Construction and validation of an autophagy-related long non-coding RNA signature to predict the prognosis of kidney renal papillary cell carcinoma

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ABSTRACT

To identify the autophagy-related long non-coding RNAs (ARlncRNAs) associated with the prognosis of kidney renal papillary cell carcinoma (KIRP), thereby establishing a clinical prognostic model. The gene expression matrix and clinical survival information of patients with KIRP were downloaded from The Cancer Genome Atlas database, and were divided into the training and testing groups. ARlncRNAs associated with the KIRP prognosis were analyzed by univariate, Least Absolute Shrinkage and Selection Operator (LASSO(, and multivariate Cox regression to construct a signature. We combined clinical factors associated with the prognosis with ARlncRNAs to establish a prognostic model of patients with KIRP. A nomogram was established to predict 1-year, 3-year, and 5-year survival of patients with KIRP. Besides, we built the lncRNA-messenger RNA co-expression network and used Kyoto Encyclopedia of Genes and Genomes and Gene Set Enrichment Analysis to detect the biological functions of ARlncRNAs. LEF1-AS1, CU634019.6, C2orf48, AC027228.2, and AC107464.3 were identified. A prognosis-related ARlncRNAs signature was constructed in the training group and validated in the testing group. Patients with KIRP with a low risk score had significantly longer survival time than those with a high risk score. The risk score significantly affected the prognosis of patients, thereby being used for modeling. The area under the receiver operating characteristic curve values of 1-year, 3-year, and 5-year overall survival were 0.80, 0.78, and 0.84 in the training group, respectively. The signature had high concordance index and good accuracy in predicting the prognosis, which were confirmed by the nomogram. The prognosis-related ARlncRNAs signature we identified had a more accurate prediction for the prognosis of patients with KIRP.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Long non-coding RNA (lncRNA) is a key regulator of gene expression.
⇒ It can affect the occurrence and development of diseases through different mechanisms.
⇒ Its role in the occurrence and development of cancer has also been paid more and more attention to.

WHAT THIS STUDY ADDS

⇒ Our presented paper used bioinformatics methods to construct a prognostic model of autophagy-related lncRNAs (ARlncRNAs).
⇒ We used the identified five ARlncRNAs to establish a prognostic model of kidney renal papillary cell carcinoma (KIRP).
⇒ We carried out relevant tests and microgenetic analysis.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ It was designed to provide a theoretical basis for predicting the prognosis of KIRP.
⇒ The existing database was used to fill the gap of the lack of KIRP-related prognostic model as much as possible.
⇒ It might provide a new insight for the treatment of KIRP in the future.

INTRODUCTION

Renal cell cancer (RCC) is one of the most common cancers worldwide, killing about 170,000 people per year.[1] The typical clinical manifestations of renal cancer include hematuria, mass, and pain, but most of patients have no specific signs in the early stage, and half of the patients were discovered during physical examination or other medical examinations.[2–4] Early stage renal tumor can be surgically removed, and the prognosis is good. However, when renal tumor recurs and metastasizes, chemotherapy and immunotherapy are main therapeutic options, risk models associated with clinical prognosis can help predict survival for patients with RCC experiencing different therapeutic options. Currently, commonly used criteria include Memorial Sloan Kettering Cancer Center (MSKCC) criteria,[5] and International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) criteria.[6] The prognosis of patients with different risk levels is different, and the treatment plan changes accordingly. With the advent of the era of precision medicine, individualized treatment is

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gradually becoming the mainstream trend, and individual differences and tumor heterogeneity will affect the treatment outcomes. However, MSKCC and IMDC criteria fail to take this into consideration. Among the pathological types of renal cancer, clear cell renal cell carcinoma (ccRCC) is the most common subtype, accounting for about 70%–80% of the total renal cancer. Therefore, most of past studies focused on ccRCC. There are relatively few studies on other types of RCC. Kidney renal papillary cell carcinoma (KIRP) is the second most common subtype, accounting for 15%–20% of all RCCs.[7] Compared with ccRCC, KIRP has a better clinical prognosis due to its lower malignancy. If the prognosis of these patients could be accurately predicted by gene checking, it can effectively guide clinical diagnosis and treatment, and improve clinical survival. However, so far, there is few research on the specific clinical prognostic biomarkers and prognostic models for KIRP.

Autophagy, known as the gene-related endogenous autophagy, is under the strict control of highly adaptive cell metabolic process itself, and is responsible for aggregation, degradation, and recycling of the damaged or dysfunctional organelles and protein to adapt to changes of nutritional environment and maintain cell steady state, which is the important mechanism for the cell development, differentiation, and survival.[8] Cancer cells, under stress conditions, can use this autophagy process to generate alternative energy through nutrient recycling, but excessive activation of autophagy might cause cancer cells death.[9] Autophagy is also closely related to RCC. Bray *et al* found that kidney cancer has a high level of autophagy, and cells with basic autophagy account for about 30%–60%, while in normal kidney tissue, only 1%–5% of cells have obvious autophagy markers.[10] Therefore, autophagy inducers or inhibitors might be promising for the treatment of renal cancer. For example, sunitinib can inhibit the AKT/mammalian target of rapamycin (mTOR) signaling pathway and cause autophagy in renal cancer cells, thereby delaying the development of cancer.[11 12] Previous studies have confirmed that autophagy-related genes can be used as prognostic biomarkers for RCC, verified the correlation between the survival status of patients with renal cancer and autophagy, and constructed a prognostic model of renal caner.[13 14] However, there is no relevant research on KIRP, and how the autophagy regulates the occurence and development of KIRP is still unclear.

