edu. cn; Wang Tingjian, E-mail: Tingjian_wang@ dfci. harvard. edu
This study was supported by National Natural Science Foundation of China (No. 81430099), and by grants from Beijing University of Chinese Medicine (No. BUCM-2019-JCRC006, No. 2019-JYB-TD013).

[**Abstract**] **Objective**: Kidney cancer is a group of cancers occurred in the kidney. Hypoxia is a condition characterized by insufficient oxygen supply in the body or specific organs. It has been proven to play an essential role in the pathogenesis and development of various cancer. However, the roles and mechanisms of hypoxia in kidney cancer have not been investigated clearly. In this study, we comprehensively analyzed the roles of hypoxia in kidney cancer. **Methods**: The RNA-Seq data of kidney cancer were downloaded from TCGA dataset. The survival of patients with differentially expressed hypoxia-related genes was analyzed with the survival package. The prognostic value of hypoxia-related genes was evaluated with univariate Cox regression analysis. The correlation of hypoxia-related genes to immune cells infiltration and gene mutation in kidney cancer was assessed. We identified 7 hypoxia-related genes of kidney cancer, with which we developed a hypoxia-related genes-based prognostic index using multivariate Cox regression analysis to establish the prognostic model. **Results**: The high-risk group showed lower survival compared with the low-risk group. HRGPI was an independent predictor of kidney cancer, and it was associated with malignant stages. Hypoxia-related genes were correlated to activation of inflammatory pathways, infiltration of inflammatory cells, and expression of inhibitory immune checkpoints, indicating that hypoxia was related to immune response in kidney cancer. Lastly, we found that hypoxia was related to missense mutation in kidney cancer. **Conclusion**: Hypoxia is a risk factor of kidney cancer. It can be regarded as the prognostic indicator and therapeutic target for the treatment of kidney cancer in the future.

[**Key words**] Kidney cancer; Hypoxia; Risk score; Inflammation; Mutation
Received April 2, 2021
Accepted May 26, 2021
doi: 10. 3969/j. issn. 1674-0904. 2021. 11. 002

Cite this article as: Wang HS, Feng YY, Wang T, *et al*. Development of a prognostic index for kidney cancer based on hypoxia landscape[J]. J Cancer Control Treat, 2021, 34(11): 992 – 1004.

INTRODUCTION

A series of cancers occurred in the kidney can be called kidney cancer[1]. Almost 350,000 new cases of kidney cancer are diagnosed every year in the world. This cancer is responsible for the 7th most common

cancer, leading to 140,000 deaths every year[2]. The risk factors of kidney cancer include obesity, hypertension, smoking, etc. Hematuria, lumbodynia, and weight loss are typical symptoms of kidney cancer. According to histological features and molecular alterations, the sub-types of kidney cancer involve clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (PRCC), chromophobe renal cell carcinoma (chRCC), etc. The treatment of kidney cancer depends on its sub-type and stage[3]. Surgery, chemotherapy and radiotherapy are common strategies. In recent decades, gene abnormalities have frequently been identified to correlate with kidney cancer. Previous studies have discovered that the Von Hippel - Lindau (*VHL*) gene mutation occurred in most ccRCC patients[4]. *VHL* mutation can up-regulate hypoxia-inducible factors (HIFs). *VHL* tumor suppressor protein is critical in the oxygen regulation of kidney cancer. Mutation of other genes other than *VHL*, including *TCEB1*, *SETD2* and *PBRM1*, were also involved in developing and progressing kidney cancer[5]. Thus, gene alteration which could offer novel therapeutic targets should be investigated in the future.

Hypoxia is a condition characterized by insufficient oxygen supply in the body or specific organs. It has been proven that hypoxia plays an important role in the pathogenesis and development of cancer[6]. In the tumor microenvironment (TME) of hypoxia, the oxygen concentration is often lower in tumor tissue than in healthy tissue. Hypoxia also involves in extracellular matrix remodeling and the metastasis of tumor[7]. Hypoxia could influence the apoptosis and angiogenesis of tumors by altering TME[8], also induce the differentiation and maturation of inflammatory cells such as macrophages, dendritic cells in tumor tissue[9]. Hypoxia in TME includes 4 types, chronic, acute, anemic and toxic hypoxia, which leads to different consequences of tumor progression. Various proteins, including glucose transporter, basic fibroblast growth factor, hypoxia-inducible factor 1 (HIF-1), are involved in effects of hypoxia[10]. Apart from pathogenesis and carcinogenesis, hypoxia is also conducive to cancer therapy resistance. Specifically, hypoxia is responsible for chemoresistance by increasing the level of HIF-1[11]. It also induces radioresistance by activating HIF-1 signaling pathway. HIF-1 could influence radiotherapy and chemotherapy sensitivity in solid tumors by regulating cell proliferation, metabolism, and apoptosis. However, the roles and mechanisms of hypoxia in kidney cancer have not been investigated clearly.

In this study, we have created a hypoxia-related genes - based prognostic index (HRGPI) for kidney cancer based on a hypoxia landscape analysis. The survival of patients with hypoxia-related genes is analyzed, and the HRGPI is further developed. We have also calculated the correlation of hypoxia-related genes to immune cells infiltration and gene mutation in kidney cancer, and investigated the potential mechanisms of hypoxia, as an independent biomarker, in the prognosis of kidney cancer patients.

METHODS

Clinical samples and data acquirement

RNA-Seq and somatic mutation data of kidney cancer, including ccRCC, PRCC, and chRCC, as well as clinical data of patients were acquired from TCGA dataset, comprising 893 tumor samples and 128 adjacent normal samples. A total of 820 tumor samples were finally enrolled after deleting cases with survival less than 90 days. Differentially expressed genes (DEGs) of kidney cancer were analyzed with the Deseq2 package. Significant genes were defined as abs (logFC) > 1 and $FDR < 0.001$. The data were then analyzed with normalized FPKM (Fragments Per Kilobase of transcript, per Million mapped reads) values, and the batch effect was removed with the SVA package. Heatmaps and volcano plots of DEGs were produced with the R package. In addition, information of transcript factors was downloaded from Cistrome (http://cistrome.org/), a bioinformatics platform for transcriptional regulation studies. Differentially expressed transcription factors (DETFs) were recognized from DEGs. Spearman's correlation analysis was used to identify the correlation between DETFs and hypoxia-related genes, with the Spearman's rank correlation coefficient > 0.5 and $P < 0.05$ for statistical significance.

Prognostic analysis

The prognosis of patients with differentially ex-

pressed hypoxia-related genes was analyzed with the survival package. The prognostic value of hypoxia-related genes was evaluated with univariate Cox analysis. Forest plots of hypoxia-related genes were described with hazard ratio (*HR*).

Functional enrichment analysis

Functional enrichment analysis, including GO (analyses on biological process, molecular function, cellular component and biological process), KEGG, and GSEA analyses, were conducted in this study. All significant KEGG signaling pathways were visualized with a bubble chart. GSEA analysis was used to evaluate the significance of the biological states of the specific genes or pathways.

HRGPI

Multivariate analysis by the Cox proportional hazard model was used to develop HRGPI which was considered as an independent prognostic factor of kidney cancer. The prognostic index was constructed with Cox regression coefficient. In this study, we divided the patients into two groups, the high-risk group and the low-risk group, according to the median of HRGPI. Kaplan-Meier survival curves were drawn to investigate the survival of two groups, and the receiver operating characteristic (ROC) curve was used to evaluate the specificity and sensitivity of this prognostic index. Furthermore, univariate analysis was conducted to evaluate the prognostic value of HRGPI and other clinicopathologic factors, such as age, gender, grades and stages. The survival package was used to explore the survival prognosis of the prognostic index.

