

# Early Diagnostic and Prognostic Value of BIRC5 in Clear Cell Renal Cell Carcinoma Based on TCGA Data

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## Research article

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# Abstract

**Purpose:** Clear cell renal cell carcinoma (ccRCC) is a highly lethal cancer that would benefit from non-invasive innovative markers providing early diagnostic and prognostic detection. Increased BIRC5 (baculoviral inhibitor of apoptosis repeat containing 5) expression is associated with negative outcomes or survival in various cancers. Our study aims to investigate the role of BIRC5 of early diagnosis and prognosis in ccRCC by studying the expression of BIRC5 and the correlation between BIRC5 expression and clinicopathological parameters, prognosis in ccRCC.

**Methods:** The BIRC5 expression in ccRCC tissues and normal kidney tissues was measured using the Cancer Genome Atlas (TCGA) database and The Human Protein Atlas database. The correlation between BIRC5 expression and clinicopathological parameters, prognosis in ccRCC was analyzed using UALCAN, Kaplan Meier plotter, GEPIA and SurvExpress.

**Results:** BIRC5 expression is significantly higher in ccRCC than in normal kidney tissues, and is correlated with the clinical stage and pathological grade of ccRCC ( $P < 0.05$ ). The result of analyzing the relationship between BIRC5 expression and outcomes in ccRCC indicates that high BIRC5 expression is an independent prognostic factor affecting overall survival and disease-free survival of ccRCC ( $P < 0.05$ ). Compared with normal kidney tissues, the immunohistochemical test shows that BIRC5 is significantly upregulated in ccRCC tissues.

**Conclusions:** The high expression of BIRC5 is an important indicator of the prognosis of ccRCC, which makes BIRC5 be an effective biomarker for predicting the prognosis of patients in ccRCC. BIRC5 may be a great potential biomarker for early diagnosis of ccRCC.

## Introduction

Renal cell carcinoma (RCC) is the third most frequent urological malignant neoplasms worldwide, among which, clear cell renal cell carcinoma (ccRCC) accounts for approximately 80–90% [1], being responsible for the most common type of renal malignancy. Because of its high rates of local invasion, metastasis, and acquired chemo-resistance, ccRCC is the most lethal RCC histological subtype [2]. If diagnosed at an early stage, ccRCC can be cured by surgery. However, the 5-year disease-specific survival rate of ccRCC is only 12%. What's more, due to the asymptomatic nature of the early ones, ~ 16% of patients with ccRCC are identified with lymph node metastasis or distant metastasis at first diagnosis, and 20–30% of localized ccRCC patients experience recurrence or metastasis even following surgery and no effective therapies are found to reduce the risk of recurrence, progression or death [3, 4]. Thus, it is important to find reliable biomarkers to facilitates early diagnosis and screen out disease progression for ccRCC.

BIRC5 (also known as survivin) is a cancer-associated protein that inhibits cell death, which is a key member of the inhibitor of apoptosis protein (IAP) family, encoded by the BIRC5 (baculoviral inhibitor of apoptosis repeat containing 5) gene. Biological function of BIRC5 is involved in the regulation of both apoptosis and cell division when BIRC5 is usually highly expressed in several cancers but not expressed

in normal differentiated tissues. BIRC5 expression allows tumor cells to overcome apoptotic checkpoints, while several antitumor agents function through apoptosis activation, BIRC5 expression may contribute to the resistance to anticancer agents [5, 6]. Increased BIRC5 expression is associated with negative outcomes or survival in various cancers, such as breast, lung, colorectal, prostate, and ovarian cancer [7]. All of these features make BIRC5 a famous molecule among cancer research, however very few researches have been investigated regarding BIRC5 expression and ccRCC.

The Cancer Genome Atlas (TCGA), having huge data resources of cancers, plays an irreplaceable role in cancer research when providing an opportunity to analyze the associations of various clinicopathologic factors with tumor initiation, progression to cancer researchers [8]. Various computational tools have been developed to aid researchers in carrying out specific TCGA data analyses and facilitate the study of gene expression variations and survival associations across tumors. In our study, we aimed to examine BIRC5 expression and identify BIRC5 of prognostic value in the ccRCC using developed computational tools of TCGA; try to explore the possible mechanisms behind ccRCC development and reveal reliable early diagnosis and prognostic biomarkers and therapeutic targets for ccRCC.