Autophagy is mainly divided into four stages: activation and initiation, free membrane formation of autophagosome, fusion of autophagosome and lysosome, and degradation; each stage depends on the protein action elements produced during the transcription and translation of various autophagy-related genes; the nucleotide length of long non-coding RNA (lncRNA) can regulate the function and activity of autophagy-related DNA, RNA, or protein, or affect autophagy-related stress factors and energy receptors, so as to participate in the regulation of autophagy.[15] The nucleotide length of lncRNA exceeds 200. In the past, it was believed that lncRNA had no ability of coding protein. However, with in-depth study of biological functions and clinical significance of these lncRNAs, it is found that lncRNA, as a key regulator of gene expression, can affect the occurrence and development of diseases through different mechanisms, and its role in the occurrence and development of cancer has also been paid more and more attention to.[16 17]

Therefore, our presented paper used bioinformatics methods to construct a prognostic model of autophagy-related lncRNAs (ARlncRNAs), which was designed to provide a theoretical basis for predicting the prognosis of KIRP. It is assumed that lncRNA might affect tumor progression by regulating autophagy, and ARlncRNAs might also be used to predict the progression and prognosis of KIRP.[18]

In order to gain insight into the clinical efficacy of lncRNA in evaluating the prognosis of KIRP, we screened ARlncRNAs to predict the survival of patients with KIRP, and combined with their clinicopathological characteristics, we established a more personalized and precise predictive model for the prognosis of patients with KIRP, which was expected to provide new ideas for clinical decision-making.

MATERIALS AND METHODS

Data collection and preparation

We downloaded fragments per kilobase of exon model per million mapped fragments (FPKM) standardized RNA-sequencing data and clinical information from the KIRP cohort in The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) database. The original data were normalized and processed into gene matrix by the R program, and then we obtained a messenger RNA (mRNA) matrix, of which the abscissa was the specimen name, and the ordinate was the gene name. To select autophagy-related genes, we visited The Human Autophagy Database (http://autophagy.lu/clustering/index.html), it is the first human autophagy-dedicated database, and is a public repository containing information about the human genes involved in autophagy. We entered this website, then clicked 'clustering' option, and finally obtained 232 autophagy related genes (ARGs) that were used for the subsequent analysis. Based on the $|R|^2 > 0.5$ and $p<0.001$ of Pearson's correlation coefficient, ARlncRNAs were selected. The clinical data of 291 patients with KIRP were downloaded from TCGA, but only 285 patients were recorded with clear survival and gene expression information, which were combined with the data of ARlncRNA expression for subsequently constructing prognostic model. Among these 285 patients, 256 patients had complete clinicopathological data records.

Constructing a prognosis-related ARlncRNA signature

The clinical prognostic model was constructed by combining gene expression data with clinical data. The subjects were randomly assigned into the training group (144 cases) and the testing group (141 cases). The χ^2 test was used to detect the difference of the clinical characteristics between each case. We used the training group to construct the clinical prognostic model and used the testing group to validate this model. ARlncRNAs significantly associated with prognosis ($p<0.05$) were identified in the training group using Cox univariate regression analysis, and the overcorrelated ARlncRNAs were removed by LASSO regression. Then, the risk scores formula of the patients were established by multivariate Cox proportional hazards regression for the identification of the independent prognostic genes. Before constructing the Cox regression model, Schoenfeld residuals was used to test proportional hazard regression model.

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If the data met the Cox regression model, there was no obvious correlation between the Schoenfeld residuals and the rank of survival time, which indicated that there was no obvious consistent trend in the changes of Schoenfeld residual error and survival time ($p>0.05$), and the judgment was made here by the cox.zph() function of the survival package. Finally, five prognosis-related ARlncRNAs were identified. The calculation of risk scores was performed based on the risk coefficients of different prognostic genes, and the calculation formula was: risk score=$\sum$ ni=1coef(i)×lncRNA (i) expression (formula A).

Evaluating the prognostic signature in the training group and validation in the testing group

We used the maxstat package of R software (maximally selected rank statistics with several p value approximations version: 0.7–25) to calculate the best cut-off value of riskScore. Based on this cut-off value, we divided the patients into high-risk and low-risk groups, and further used the survfit function of survival package of the R software to do the analysis of the prognostic difference between the two groups. The logrank test method was used to evaluate the significance of the prognostic difference between samples in different groups. The Kaplan-Meier survival curve was used to do the comparison of the survival results between the both groups. According to the risk score, we ranked the patients with KIRP of the training group, and the relationship between the risk score and the risk grade was represented by a distribution curve; scatter dot plots were drawn to show the association between different risk scores and survival time; heat maps were used to show the distribution of risk scores, the number of patients in the high-risk and the low-risk groups, and the relationship between lncRNAs associated with prognosis. Finally, according to the cut-off value, the same grouping criteria and comparison method were performed on 141 patients in the testing group to detect the accuracy of prognostic risk-related genes in different groups.