Correlation between hypoxia-related genes and immune status

In this study, we evaluated the correlation between hypoxia-related genes and the infiltration of immune cells, including type 2 T helper cells, regulatory T cells, activated $CD4^+$ T cells, natural killer cells, etc., by using ssGSEA[in the R package [12 - 13]]. In addition, we evaluated the correlation between hypoxia-related genes and inhibitory immune checkpoints such as *PDCD1*, *CTLA4*, *TGFβ1*, *LAG3* and *TIGIT*.

Statistical analysis

The R package (version 3.5.3) was used to conduct the analyses in our study. The area under the curve (AUC) was calculated with the survivalROC package. Independent t-test was used to analyze the relationship between HRGPI and clinical factors, as well as the relationship between HRGPI and mutant genes. Wilcoxon test was used to analyze the difference in HRGPI between groups. $P < 0.05$ was considered as statistically significant.

RESULTS

Identification of DEGs

In our study, DEGs in kidney cancer were analyzed with the DESeq2 package. 893 tumor samples and 128 adjacent normal samples were analyzed. The results showed that a total of 4,879 genes were up-regulated, while 1,563 genes were down-regulated in kidney cancer (Figure 1A and 1C). 28 up-regulated and 8 down-regulated hypoxia-related DEGs were identified from a list of 99 hypoxia-related genes (Figure 1B and 1D). The role the 36 hypoxia-related DEGs play in the prognosis of patients was also evaluated. As is shown in Figure 2, 28 hypoxia-related DEGs were associated with the prognosis of kidney cancer patients, in which 24 were high-risk genes for death, whereas 4 were low-risk genes.

Identification of DETFs

A total of 318 transcription factors were downloaded from the Cistrome dataset. After a comparison between up-regulated or down-regulated genes in kidney cancer was made, 48 DETFs were recognized in kidney cancer (Figure 3). Spearman's correlation analysis was conducted to discover the correlation between DETFs and hypoxia-related genes (Figure 4A). GO and KEGG analyses were conducted to discover the functional enrichment of the 75 DETFs and hypoxia-related genes. KEGG signaling pathway analysis showed that those genes were correlated to HIF-1 signaling pathway, transcriptional misregulation in cancer, acute myeloid leukemia, central carbon metabolism in cancer, and bladder cancer, respectively (Figure 4B). GO analysis indicated that the biological process was enriched in 4 clusters: metabolism pathway, immune-associated functions, kidney development as well as angiogenesis, cell proliferation and cell differentiation (Figure 4C). 17 hypoxia-related genes were correlated to DETFs ($r_s > 0.5$, $P < 0.05$), including *LDLR*,

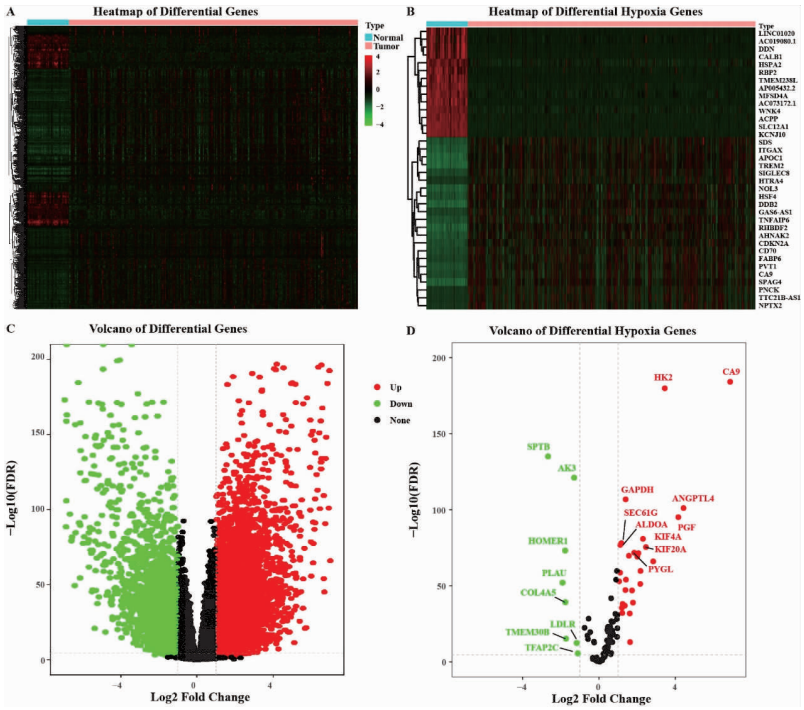

Figure 1. Identification of DEGs

A. The heatmap of DEGs in kidney cancer tissue and adjacent normal tissue; B. The heatmap of hypoxia-related DEGs in kidney cancer; C. The volcano plot of DEGs in kidney cancer tissue and adjacent normal tissue (up-regulated genes as indicated by the red dots, while the down-regulated by the green dots); D. The volcano plot of hypoxia-related DEGs in kidney cancer tissue and adjacent normal tissue (up-regulated genes as indicated by the red dots, while the down-regulated by the green dots).

DEGs: Differentially expressed genes.

Genes	*Pvalue*	Hazard ratio
SLC16A1	*<0.001*	1.028(1.018−1.038)
CORO1C	*0.004*	1.017(1.005−1.029)
KIF4A	*<0.001*	1.213(1.161−1.268)
ANLN	*<0.001*	1.141(1.109−1.174)
TUBA1B	*<0.001*	1.011(1.007−1.015)
CDKN3	*<0.001*	1.086(1.061−1.111)
VEGFA	*<0.001*	1.003(1.001−1.004)
KIF20A	*<0.001*	1.165(1.134−1.197)
ADM	*0.002*	1.003(1.001−1.006)
HK2	*0.002*	1.018(1.007−1.030)
SLC2A1	*<0.001*	1.005(1.003−1.007)
CA9	*0.005*	1.002(1.001−1.004)
P4HA1	*0.001*	1.008(1.003−1.012)
SEC61G	*<0.001*	1.015(1.008−1.021)
LDHA	*<0.001*	1.002(1.001−1.003)
DDIT4	*<0.001*	1.003(1.001−1.004)
GAPDH	*<0.001*	1.001(1.000−1.001)
AK3	*<0.001*	0.919(0.893−0.947)
ANGPTL4	*0.001*	1.001(1.000−1.001)
PGF	*<0.001*	1.004(1.002−1.007)
LDLR	*0.044*	0.965(0.933−0.999)
NME1	*<0.001*	1.023(1.013−1.034)
PFKFB4	*<0.001*	1.122(1.076−1.170)
COL4A5	*<0.001*	1.090(1.041−1.141)
PYGL	*0.004*	1.024(1.008−1.040)
SLC6A8	*0.038*	1.002(1.000−1.004)
TFAP2C	*0.040*	0.894(0.803−0.995)
TMEM30B	*<0.001*	0.964(0.945−0.984)

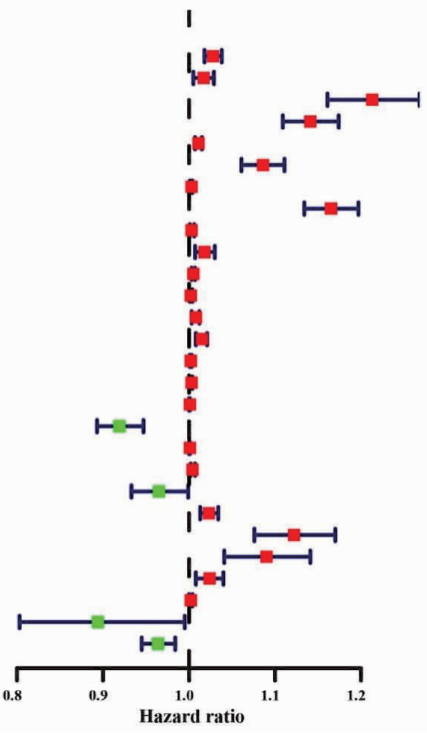

Figure 2. Prognostic Values of Hypoxia Related DEGs

In 28 hypoxia-related genes relevant to survival, 24 genes were high-risk genes and 4 genes were low-risk genes (*HR* > 1 was considered as high risk, and *HR* < 1 was considered as low risk).