## **Materials And Methods**

### **Relationship between BIRC5 expression and clinicopathological features**

UALCAN (<http://ualcan.path.uab.edu>) is an easy to use and interactive web-portal to perform in-depth analyses of TCGA gene expression data. The gene expression and clinical patient data were downloaded from TCGA and the analysis results are represented by box plots, KM-plots, and heatmaps. Through its links, queried gene expression analysis and chose cancer type survival analysis results can be acquired [9]. We used UALCAN to analyze relative expression of BIRC5 across tumor and normal samples, also in various tumor sub-groups based on individual cancer stages, tumor grade and nodal metastasis.

### **Relationship between BIRC5 expression and outcome of ccRCC**

The Kaplan Meier plotter (<http://kmplot.com/analysis/>) is capable to assess the effect of 54 k genes on survival in 21 cancer types (breast, ovarian, lung, gastric cancer, ccRCC and so on ). Its gene expression data and relapse-free and overall survival information are downloaded from GEO, EGA and TCGA. The patient samples are split into two groups according to various quantile expressions of the proposed biomarker. The two patient cohorts are compared by a Kaplan-Meier survival plot, and the hazard ratio with 95% confidence intervals and the log-rank P-value is calculated. We used The Kaplan Meier plotter to analyze the association between BIRC5 expression and overall survival (OS) of ccRCC.

GEPIA (<http://gepia.cancer-pku.cn/index.html>) is a web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects. It

provides multiple customizable functions such as tumor/normal differential expression analysis, patient survival analysis, similar gene detection [10]. We used GEPIA to analyze the association between BIRC5 expression and disease-free survival (DFS) of ccRCC.

SurvExpress (<http://bioinformatica.mty.itesm.mx/SurvExpress>) is a comprehensive gene expression database and web-based tool providing

survival analysis and risk assessment in cancer datasets. It can facilitate performance comparisons of Kaplan Meier plotter, PrognoScan or others and validations of prognostic and predictive biomarkers for cancer outcomes [11]. To further validate the association between BIRC5 expression and outcome of ccRCC, we used SurvExpress to analyze 468 ccRCC data from TCGA, using COX regression analysis.

## **Protein expression of BIRC5 between tumor tissues of ccRCC and normal kidney tissues**

The Human Protein Atlas database (HPA, <https://www.proteinatlas.org>) is capable of detecting 84% of the human proteome through antibody-based proteomics and can be used to study gene expression between the tissue of normal and tumors. We used the HPA database to test the immunohistochemistry of BIRC5 between the tissue of normal and ccRCC.

## **Results**

### **BIRC5 expression is up-regulated in ccRCC tissues**

Analyzing the BIRC5 mRNA expression in normal kidney tissue and ccRCC tumor tissue of TCGA, through UALCAN, we found the level of BIRC5 expression reported to be significantly increased in ccRCC tissues compared with normal kidney tissues, BIRC5 expression is significantly up-regulated in ccRCC tissues ( $p < .05$ ) (Fig. 1).

### **High BIRC5 expression is significantly related to clinical parameters**

Analyzing the expression of BIRC5 in various tumor sub-groups based on individual cancer stage, tumor grade and nodal metastasis using UALCAN, we found that the clinical parameters (clinical stage, histological grade, nodal metastasis) increased with higher BIRC5 expression ( $p < .05$ ) (Fig. 2). Compared with normal people, BIRC5 mRNA expression is significantly higher in the early stage and grade of ccRCC ( $p < .05$ ). These results show that a high BIRC5 mRNA expression was associated with disease progression in ccRCC and BIRC5 may be a biomarker of ccRCC early diagnosis.

### **Correlation between BIRC5 expression and outcome of ccRCC**

The correlation between higher BIRC5 expression and ccRCC progression inspired us to consider whether BIRC5 can be used as an independent prognostic predictor for ccRCC patients. Analyzing the association between BIRC5 expression and OS using The Kaplan Meier plotter, we found the higher BIRC5 expression was significantly related to patients' overall survival ( $p < .05$ )(Fig. 3a). Then analyzing the association between BIRC5 expression and DFS using GEPIA, we found the higher BIRC5 expression was significantly related to patients DFS( $p < .05$ ) [Fig. 3b]. Also, to further verify the role of BIRC5 in ccRCC, we used the SurExpress to analyze the data of 468 ccRCC from TCGA using COX regression analysis. The prognostic index was calculated regard BIRC5 mRNA expression level as a dichotomous risk factor. We found that in high-risk ccRCC patients, the expression level of BIRC5 is significantly high[Fig. 3d], and high expression of BIRC5 is an independent poor prognostic indicator of ccRCC[Fig. 3c].