Analysis of independent prognostic factors and correlation analysis of clinical characteristics

The risk score and different clinical characteristics (stage, gender, and age) were taken as possible independent prognostic factors. Before Cox regression, similarly, Schoenfeld residuals was used to test proportional hazard regression model for three clinical characteristics of patients. After that, univariate Cox regression analysis and multivariate Cox regression analysis were performed to assess the association between these factors and patients' prognosis. The selected clinical factors and prognostic risk-related genes were combined to establish a prognostic model. Then we used the pROC package of R software (V.1.17.0.1) to perform receiver operator characteristic (ROC) curve analysis to obtain the area under ROC curve (AUC). Specifically, we used the ROC function of pROC package to do the ROC analysis at 1 year, 3 years, and 5 years, and used the ci function of pROC package to calculate the AUC and CI.

Establishment of a prognostic nomogram

A nomogram was established using the training group to do the prediction of 1-year, 3-year, and 5-year survival for patients with KIRP by the risk score. Then, the consistency between predicted survival results and observed survival results was assessed by concordance index (C-index) and the calibration chart in the training group.

Constructing mRNA-ARlncRNA co-expression network, Kyoto Encyclopedia of Genes and Genomes pathway analysis, and Gene Set Enrichment Analysis of all genes in patients with KIRP

The mRNA-ARlncRNA co-expression network was visualized by Cytoscape to better demonstrate the relationship between the prognosis-related ARlncRNAs and targeted mRNA, and the evaluation standard was the Pearson's correlation coefficient, with the correlation coefficient of $|R|^2>0.5$ and $p<0.001$. Sankey plots were used to detect the association between gene co-expression networks and patient risk assessment. For Gene Set Enrichment Analysis (GSEA), we used Kyoto Encyclopedia of Genes and Genomes (KEGG) rest API (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest KEGG Pathway gene annotations, and based on this, the genes were mapped into the background set. The clusterProfiler package (V.3.14.3) of R software was used for enrichment analysis to obtain the results of gene set enrichment. The minimum gene was set to 5 and the maximum gene to 5000. $P<0.05$ and false discovery rate (FDR) <0.25 were considered statistically significant. In order to understand the whole gene set enrichment pathway in high-risk and low-risk patients, KEGG enrichment analysis was performed using the GSEA_4.1.0 software, and $|$ normalized enrichment score (NES) $|>1$, NOM p value <0.05, and FDR q value <0.25 under the pathways of gene set were thought to be significant.

Statistical analysis

R software (V.4.0.5) was used to do the statistical analysis. The Perl programming language (V.5.30.2) was used to process the data. $P<0.05$ meant statistically significant.

RESULTS

Identification of five ARlncRNAs

First, 56,753 genes expressed in patients with KIRP were downloaded from TCGA database, and among them, 14,143 lncRNAs were selected from them. Then, we obtained 3317 ARlncRNAs. Finally, the ARlncRNAs expression was combined with the clinical information (survival status, survival information) in the form of matrix. After obtaining the gene-clinical expression matrix of all samples, we divided them into the training group and the testing group randomly, and there was no significant difference in the clinical characteristics (age, gender, stage) between the two groups (table 1). Then, univariate Cox proportional hazards regression analysis of the expression of 3317 ARlncRNAs in the training group was performed, and it was found that the expressions of 78 lncRNAs were significantly correlated with the KIRP prognosis ($p<0.05$). As shown in figure 1A,B, through the LASSO regression analysis, we obtained 11 independently correlated lncRNAs. Ultimately, multivariate Cox proportional hazards regression analysis was performed (figure 1C), there was no significant correlation between the Schoenfeld residuals of all

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Table 1 Clinical features of patients with KIRP

Variables	Type	Entire group (n=256)	Training group (n=132)	Testing group (n=124)	P value
Age (years)	≤65	157	78	79	0.448
	>65	99	54	45	
Gender	Female	67	37	30	0.485
	Male	189	95	94	
Stage	Stage I–II	191	100	91	0.663
	Stage III–IV	65	32	33	

KIRP, kidney renal papillary cell carcinoma.

ARlncRNAs and the rank of survival time ($p>0.05$), and finally five ARlncRNAs were identified to establish the prognostic risk score model by the 'Formula A' as follows: risk score=(0.136481)×LEF1-AS1+(0.013083)×AC027228.2+(0.133607)×CU634019.6+(0.021938)×C2orf48+(−0.007731)×AC107464.3. Therefore, LEF1-AS1, CU634019.6, C2orf48, and AC027228.2 were thought to be high-risk gene factors, as well as major factors affecting KIRP prognosis. These genes were expressed to varying degrees in all patients, so based on the median value of gene expression (figure 1D–G), the patients were divided into high gene expression group and low gene expression group. When the above four genes highly expressed, the survival time of patients decreased ($p<0.05$). In contrast, AC107464.3 (figure 1H) was a protective factor ($p<0.05$). In addition, the cross of two lines in figure 1D might indicate that only LEF1-AS1 was not reliable to predict the survival situation, but the $p<0.05$ showed us that this gene could be used to construct the prognostic model. The essence of Kaplan-Merier curve is a single factor analysis, but survival is affected by multiple factors. Therefore, finally, we used five genes to establish a prognosis model.