Abbreviations as indicated in Figure 1.

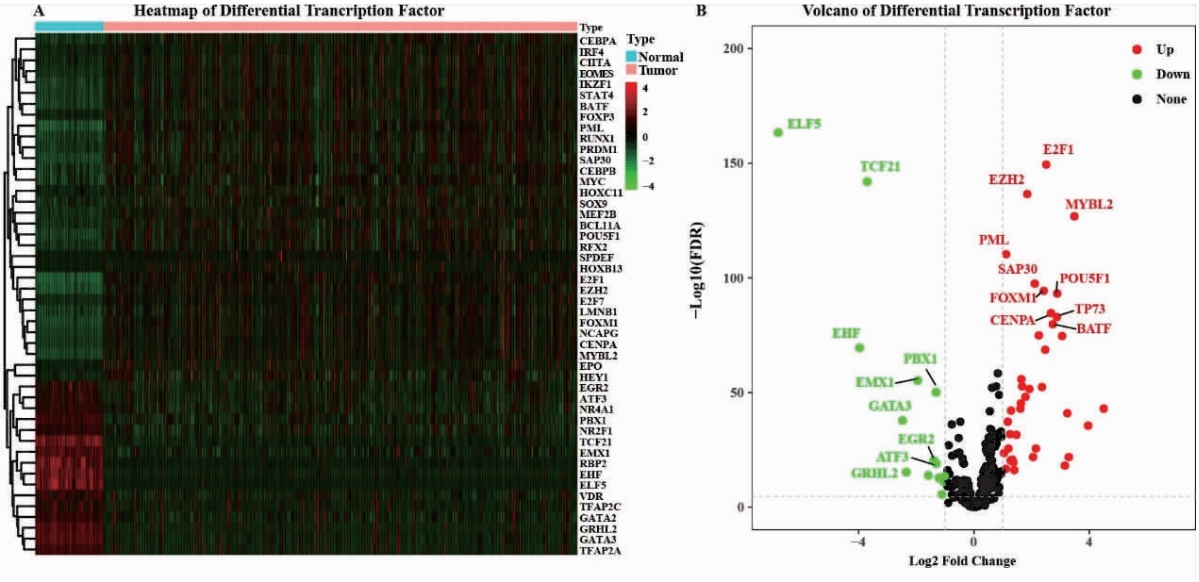

Figure 3. Identification of DETFs

A. The heatmap of DETFs; B. The volcano plot of DETFs.

Up-regulated transcription factor genes as indicated by the red dots, while the down-regulated by the green dots.

DETFs: Differentially expressed transcription factors.

ADM, *ANLN*, *CDKN3*, *KIF20A*, *KIF4A*, *VEGFA*, *ANGPTL4*, *PFKFB4*, *DDIT4*, *PYGL*, *TFAP2C*, *CA9*, *TMEM30B*, *SLC16A1*, *P4HA1*, and *LDHA*. After filtering the highly interrelated genes, 7 hypoxia-related genes, including *SLC16A1*, *VEGFA*, *KIF20A*, *CA9*, *PFKFB4*, *PYGL* and *TMEM30B*, were maintained for further analysis.

Calculation of HRGPI

HRGPI was developed with multivariate Cox regression analysis. We built a prognostic signature to divide the kidney cancer patients into two groups, the high-risk group (HRGPI > the median level) and the low-risk group (HRGPI < the median level). The formula for evaluating the risk level was as follows: HRGPI = [the expression level of *SLC16A1* × 0.0157681] + [the expression level of *VEGFA* × 0.0030014] + [the expression level of *KIF20A* × 0.2133521] + [the expression level of *CA9* × (−0.001576)] + [the expression level of *PFKFB4* × 0.0700786] + [the expression level of PYGL × (−0.050195)] + [the expression level of *TMEM30B* × (−0.043569)]. Figure 5A indicated that the survival in the high-risk group was lower than that in the low-risk group ($P < 0.001$). There are similar results in ccRCC, PRCC, and chRCC (Figure 5C, 5D and 5E). The AUC was 0.754 (Figure 5B). In addition, Figure 6 indicated that the survival time shortens as HRGPI increases. And the heat map of 7 hypoxia-related genes including *SLC16A1*, *VEGFA*, *KIF20A*, *CA9*, *PFKFB4*, *PYGL* and *TMEM30B* was also shown in Figure 6C.

Furthermore, we used univariate and multivariate Cox regression analyses to evaluate the HRGPI as an independent predictor of kidney cancer. The results was proved to be affirmative when other clinical factors (including age, gender, grade, clinical stage, stage T, stage N and stage M) were also included in the computational formula (Figure 7). Table 1 showed the relationship of clinical factors to HRGPI and 7 hypoxia-related genes; HRGPI was positively related to grade, clinical stage and stage T. As is shown in Figure 8, a high HRGPI was usually related to advanced stages of kidney cancer, including grade 3 & 4, clinical stage III & IV, and stage T3 & T4.

Functional enrichment analysis of hypoxia-related genes

In this study, we analyzed the correlation between hypoxia-related genes and immune cells as well as the correlation between hypoxia-related genes and inhibitory immune checkpoints. As is shown in Figure 9A, hypoxia-related genes were positively correlated to type 2