## Protein expression of BIRC5 is higher in tumor tissues of ccRCC

Analyzing immunohistochemical results of BIRC5 protein in normal and ccRCC kidney tissue using HPA, we found that antibody for BIRC5 protein is "not detected"- "low" level in normal tissue when is "medium"- "high" level in ccRCC(Fig. 4). This further confirms that the expression of BIRC5 in ccRCC is higher than in normal kidney tissue.

## Discussion

In this study, we used several web-portal to explore the early diagnostic and prognostic value of BIRC5 in ccRCC patients by integrated bioinformatics analysis. Compared with a healthy population, the BIRC5 expression level in patients with early-stage ccRCC is significantly higher, which means that BIRC5 may be an early diagnostic biomarker for ccRCC. Moreover, BIRC5 expression is highly correlated with the ccRCC clinical and pathological stage, and BIRC5 expression is significantly increased in the higher clinical and pathological stage. We analyzed the relationship between BIRC5 expression and prognosis of ccRCC and found patients with high BIRC5 expression had a worse overall survival or disease-free survival. These results suggested that the high expression of BIRC5 prominently correlated with the prognosis and development of ccRCC, which means that BIRC5 is an important predictor of ccRCC patient prognosis.

ccRCC is a very invasive and chemoresistant disease which is often treated by surgical resection [2]. ccRCC can be cured by surgery when diagnosed at an early stage. However, it's usually asymptomatic at early stage and hard to diagnose in the early days [3, 4]. Unfortunately, ccRCC has high rates of local invasion and metastasis [2] and there are no effective therapies to reduce the risk of recurrence, progression or death. Therefore, there is an urgent need for reliable biomarkers of ccRCC, enabling early diagnosis, prognosis, and monitoring of potential relapse of the disease.

However, there is no simple and rapid method to early detect ccRCC. For example, as a gold standard for ccRCC diagnosis, renal biopsy has a high sensitivity of diagnosis with a low complication rate of less

than 5% [12], however due to the asymptomatic nature of ccRCC at an early stage, it cannot be diagnosed at the early days through renal biopsy. What's more, M. SadatKhonsari et al found that patients with suspicious renal masses cannot be precluded from being diagnosed with malignancies although, with an unsuspecting histology in CT-guided renal tumor biopsy and almost 30% of these patients, further diagnostic or therapeutic workup demonstrated a cancer diagnosis [13]. Previous several studies in other cancer types already showed that survivin can also be measured in the serum using a human surviving enzyme-linked immunosorbent assay (ELISA) [14, 15, 16, 17, 18] which makes it a noninvasive method for detecting ccRCC. In our study, the BIRC5 expression level in patients with early-stage ccRCC is significantly higher than in a healthy population. So we assume survivin may be an early diagnostic biomarker for ccRCC, but its sensitivity and specificity need to be clear by further clinical trials.

Previous studies have suggested increased BIRC5 expression contributes to the negative outcomes of various cancers, such as breast, lung, colorectal, prostate, and ovarian cancer [7]. CAO et al [19] found that high BIRC5 expression is related to tumor progression and poor prognosis of lung adenocarcinoma. Narimani M et al [20] found that knock-down of BIRC5 induces apoptosis in acute myelocytic leukemia (AML). What's more, two meta-analyses suggested that high BIRC5 expression was associated with poor prognosis and a more advanced pathological stage of renal cell carcinoma [21, 22]. In this study, we certify that high BIRC5 expression is associated with worse prognosis in ccRCC patients. Our findings are consistent with existing investigations. Therefore, we hypothesize that BIRC5 is a potential biomarker for progression and therapeutic target of ccRCC patients.