Validation of the prognostic model

For further verifying the reliability and accuracy of our prognostic model, first we calculated the risk score according to the model. We used the 'maxstat' package of R software (maximally selected rank statistics with several p value approximations version: 0.7–25) to calculate the best cut-off value of riskScore, and set the minimum number of grouping samples to be >25%, and the maximum number to be <75%. Finally, 0.3973808 of the best cut-off value was obtained. Based on this best cut-off value, patients in the training group were divided into high-risk and low-risk groups. The survfit function of the survival package of R software was further used to analyze the prognostic differences between the two groups. The logrank test method was used to evaluate the significance of differences between different groups, the results of which showed the prognostic difference between the two groups was significant, and finally we observed a significant prognostic difference ($p=1.6e-10$) (figure 2A). Based on the same cut-off value, patients in the testing set were divided into the high-risk group and the low-risk group. The overall survival (OS) of patients with KIRP with low risk score were significantly better than those with high risk score ($p=2.5e-5$) (figure 2B).

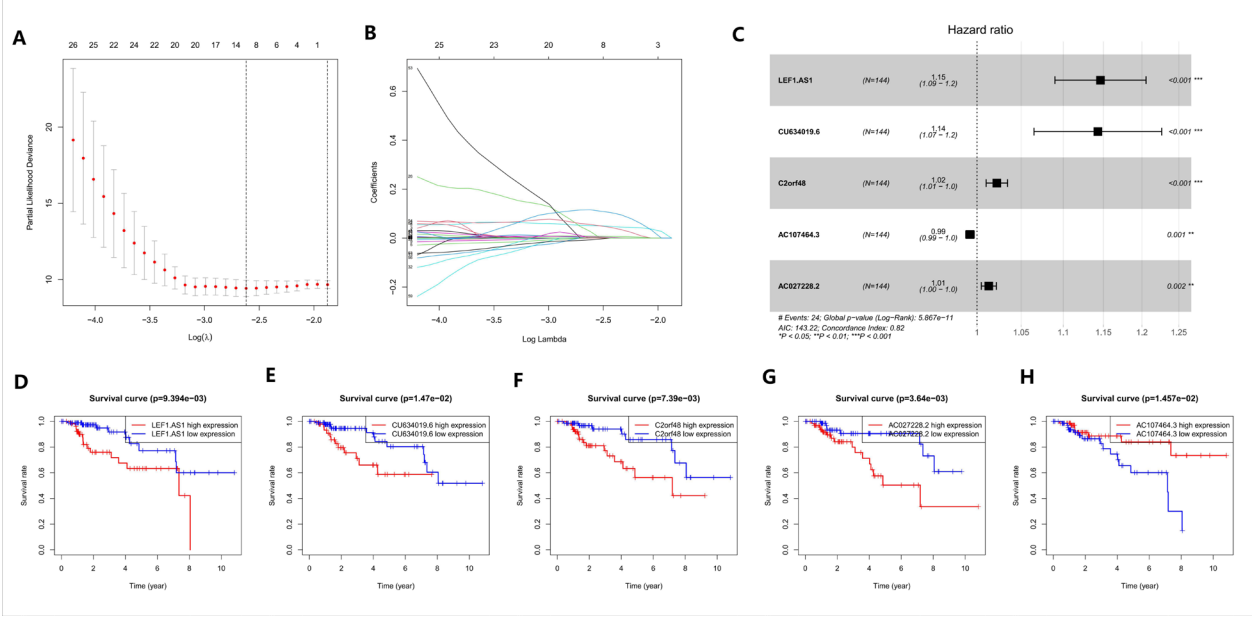

Figure 1 In the training group, LASSO analysis was conducted on 11 genes selected by univariate Cox regression analysis, and finally 5 autophagy-related long non-coding RNAs (ARlncRNAs) were identified. (A) LASSO coefficient curves were selected, with simulation parameters set to 1000, (B) partial likelihood function deviation and log (λ), and the cross validation parameter was set to 1000, λ=−2.8 the minimum number of genes, (C) the forest map showing the HR and 95% CIs of the identified nine ARlncRNAs. (D–G) Kaplan-Meier curve of relationship between high-risk gene expression and survival time. (H) Kaplan-Meier curve of the survival time of the cases increased with the high expression of protective factors. *P<0.05; **p<0.01; ***p<0.001.