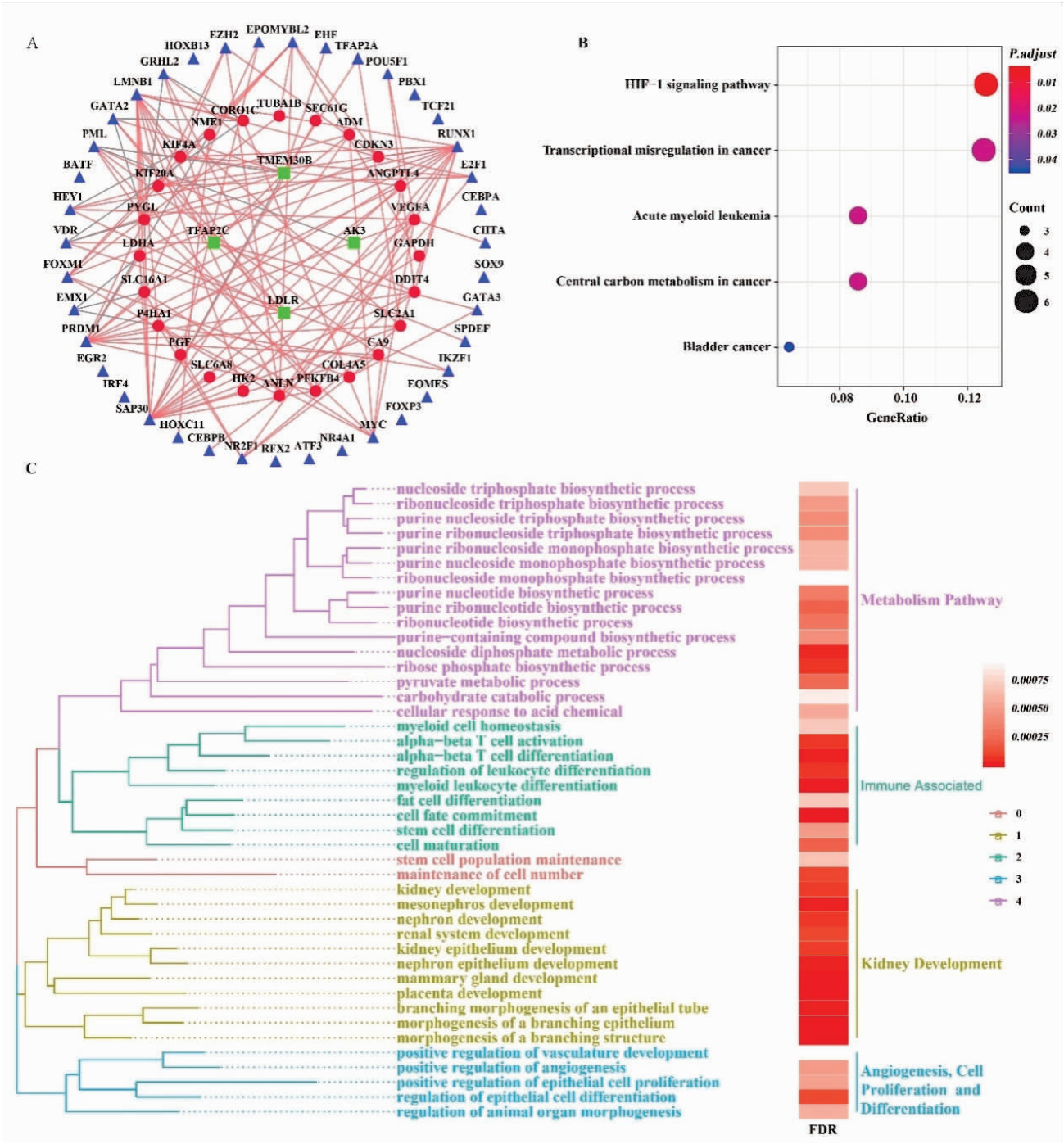

Figure 4. Correlation between and Functional Enrichment Analysis of DETFs and Hypoxia Related Genes

A. Correlation between DETFs and hypoxia-related genes by Spearman's correlation analysis (DETFs as indicated by the blue dots, up-regulated hypoxia-related DEGs by the red dots, and down-regulated hypoxia-related DEGs by the green dots; the genes were linked by lines only when Spearman's rank correlation coefficient > 0.3 (a positive correlation as indicated by the red lines, while a negative correlation by grey lines); B. KEGG analysis of DETFs and hypoxia-related genes showed that DETFs and hypoxia-related genes were correlated to HIF-1 signaling pathway, transcriptional misregulation in cancer, acute myeloid leukemia, central carbon metabolism in cancer, and bladder cancer, respectively; C. GO analysis of DETFs and hypoxia-related genes showed that the biological process was enriched in 4 clusters including metabolism pathway (as indicated by the pink cluster), immune-associated functions (as indicated by the green cluster), kidney development (as indicated by the yellow cluster) as well as angiogenesis, cell proliferation and cell differentiation (as indicated by the blue cluster).

DETFs: Differentially expressed transcription factors; DEGs: Differentially expressed genes; HIF-1: Hypoxia-inducible factors 1.

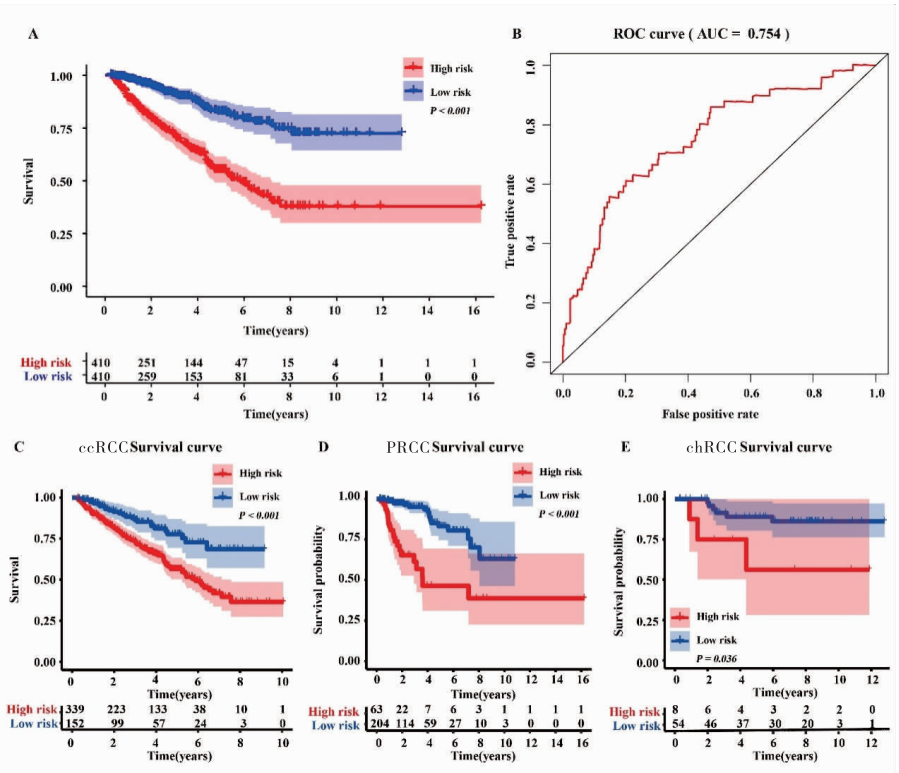

Figure 5. Evaluation of HRGPI

A. The survival in the high-risk group was lower than that in the low risk group according to HRGPI ($P<0.001$); B. The value of AUC (0.754) was used to validate the model of HRGPI; C. The high ccRCC risk group showed lower survival compared with the low ccRCC risk group ($P<0.001$); D. The high PRCC risk group showed lower survival compared with the low PRCC risk group ($P<0.001$); E. The high chRCC risk group showed lower survival compared with the low chRCC risk group ($P=0.036$).

HRGPI: Hypoxia-related genes-based prognostic index; AUC: Area under the curve; ccRCC: Clear cell renal cell carcinoma; PRCC: Papillary renal cell carcinoma; chRCC: Chromophobe renal cell carcinoma.

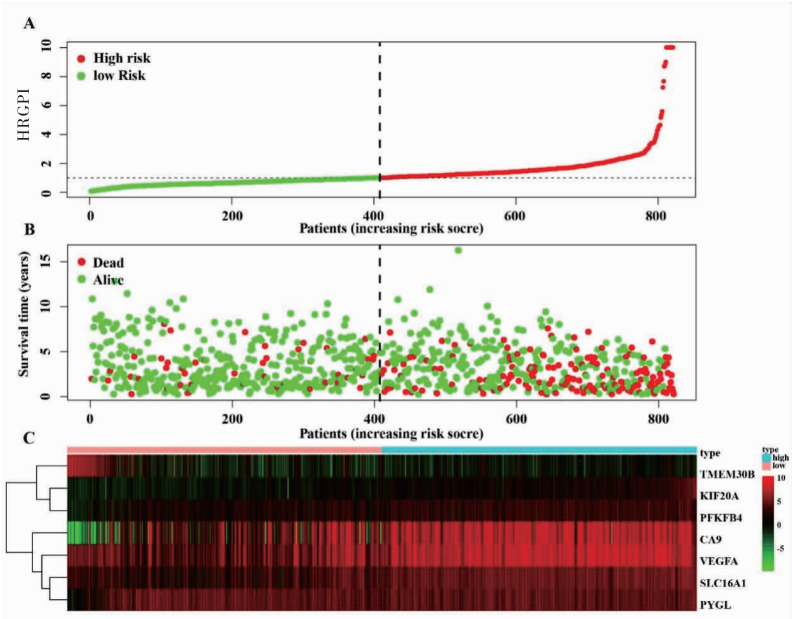

Figure 6. Development of HRGPI

A. HRGPI in high- and low-risk groups; B. Survival of kidney cancer patients in high- and low-risk group; C. Heat map of 7 hypoxia-related genes including *SLC16A1*, *VEGFA*, *KIF20A*, *CA9*, *PFKFB4*, *PYGL* and *TMEM30B*.