BIRC5 is a mitotic spindle checkpoint gene and is located near the telomeric end of chromosome 17 [23]. It plays an important role in the regulation of mitosis and apoptosis of cell, also involved in pathological processes [5]. Its encoded protein, survivin, has two phosphorylation sites on its different domains (Thr34 and Thr117) which determine its bifunctional molecule effect on apoptosis and cell proliferation. Thr34 is a site on the regulation of cell apoptosis when the Thr117 is involved in proliferation and cell cycle [24]. Previous studies demonstrate that BIRC5 is a key target involved in a variety of cancer cell signaling pathways and the up-regulation of BIRC5 may play a role in the following several mechanisms. First, it can antagonize caspase-dependent apoptosis and activate P53 and its downstream target P21 to achieve the purpose of stalling cell cycle progression [25]. Second, BIRC5 facilitates the invasion and migration of tumor cells mediated through the PI3K/AKT pathway [26] or the TGF- $\beta$  pathway [27]. Another, it can regulate tumor cell proliferation mediated by the  $\beta$ -catenin pathway [28]. These results may explain BIRC5 was mainly involved in the regulation of cell cycle and apoptosis. Meanwhile, our study indicates that the high expression of BIRC5 promoted the development of ccRCC which may be through regulating the cell cycle signaling pathway.

The main limitation of our study is that our study was conducted using data from public databases that were not verified in prospective clinical trials. These findings need to be validated in prospective clinical trials. Moreover, as a potential biomarker for early diagnosis, the specific concentration of survivin to diagnose ccRCC needs to be further determined by clinical trials, also its sensitivity and specificity need to be clear. Also, the mechanisms through which BIRC5 promotes the progression of ccRCC require

further investigation and the functions of the BIRC5 that impact the development of ccRCC need to be investigated further through in vivo and in vitro experiments.

In conclusion, for ccRCC, BIRC5 may serve as a promising early diagnostic or prognostic predictor and therapeutic target. However further investigations are necessary to confirm the findings of our study.

## Abbreviations

ccRCC

clear cell renal cell carcinoma

BIRC5

baculoviral inhibitor of apoptosis repeat containing 5

TCGA

the Cancer Genome Atlas database

HPA

The Human Protein Atlas database

## Declarations

### Ethics declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethics approval** This article does not contain any studies with human participants or animals performed by any of the authors. No ethical approval is required.

**Informed consent** For this type of study, formal consent is not required.

**Authors' contributions** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by, Xin Wang, Jianhao Yin and Rui Jia. The first draft of the manuscript was written by Jingyuan Wang, Min Chen and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

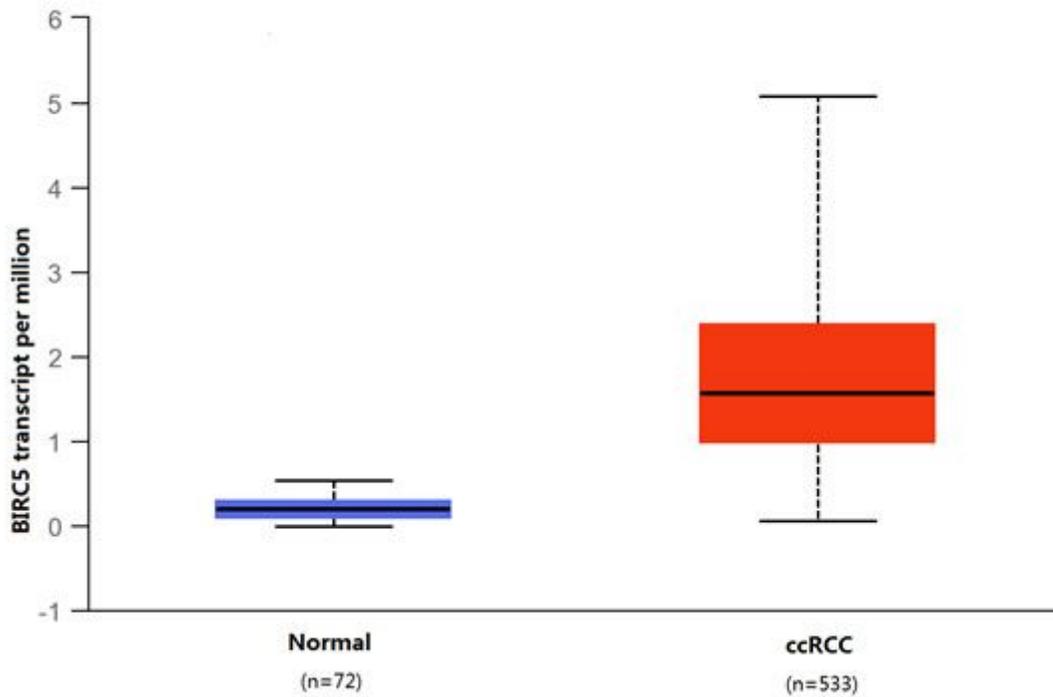
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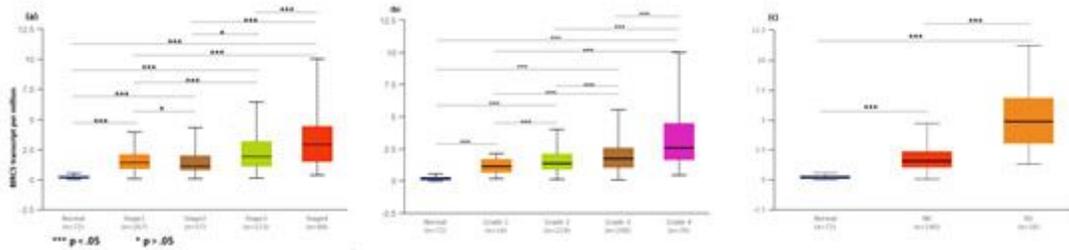
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## Figures