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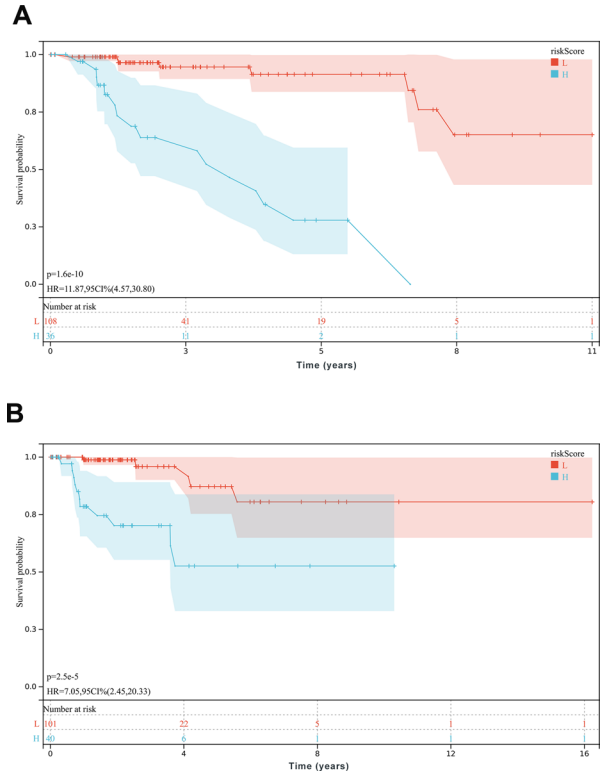

Figure 2 (A) Kaplan-Meier curves of the high-risk group and the low-risk group in the training group; (B) Kaplan-Meier curves of the high-risk group and the low-risk group in the testing group.

The gene level and clinical feature level of different groups

According to the cut-off value, the patients were evenly distributed in the high-risk group and the low-risk group (figure 3A,B). The scatter dot plot revealed that in the training group (figure 3C) and the testing group (figure 3D), the high-risk group both had more deaths. The heat map (figure 3E,F) indicated that KIRP cases with the high risk score had higher-level expression of risk factors, whereas KIRP cases with the low risk score had higher-level expression of protective factors. The expression level of risk genes was higher in the high-risk KIRP cases, including LEF1-AS1, CU634019.6, C2orf48, and AC027228.2, whereas patients with low-risk KIRP had higher-level expression of protective genes, including AC107464.3.

Clinical significance of the prognosis-related ARlncRNA signature

There was no significant correlation between the Schoenfeld residuals of two clinical factors (gender and age) and the rank of survival time ($p>0.05$), while there was significant correlation between the Schoenfeld residuals of the stage and the rank of survival time ($p<0.05$). Therefore, the stage was not used for the Cox regression analysis. Univariate and multivariate Cox regression analysis was performed to verify the associations between the survival time and the clinical characteristics (age and gender). The results showed that only the risk score was the independent prognostic factor in the training group (figure 4A,B). Multivariate Cox analysis suggested that risk score was significant factor for KIRP survival status. Therefore, we used the risk score to predict the prognosis of patients with KIRP, and used the linear regression model to establish a prognostic model. The ROC curve showed that the model had good predictive power and high accuracy to predict the survival status of patients at 1, 3, and 5 years (figure 4C). Based on this, we also constructed a nomogram with a C-index value of 0.805 (figure 4D) to broaden the clinical applicability of the prognostic model. In addition, the calibration chart showed that the nomogram had similar performance with the ideal model (figure 4E).

Construction of the ARlncRNA-mRNA co-expression network and functional enrichment analysis

An lncRNA-mRNA co-expression network containing 15 lncRNA-mRNA pairs was constructed to explore the potential biological function of the 5 prognosis-related ARlncRNAs (figure 5A). The Sankey diagram showed the association between the five prognosis-related ARlncRNAs and targeted mRNAs as well as risk types included risk or protective factors (figure 5B). KEGG pathway analysis of targeted mRNA revealed that ATG16L2, ATG4B, and ATG9B were involved in autophagy, GRID2 and ITPR1 were involved in long-term depression, and BIRC5 and CASP3 were involved in the growth of colorectal cancer (figure 5C).

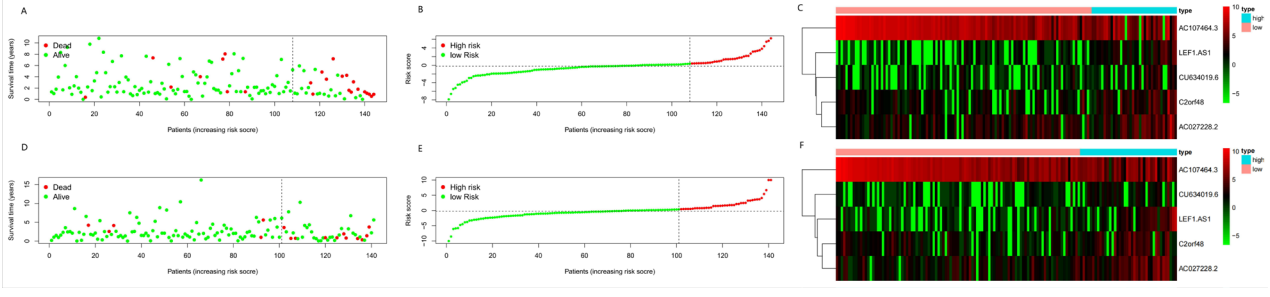

Figure 3 Assessment of the prognostic signature in the training group and validation in the testing group. The risk score distribution of patients with kidney renal papillary cell carcinoma (KIRP) in the training group (A) and the testing group (B). Scatter dot plot displayed survival outcomes of the high-risk group and the low-risk group in the training group (C) and the testing group (D). Heat map suggested the expressions of five prognosis-related autophagy-related long non-coding RNAs (ARlncRNAs) of patients with KIRP with high risk score and the low risk score in the training group (E) and the testing group (F).