HRGPI: Hypoxia-related genes-based prognostic index.

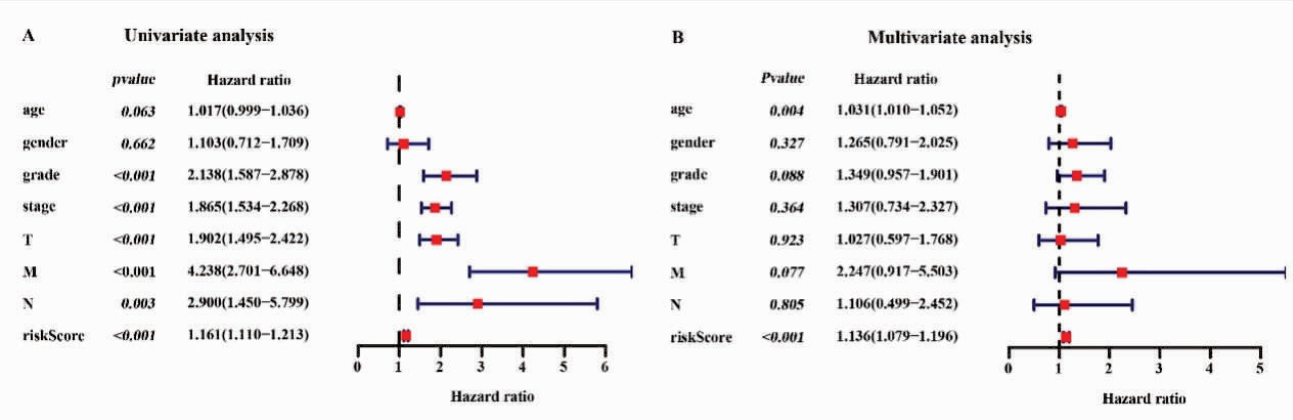

Figure 7. Univariate and Multivariate Analyses of Kidney Cancer

A. HRGPI is an independent predictor of kidney cancer by univariate analysis; B. HRGPI is an independent predictor of kidney cancer by multivariate analysis.

Table 1. Relationship of Clinical Factors to HRGPI and 7 Hypoxia-Related Genes [*t*-test (*P*)]

Variable	Age(≥ 60/ < 60)	Gender (male/female)	Grade (G4/G2-G3)	Stage (Ⅲ-Ⅵ/ Ⅰ-Ⅱ)	T (T3-T4/T1-T2)	M(M1/M0)	N(N1/N0)
SLC16A1	−1.258 (0.210)	0.79 (0.431)	0.087 (0.930)	−1.842 (0.067)	−2.251 (0.026)	−0.962 (0.340)	−0.057 (0.955)
VEGFA	0.468 (0.640)	2.149 (0.033)	1.983 (0.049)	1.071 (0.286)	0.326 (0.745)	1.142 (0.258)	3.214 (0.006)
KIF20A	1.69 (0.093)	−2.526 (0.012)	−3.661 (<0.001)	−4.174 (<0.001)	−3.754 (<0.001)	−2.336 (0.024)	−2.451 (0.030)
CA9	0.248 (0.804)	−0.36 (0.720)	0.484 (0.629)	0.072 (0.943)	1.142 (0.255)	0.187 (0.852)	−0.339 (0.740)
PFKFB4	−1.132 (0.259)	1.635 (0.104)	−1.206 (0.229)	−1.41 (0.160)	−1.259 (0.210)	−0.399 (0.692)	−0.344 (0.737)
PYGL	−0.351 (0.726)	−0.314 (0.754)	0.478 (0.633)	−0.395 (0.693)	−1.086 (0.279)	0.501 (0.618)	0.188 (0.854)
TMEM30B	−0.565 (0.573)	−1.222 (0.223)	0.546 (0.586)	0.701 (0.484)	0.419 (0.676)	0.422 (0.674)	2.355 (0.019)
HRGPI	1.438 (0.153)	−1.173 (0.242)	−2.763 (0.006)	−3.139 (0.002)	−3.02 (0.003)	−1.877 (0.068)	−1.568 (0.143)

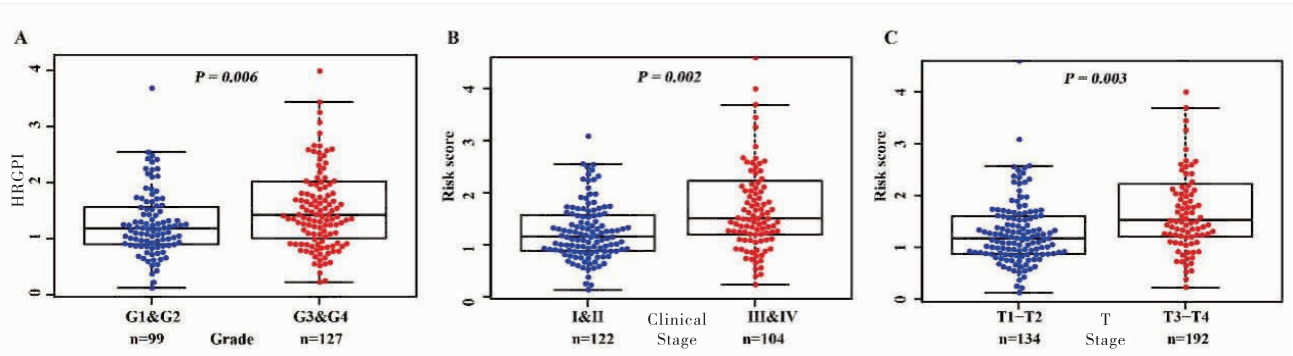

Figure 8. Relation of HRGPI to Grade, Clinical Stage and Stage T in Kidney Cancer

A. High HRGPI was related to grade 3 & 4 ($P=0.006$); B. High HRGPI was related to clinical stage III & IV ($P=0.002$); C. High HRGPI was related to stage T3 & T4 ($P=0.003$).

T helper cells, regulatory T cells, activated $CD4^+$ T cells, natural killer cells, effector memory $CD8^+$ T cells, type 1 T helper cells, effector memory $CD4^+$ T cells, neutrophils, natural killer T cells, etc. As is shown in Figure 9B, hypoxia-related genes were positively correlated to *TGFβ1*, *TIGIT*, *LAG3*, *CTLA4* and *PDCD1*.

GSEA analysis was then used to conduct the functional enrichment analysis of hypoxia-related genes. Hypoxia-related genes were positively correlated to hypoxia, tumor necrosis factor (TNF)-α signaling pathway, IL2-STAT5 signaling pathway, IL6-JAK-STAT3

signaling pathway, inflammatory response, interferon-alpha response, interferon-gamma response, etc. (Figure 9C). All these pathways were involved in inflammation.

Relationship between HRGPI and mutation landscape

In this study, we conducted a genetic alteration analysis of kidney cancer. The top 10 mutated genes in kidney cancer were *VHL*, *PBRM1*, *TTN*, *SETD2*, *MUC16*, *BAP1*, *KMT2C*, *TP53*, *LRP2* and *PKHD1*. Missense mutation and frameshift mutation were the two most common types of mutation (Figure 10). We also analyzed the relationship between HRGPI and the mutated genes. HRGPI was higher in patients with top 10 mutated genes than in patients with wildtype genes (Figure 11A), and HRGPI in patients with *VHL*, *PBRM1* and *BAP1* were significantly higher than those in patients with wildtype genes (Figure 11B-D). Furthermore, we also evaluated the relationship between HRGPI and tumor mutation burden (TMB) in pan-kidney cancer, ccRCC, PRCC and chRCC, respectively. We found that a high HRGPI was related to high TMB in pan-kidney cancer and ccRCC, respectively (Figure 11E-H).