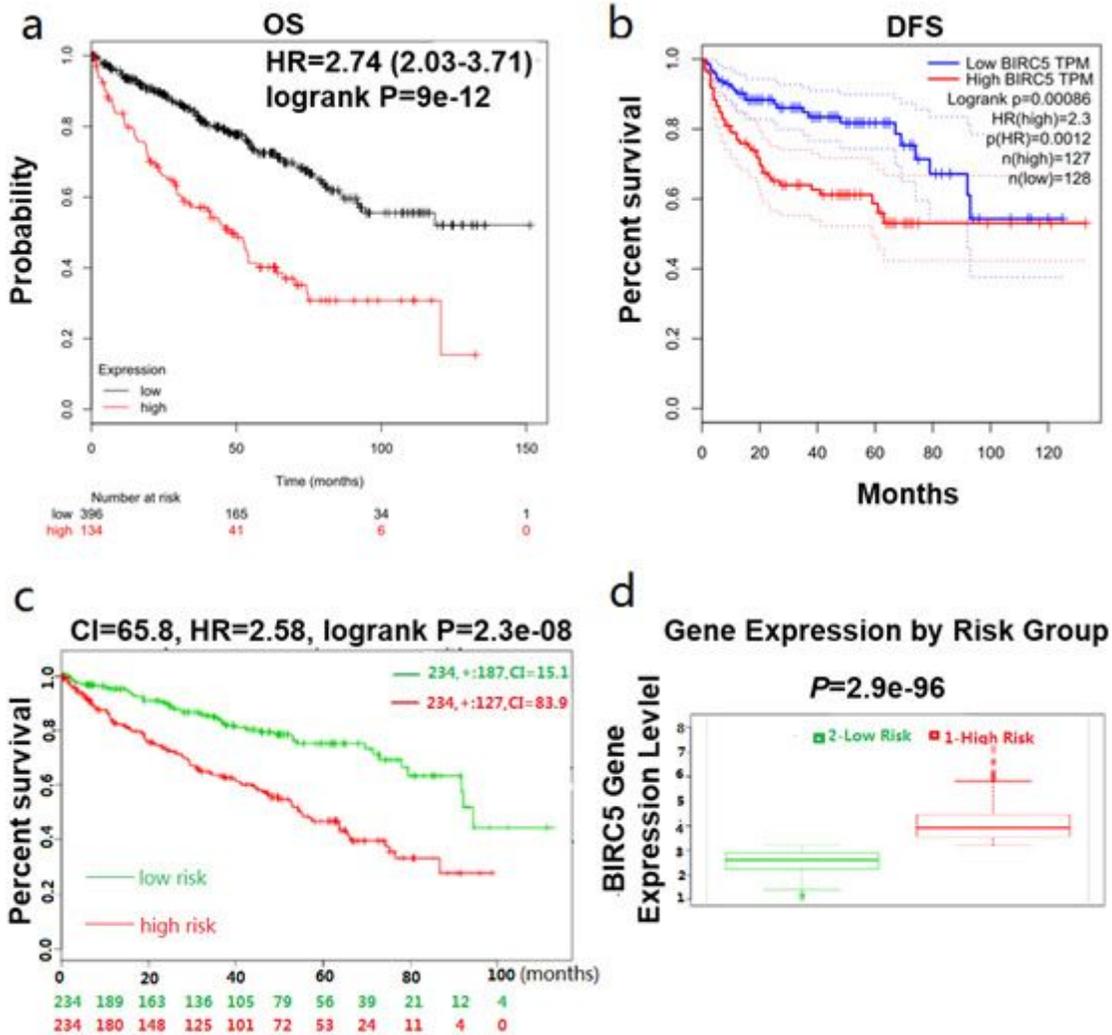
**Figure 1**

Expression of BIRC5 in ccRCC based on TCGA samples ( $p < .05$ )



**Figure 2**

The relationship between BIRC5 expression of ccRCC and clinical parameters. BIRC5 expression was differential in clinical stage, grade and nodal metastasis parameters ( $p < .05$ ). (a) BIRC5 expression and stage; (b) BIRC5 expression and grade; (c) BIRC5 expression and nodal metastasis.