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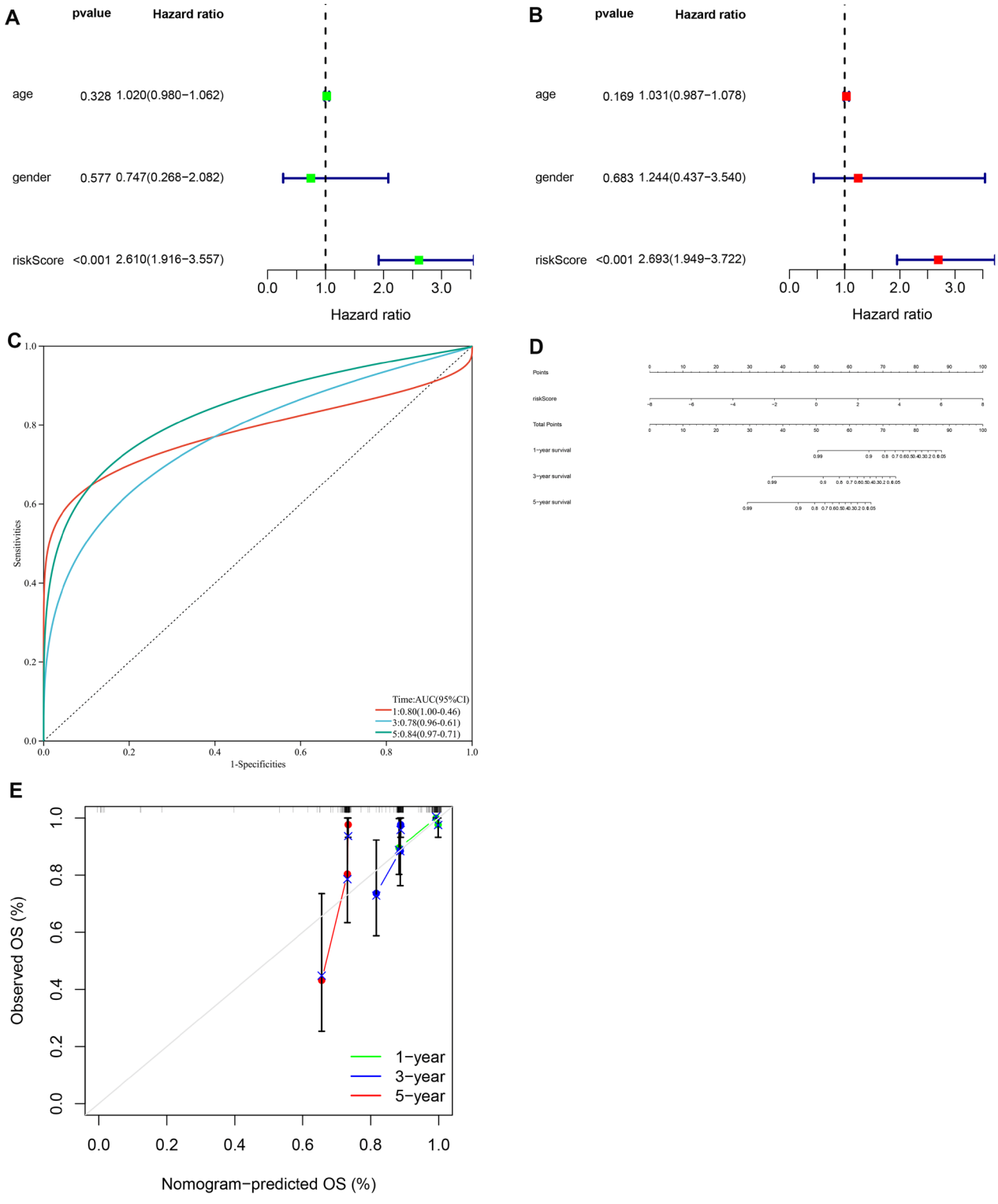

Figure 4 Correlation analysis between risk factors and patient prognosis in the training group (A, B); receiver operating characteristic (ROC) curve of clinical stage combined with risk score to predict patient survival in 1, 3, and 5 years (C); nomogram for multi-index joint prediction of kidney renal papillary cell carcinoma (KIRP) (D); calibration chart for joint prognostic model (E). OS, overall survival.

For the training group, in order to better understand the significant gene enrichment pathway of KIRP, we conducted GSEA on the genes in the high-risk group and low-risk group, respectively. A total of 55,269 genes were included in the training group, of which, 28,731 (52.0%) genes were included in the high-risk group and 26,538 (48.0%) genes in the low-risk group. The heat map (figure 5D) shows the differentially expressed genes among patients in different risk groups. We performed GSEA for the different risk groups, which showed that in the high-risk group, gene set