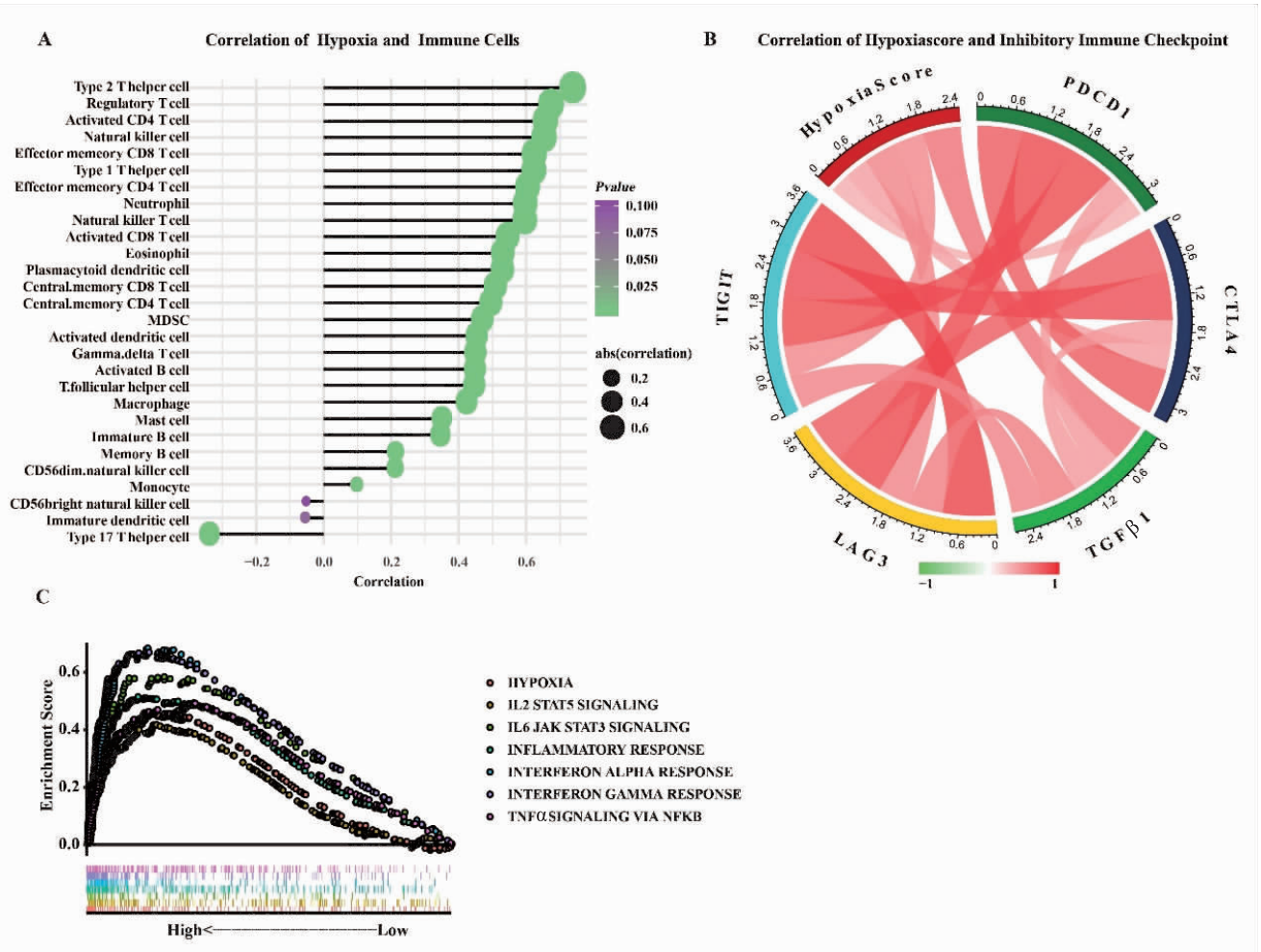

Figure 9. Correlation between Hypoxia-Related Genes and Immune Cells/Inhibitory Immune Checkpoint Genes

A. Hypoxia related genes were positively correlated to type 2 T helper cells ($r_s = 0.73, P < 0.05$), regulatory T cells ($r_s = 0.66, P < 0.05$), activated CD4 T cells ($r_s = 0.65, P < 0.05$), natural killer cells ($r_s = 0.64, P < 0.05$), effector memory CD8 T cells ($r_s = 0.61, P < 0.05$), Type 1 T helper cells ($r_s = 0.61, P < 0.05$); B. Hypoxia related genes were positively correlated to *TGFβ1*, *TIGIT*, *LAG3*, *CTLA4* and *PDCD1*; C. GSEA analysis of hypoxia-related genes showed that hypoxia-related genes were positively correlated to hypoxia, IL2-STAT5 signaling pathway, IL6-JAK-STAT3 signaling pathway, inflammatory response, interferon-alpha response, interferon-gamma response.