**Figure 3**

The relationship between BIRC5 expression and outcome of ccRCC. (A) BIRC5 expression and OS; (B) BIRC5 expression and DFS; (C) survival across high- and low-risk group (CI: concordance index; the numbers below horizontal axis represent the number of individuals not presenting the event of the corresponding risk group along time); (D) BIRC5 expression across high- and low- risk group.

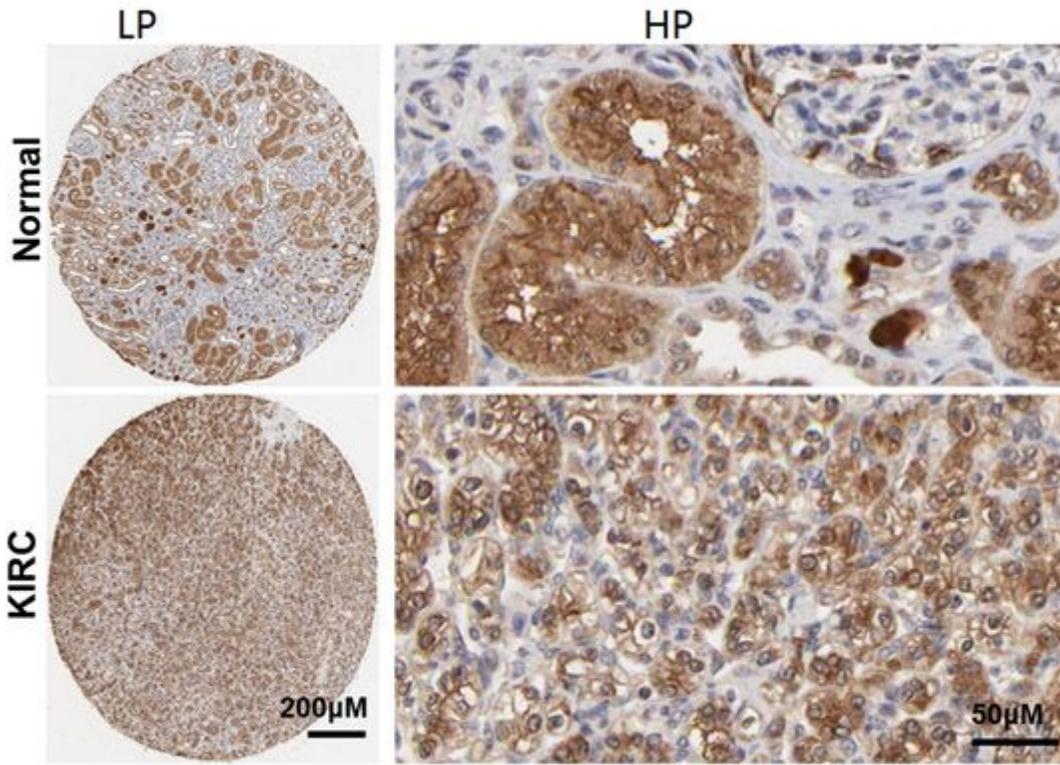


Figure 4

Expression of BIRC5 protein of normal and ccRCC kidney tissue in HPA(magnification: LP×85; HP×240)