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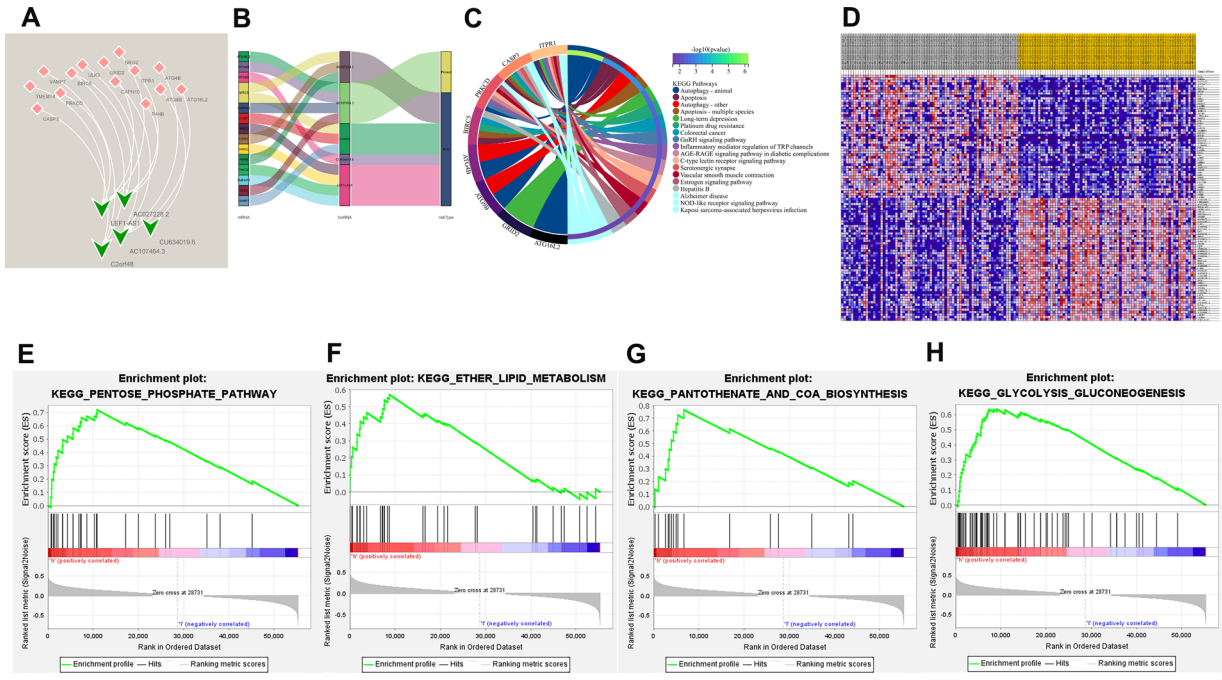

Figure 5 Co-expression network analysis of ARlncRNA-mRNA and gene enrichment analysis of patients with KIRP. Eighteen pairs of ARlncRNA-mRNA co-expression network (A) and the relationship between gene pairs and patients' risk (B); KEGG significant pathway analysis about target mRNA (C); in the training group, the high-risk (gray) and low-risk (yellow) patients were analyzed by differential genes analysis (D), and the high-risk patients in the training group were analyzed by GSEA, showing some upregulated gene sets (E–H). ARlncRNAs, autophagy-related long non-coding RNAs; GSEA, Gene Set Enrichment Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; KIRP, kidney renal papillary cell carcinoma.

size filters (min=15, max=500) resulted in filtering out 8 from 186 gene sets, and the remaining 178 gene sets were upregulated in the high-risk group, and were used for analysis, then 164 gene sets were at FDR <25%, and finally, 15 gene sets were significantly enriched at nominal p value <0.01 (online supplemental table 1). The gene sets of the patients with high-risk KIRP were mainly enriched in the pentose phosphate pathway (figure 5E), ether lipid metabolism pathway (figure 5F), pantothenate and coenzyme A (CoA) biosynthesis (figure 5G), glycolysis gluconeogenesis (figure 5H), and so on. However, there was no significant enrichment pathway that met the requirements in the low-risk group (p<0.05).

DISCUSSION

Clinically, the survival status and time of patients are usually paid more attention to. The establishment of a clinical prognostic model might help doctors know well about the survival status and time of patients to a certain extent. Different analytical methods and data sources often generate different prognostic models. In our present study, based on the ARlncRNAs, we established a prognostic model of KIRP. The purpose, significance, and prospect of our research were elaborated from the following three aspects.

First, autophagy genes are closely related to cancer, so there is a solid theoretical basis for modeling based on the ARlncRNAs. Autophagy can provide energy for cells under stress, but it is a double-edged sword, which can maintain normal cell functions and act as an important protective mechanism for cancer cells.[9] Autophagy plays different roles depending on cell type, tumor microenvironment, disease stage, and external stimulation. Therefore, autophagy is a very attractive target for tumor therapy, and investigators have been using regulators of autophagy as adjuvant therapy for tumor.[19 20] Since autophagy plays a role in regulating tumor growth, and some autophagy-related genes have been discovered, some tumor-related prognostic models have been built.[21 22] Therefore, theoretically we also can establish a prognostic model of KIRP.