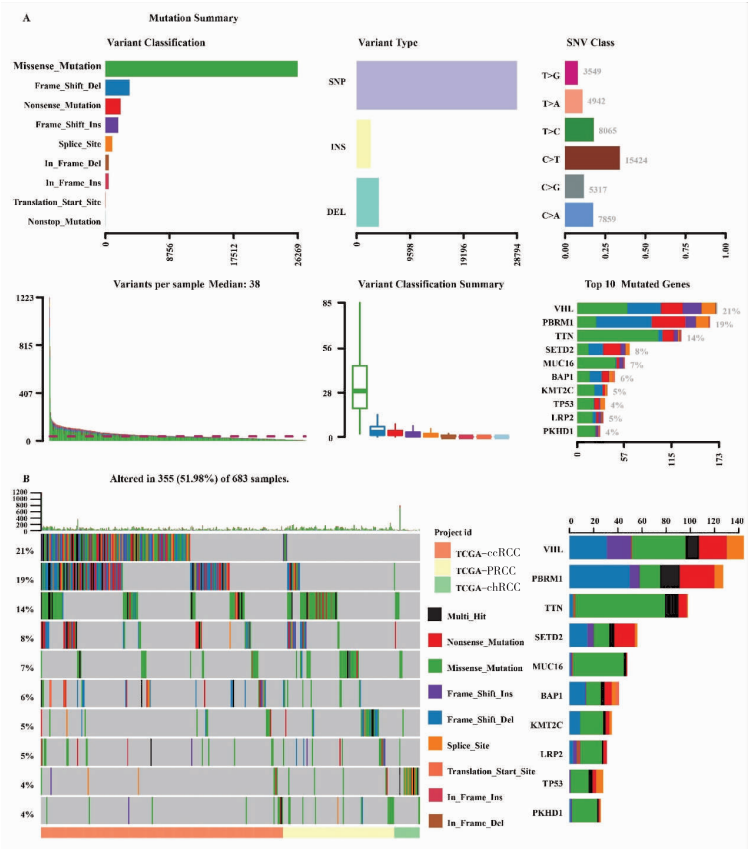

Figure 10. Mutation Landscape of Kidney Cancer

A. The total mutation frequency in kidney cancer; B. The landscape of genetic alteration of kidney cancer.

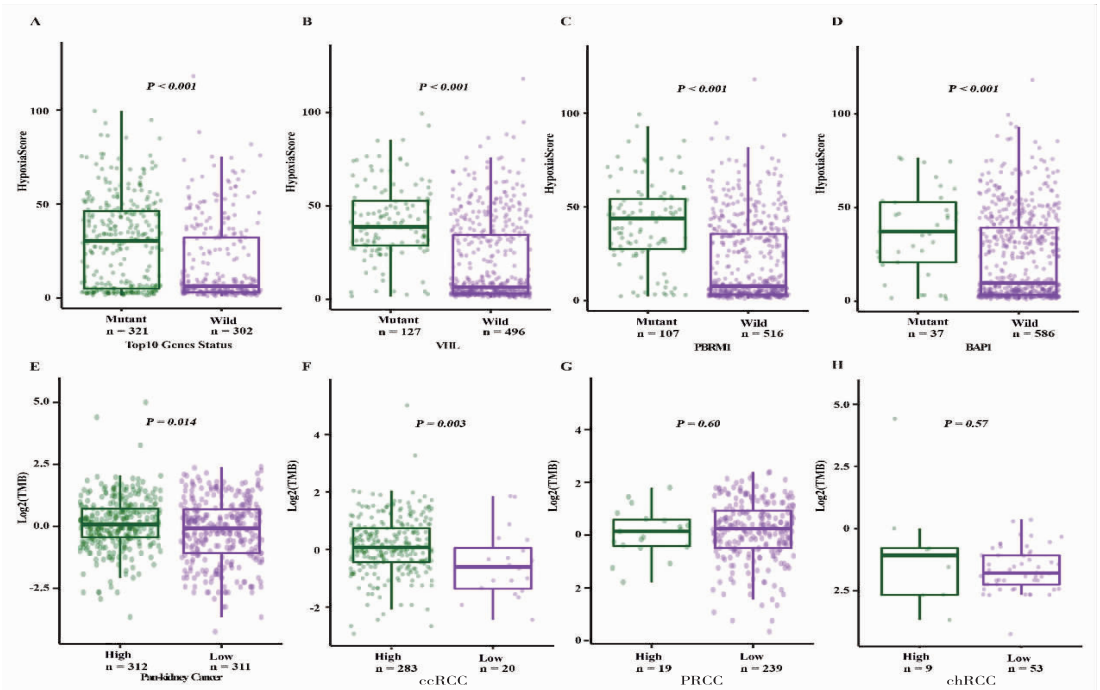

Figure 11. Relationship between HRGPI and Gene Mutation

The relation of HRGPI to top 10 mutated genes ($P < 0.001$), *VHL* ($P < 0.001$), *PBRM1* ($P < 0.001$) and *BAP1* ($P < 0.001$), respectively, as indicated by the first row (Panel A, B, C and D); a high HRGPI was related to high TMB in pan-kidney cancer ($P = 0.014$) and ccRCC ($P = 0.003$) as indicated by the second row (Panel E, F, G and H).

TMB: Tumor mutation burden.

DISCUSSION

Hypoxia is a common phenomenon of oxygen tension in solid cancers[14]. Hypoxia plays important roles in the development and metastasis of solid cancer[15]. In the TME of cancer, hypoxia leads to dis-balance of pro-angiogenic factors and anti-angiogenic factors, and results in increased formation of blood vessels in the tumor[16]. In addition, hypoxia induces cancer cell invasion and migration by epithelial-mesenchymal transition[17]. Furthermore, hypoxia causes immune suppression and resistance, leading to immune surveillance escape[18]. This oxygen tension activates a complex network of signaling pathways such as NF-κB, MAPK and JAK-STAT. Hypoxia in the tumor also contributes to anti-cancer chemotherapy and radiotherapy[19]. So, it is important to investigate the roles and mechanisms of hypoxia in solid cancer and propose new strategies for cancer treatment.

Hypoxia is considered as a common biological feature in kidney cancer. The hypoxia factor, HIF-1, is a critical factor in the carcinogenesis and progression of kidney cancer[20]. The 7 hypoxia-related genes screened in our study were *SLC16A1*, *VEGFA*, *KIF20A*, *CA9*, *PFKFB4*, *PYGL* and *TMEM30B*. ① SLC16A1 encodes monocarboxylate transporter 1, which could open to the extracellular matrix[21]. ② *VEGFA* modulates vasculogenesis, angiogenesis, endothelial cell growth, and cell migration[22]. The expression of *VEGFA* is regulated by HIF-1 to invoke hypoxia response elements[23]. ③ *KIF20A* regulates ATPase activity and protein kinase binding. ④ *CA9* belongs to the family of zinc metalloenzymes involved in the biological functions of respiration, bone resorption, and calcification. It is induced by hypoxia and regarded as an endogenous biomarker in hypoxia cells[24]. ⑤ *PFKFB4* is induced by hypoxia and is highly expressed in kidney cancer, which is important in cancer cell survival. ⑥ *PYGL* is an isoform that belongs to *PYG* family and is involved in hypoxia-regulated cancer metabolic pathways. ⑦ *TMEM30B* participates in the maintenance of the asymmetric distribution of phospholipids. Thus, these 7 hypoxia-related genes were involved in multiple mechanisms in kidney cancer.

Then, we established HRGPI to evaluate the survival of kidney cancer. HRGPI could function as an independent predictor of kidney cancer. High HRGPI was found to be correlated to advanced stages of kidney cancer. The high risk group (including pan-kidney cancer, ccRCC, PRCC and chRCC patients) showed a lower survival compared with the low risk group. HRGPI was, thus, inferred as a valuable diagnostic marker in kidney cancer.

Furthermore, we investigated the mechanisms of hypoxia in kidney cancer. Hypoxia is involved in the inflammatory-related pathways, including TNF-α signaling pathway, IL2-STAT5 signaling pathway, IL6-JAK-STAT3 signaling pathway, inflammatory response, interferon-alpha response and interferon-gamma response. As for inflammatory cells, hypoxia-related genes are positively correlated to type 2 T helper cells, regulatory T cells, activated $CD4^+$ T cells, natural killer cells and effector memory $CD8^+$ T cells. Our study indicated that hypoxia is correlated to inflammatory status in the TME of kidney cancer. Inflammatory pathways and inflammatory cells are proven to be involved in the development and metastasis of kidney cancer[25]. The inflammatory mediators produced by immune cells promote tumor cell proliferation, survival and transformation. TNF-α signaling pathway is correlated to the tumorigenesis in kidney cancer. TNF-α stimulates epithelial-mesenchymal transition in kidney cancer cells by increasing the production of matrix metalloproteinase 9 and E-cadherin[26]. The ligation of TNF receptors activates *VEGFR2* and *ERK* to promote tumor cell proliferation[27]. JAK-STAT signaling pathways play pivotal roles in cytoplasmic signaling. STATs could exert anti-apoptotic effects by up-regulating the levels of survivin and B-cell lymphoma-extra large (Bcl-xL) protein[28]. Moreover, we also discovered the correlation between hypoxia-related genes and inhibitory immune checkpoints. Hypoxia-related genes are correlated to *TGFβ1*, *TIGIT*, *LAG3*, *CTLA4* and *PDCD1*. These immune checkpoints are biologically and clinically functioning in kidney cancer. *CTLA4* inhibits the costimulation via CD28 to decrease the function of inflammatory T cell response[29]. *CTLA4* blockade could increase the anti-tumor effects of T cells.

PD-1 is expressed in macrophages, B cells, and T cells[30]. PD-*1* and *TIGIT* cause immune escape in tumor cells to exacerbate kidney cancer. So, hypoxia is related to immune response in kidney cancer as an independent risk factor.

Lastly, we evaluated the correlation between HRGPI and gene mutations. Hypoxia is related to elevated genomic instability in many types of tumors, including lung cancer, kidney cancer, cervix cancer, etc. Hypoxic tumors exhibit characteristic driver-mutation signatures. Tumor hypoxia would lead to aggressive molecular characteristics. Hypoxia status is correlated to poor clinical prognosis as well as resistance to chemotherapy and radiotherapy. High HRGPI is correlated to mutations in *VHL*, *PBRM1* and *BAP1*. *VHL* is a tumor suppressor gene leading to tumor cell proliferation and growth[31]. *PBRM1* and *BAP1* are driver genes in kidney cancer[32]. HRGPI is also correlated to TMB in kidney cancer.

CONCLUSION

In this study, we have discovered the roles and mechanisms of hypoxia in kidney cancer. Firstly, we have recognized 7 hypoxia related genes of kidney cancer. Secondly, we have developed HRGPI by multivariate Cox regression analysis. There is a lower chance of survival in the high-risk kidney cancer patients compared with the low-risk patients. HRGPI is an independent predictor of kidney cancer, and is correlated to advanced stages of kidney cancer. Hypoxia-related genes are correlated to inflammatory-related pathways, inflammatory cells and inhibitory immune checkpoints. These results indicate that hypoxia is related to immune response in kidney cancer. Lastly, we have found that hypoxia is related to gene mutations in kidney cancer. In conclusion, we have revealed that hypoxia is a risk factor in kidney cancer which might serve as a therapeutic target in the treatment of kidney cancer in the future.

REFERENCES

[1] Chow W, Dong L, Devesa S. Epidemiology and risk factors for kidney cancer [J]. Nat Rev Urol, 2010, 7(5): 245-257.

[2] Capitanio U, Montorsi F. Renal cancer [J]. Lancet, 2016, 387 (10021): 894-906.

[3] Motzer R, Jonasch E, Agarwal N, *et al*. Kidney Cancer, Version 2.2017, NCCN Clinical Practice Guidelines in Oncology [J]. J Natl Compr Canc Netw, 2017, 15(6): 804-834.

[4] Schmidt L, Linehan W. Genetic predisposition to kidney cancer [J]. Semin Oncol, 2016, 43(5): 566-574.

[5] Sato Y, Yoshizato T, Shiraishi Y, *et al*. Integrated molecular analysis of clear-cell renal cell carcinoma [J]. Nat Genet, 2013, 45(8): 860-867.

[6] Khoshinani H, Afshar S, Najafi R. Hypoxia: A double-edged sword in cancer therapy [J]. Cancer Invest, 2016, 34(10): 536-545.

[7] Gilkes D, Semenza G, Wirtz D. Hypoxia and the extracellular matrix: Drivers of tumour metastasis [J]. Nat Rev Cancer, 2014, 14 (6): 430-439.

[8] Pugh C, Ratcliffe P. Regulation of angiogenesis by hypoxia: Role of the HIF system [J]. Nat Med, 2003, 9(6): 677-684.

[9] Kumar V, Gabrilovich D. Hypoxia-inducible factors in regulation of immune responses in tumour microenvironment [J]. Immunology, 2014, 143(4): 512-519.

[10] Bhandari V, Hoey C, Liu L, *et al*. Molecular landmarks of tumor hypoxia across cancer types [J]. Nat Genet, 2019, 51(2): 308-318.

[11] Karakashev S, Reginato M. Progress toward overcoming hypoxia-induced resistance to solid tumor therapy [J]. Cancer Manag Res, 2015, 7: 253-264.

[12] David B, Pablo T, Jesse B, *et al*. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1 [J]. Nature, 2009, 462: 108-112.

[13] Hubert H, Pornpimol C, Francesca F, *et al*. Computational genomics tools for dissecting tumour - immune cell interactions [J]. Nat Rev Genet, 2016, 17: 441-458.

[14] Jing X, Yang F, Shao C, *et al*. Role of hypoxia in cancer therapy by regulating the tumor microenvironment [J]. Mol Cancer, 2019, 18(1): 157.

[15] Nobre A, Entenberg D, Wang Y, *et al*. The different routes to metastasis via hypoxia-regulated programs [J]. Trends Cell Biol, 2018, 28(11): 941-956.

[16] Martin J, Fukumura D, Duda D, *et al*. Reengineering the tumor microenvironment to alleviate hypoxia and overcome cancer heterogeneity [J]. Cold Spring Harb Perspect Med, 2016, 6(12): a027094.

[17] Gonzalez D, Medici D. Signaling mechanisms of the epithelial-mesenchymal transition [J]. Sci Signal, 2014, 7(344): re8.

[18] Chouaib S, Messai Y, Couve S, *et al*. Hypoxia promotes tumor growth in linking angiogenesis to immune escape [J]. Front Immunol, 2012, 3: 21.

[19] Minassian L, Cotechini T, Huitema E, *et al*. Hypoxia-induced resistance to chemotherapy in cancer [J]. Adv Exp Med Biol, 2019, 1136: 123-139.

[20] Schödel J, Grampp S, Maher E, *et al*. Hypoxia, hypoxia-inducible transcription factors, and renal cancer [J]. Eur Urol, 2016, 69(4): 646-657.

[21] Halestrap A. The SLC16 gene family - structure, role and regula-

tion in health and disease [J]. Mol Aspects Med, 2013, 34(2-3): 337-349.
[22] Claesson-Welsh L, Welsh M. VEGFA and tumour angiogenesis [J]. J Intern Med, 2013, 273(2): 114-127.
[23] Rehn M, Olsson A, Reckzeh K, *et al*. Hypoxic induction of vascular endothelial growth factor regulates murine hematopoietic stem cell function in the low-oxygenic niche [J]. Blood, 2011, 118(6): 1534-1543.
[24] Olive P, Aquino-Parsons C, MacPhail S, *et al*. Carbonic anhydrase 9 as an endogenous marker for hypoxic cells in cervical cancer [J]. Cancer Res, 2001, 61(24): 8924-8929.
[25] de Vivar Chevez A, Finke J, Bukowski R. The role of inflammation in kidney cancer [J]. Adv Exp Med Biol, 2014, 816: 197-234.
[26] Ho M, Tang S, Chuang M, *et al*. TNF-α induces epithelial-mesenchymal transition of renal cell carcinoma cells via a GSK3β-dependent mechanism [J]. Mol Cancer Res, 2012, 10(8): 1109-1119.
[27] Al-Lamki R, Sadler T, Wang J, *et al*. Tumor necrosis factor receptor expression and signaling in renal cell carcinoma [J]. Am J Pathol, 2010, 177(2): 943-954.
[28] Aoki Y, Feldman G, Tosato G. Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma [J]. Blood, 2003, 101(4): 1535-1542.
[29] Pardoll D. The blockade of immune checkpoints in cancer immunotherapy [J]. Nat Rev Cancer, 2012, 12(4): 252-264.
[30] Patel S, Kurzrock R. PD-L1 Expression as a Predictive biomarker in cancer immunotherapy [J]. Mol Cancer Ther, 2015, 14(4): 847-856.
[31] Zhang J, Zhang Q. VHL and hypoxia signaling: Beyond HIF in cancer [J]. Biomedicines, 2018, 6(1): 35.
[32] Brugarolas J. PBRM1 and BAP1 as novel targets for renal cell carcinoma [J]. Cancer J, 2013, 19(4): 324-332.

· 读者 · 作者 · 编者 ·

关于研究生毕业论文投稿版权问题的声明

依照教育部门相关规定，研究生在读期间所撰写的学位论文，版权归属于所就读院校。据此，本刊规定凡研究生发表的与其学位论文密切相关的学术文章，均应在文章中明确写明版权单位，如作者同时具有其他单位的，可以一并列出。欢迎广大研究生将学位论文以论著、综述等形式投稿本刊，原则上稿件第一作者与学位论文完成人一致，稿件与学位论文重复率不能超过 20%。对于优秀研究生稿件，本刊将开通绿色通道，减免部分版面费，优先发表。

本刊编辑部