Second, ARlncRNA prognostic model of patients with KIRP has not been reported. In previous studies, Liu *et al*[23] and Lan *et al*[24] constructed prognostic models of KIRP based on immune-related lncRNAs. However, the models they finally built only based on immune genes (AUC=0.958) or lncRNA (AUC=0.824), did not include clinical factors; in addition, they did not classify the prediction time point. Gao *et al*[25] used five mRNAs to establish a prognostic model, but they only selected genes related to prognosis, and did not investigate their relationship with autophagy or lncRNA in depth. All the above studies lack researches on autophagy-related lncRNAs, which leaves a lot of space for our research. We first selected autophagy-related lncRNAs, and then performed multivariate Cox analysis, and found that the risk score could be used as prognostic factor. We combined autophagy-related lncRNA with the risk score to construct a more accurate prognostic model, providing clinicians with novel diagnostic and therapeutic tools from different perspectives.

Last but not least, for KIRP, the potential relationship between lncRNA and autophagy has not been explored, and it is worthy of further studying. Due to the close

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relationship between autophagy and cancer, we believed that it was necessary to construct a new prognostic model based on ARlncRNAs to explore KIRP prognosis. Therefore, we performed KEGG pathway enrichment analysis to detect the underlying biological mechanism of the genes. The ARlncRNAs, including LEF1-AS1, CU634019.6, C2orf48, AC027228.2, and AC107464.3, were identified by constructing a co-expression network of 14 mRNAs. In our present research, AC107464.3 was the only 'protective factor'. There have been no previous studies or reports on this gene. Its co-expressed mRNAs (ATG16L2, ATG4B, and ULK3) are key genes in the main autophagy pathways. ATG4B, the core autophagy protein of the ATG8/LC3 system, is upregulated in cancer tissues. It has been reported that ATG4B can promote the growth of colorectal cancer, and the expression of silencing ATG4B can reduce the colony formation of cancer cells and inhibit tumor growth.[26 27] ATG16L1 and ATG16L2 play important roles in autophagy and cell development in the bone marrow, lymphatic, and epithelial lineages.[28] The initiation of autophagy has been shown to depend on the ULK1/2 kinase complex.[29] Braden and Neufeld[30] believed that ULK3 was a new and independent pathway of autophagy initiation pathway. AC107464.3, as a protective factor, triggers autophagy after co-expression with ATG16L2, ATG4B, and ULK3, suggesting that autophagy, the 'double-edged sword', might be beneficial to patients with KIRP, which is consistent with the previous studies with a conclusion that promoting autophagy can inhibit kidney clear cell carcinoma.[11 12] As autophagy is a critical step in the process of cancer, AC107464.3, as an autophagy factor, will continuously express during the cancer development. This is also one of the potential reasons why we believe that the expression of AC107464.3 has higher value than that of the other four lncRNAs (including LEF1-AS1, CU634019.6, C2orf48, and AC027228.2) in predicting the KIRP prognosis. These four lncRNAs are theoretical 'risk factors', and their biological functions are mostly concentrated in the common pathways related to cancer cells generation, such as apoptosis, long-term depression, platinum drug resistance, vascular smooth muscle contraction, GnRH signaling pathway, etc, leading to the occurrence and development of different cancers.[31–34] These also confirmed the accuracy of our selected prognostic factors. We performed GSEA enrichment analysis and found that in addition to the autophagy mentioned above, there were many known biological pathways significantly associated with cancer in the high-risk group, such as 'pentose phosphate pathway', 'ether lipid metabolism pathway', 'pantothenate and CoA biosynthesis', 'glycolysis gluconeogenesis', 'p53 signaling pathway', and 'PPAR signaling pathway'. Among them, P53 gene is the gene with the highest correlation with human tumors,[35] and the PBRM1 gene with the second highest mutation rate in renal cancer is closely related to the inactivation of the P53 pathway.[36] Previous studies have shown that P53 can negatively regulate mTOR factor to promote autophagy.[37 38] In addition, the PARP pathway can act on DNA recombination and repair of cancer cells[39] to protect tumors. In recent years, the newly developed PARP inhibitors have been increasingly widely used in tumor treatment,[40] and for example, PARP inhibitors have been used effectively to treat renal cancer.[41] These enrichment biological functions and pathways in the high-risk group indicated that the prognostic genes identified were highly correlated with cancer development, which further confirmed the good accuracy of our prognostic model.

However, there are still some limitations in our research. First, the establishment of the model is based on only one public database, which lacks the external independent cohort and experimental validation. We tried our best to find KIRP information in different public databases, but only a small amount of incomplete records, which did not help us much in the final verification. Second, the small sample size in the training group might have some influences on the accuracy of the results. Therefore, this model was established through repeated calculation to make up for possible errors.

In conclusion, the identified five ARlncRNAs might modulate autophagy and cancer through the above detected functional pathways, thereby resulting in the differences of survival outcomes between groups defined using prognostic characteristics. We used the identified five ARlncRNAs to establish a prognostic model of KIRP, and carried out relevant tests and microgenetic analysis. The existing data were used as much as possible to fill the gap of the lack of the KIRP-related prognostic model. The accuracy and practicability of this model need to be further confirmed in clinical work.

Contributors ZK and JY conceived and designed the study; ZK and JY collected the data; ZK and JY analyzed and interpreted the data; ZK and JY wrote the manuscript; ZK and JY provided critical revisions that are important for the intellectual content; ZK and JY approved the final version of the manuscript, and the guarantor is JY.

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