Bioinformatics identification of prognostic genes and potential interaction analysis in renal cell carcinoma

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Background: Renal cell carcinoma (RCC) is one of the ten most prevalent cancers in the world and its incidence has been rising over the past decade. However, effective biomarkers to predict the prognosis of patients remains absent, and the exact molecular mechanism of the disease remains unclear. Therefore, the identification of key genes and their biological pathways are of great significance to identify the differential expressed genes associated with the prognosis for patients with RCC, and to further explore their potential protein-protein interactions (PPIs) in tumorigenesis.

Methods: The gene expression microarray data for GSE15641 and GSE40435 were extracted from the Gene Expression Omnibus (GEO) database, including 150 primary tumors and their matched adjacent non-tumor tissues. Afterwards, gene expression for fold changes (FCs) and P value for tumor and non-tumor tissues were analyzed using online tool GEO2R. Gene expression with logFCs of greater than two combined with P value of lower than 0.01 were considered as candidate targets for treatment of RCC. The survival analysis of candidate genes was performed by online software OncoLnc. The PPI network was implemented with Search Tool for the Retrieval of Interacting Genes (STRING).

Results: In total, there were 625 differentially expressed genes (DEGs) in GSE15641, including 415 increased and 210 decreased genes. A total of 343 DEGs were identified in the GSE40435 with 101 upregulated and 242 downregulated genes, the 20 genes with highest FC in high or low expression in each database were summarized. Five candidate genes were overlapped genes in the two GEO datasets. However, aldolase, fructose-bisphosphate B (ALDOB) was found to be the only gene affecting the prognosis. A number of critical genes were identified behind the mechanism, of which they interacted with ALDOB. Among them, phosphofructokinase, platelet (*PFKP*), phosphofructokinase, muscle (*PFKM*), pyruvate kinase L/R (*PKLR*), and fructose-bisphosphatase 1 (*FBP1*) showed a better prognosis, whereas only glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) rendered a bleak outcome.

Conclusions: Five genes were found to be overlappingly expressed in the top 20 greatest FC in two human GEO datasets. This is of great value in the treatment and prognosis of RCC.

Keywords: Renal carcinoma; metabolism; aldolase, fructose-bisphosphate B (ALDOB); prognosis

Introduction

Renal cell carcinoma (RCC) accounts for 2% to 3% of all types of human cancers (1). More than 338,000 individuals are diagnosed with RCC every year, and is expected to increase by 22% by 2023; with more than 140,000 RCC-related deaths occur each year (2). Over the past decade, the prognosis of RCC patients has been significantly improved with enhanced surgical procedures and the use of specific targeted drugs (3). The clinical outcome of individuals diagnosed at early stage is comparatively good. However, approximately 50% of the patients progress metastatic disease and under this setting, renal cancer remains incurable with a median survival of around of 30 months (4). The development of effective treatment regimens has been hampered by a limited understanding of the basic molecular pathways underpinning RCC carcinogenesis. However, it is still a great challenge to accurately understand the pathological changes of RCC.

Tumor progression depends on the continuation of cancer cells to metabolize biomass simultaneously and the process, at the same time, remains feasible in tumor microenvironment. It is widely believed that the change in cell metabolism would lead to the uncontrolled growth of cancer cells (5). Notably, enhanced glycolysis, first revealed by Warburg in the 1920s, provides a large number of biosynthetic precursors for the production of amino acids, lipids, nucleotides, nicotinamide adenine dinucleotide phosphate (NAPDH), and other biochemical intermediates (6). Otto Warburg observed that cancer cells metabolized glucose faster than normal cells and that the main metabolic product was lactic acid. This indicated that glucose metabolism was suspended at the last step of glycolysis. In most tissue cells, glucose transported into the mitochondria, where glucose is metabolized into carbon dioxide and water through tricarboxylic acid (TCA) cycle, with the accompanying production of 36 molecules of adenosine-triphosphate (ATP) per molecule of glucose. Thus, under hypoxic conditions, glucose metabolism stops at the end of glycolysis while producing lactate acid (7). A single glucose molecule produces 36 ATP molecules, and this produces more ATP than in the absence of oxygen. However, even sufficient oxygen is available, glucose metabolism in cancer cells produces huge amount of lactate acid. This abnormal metabolic pattern is known as "Warburg effect" (8).

Despite the discovery of oncogenes and tumor suppressors which are largely responsible for intrinsic genetic changes in cancer, little progress of cancer treatment has been made over the past 20 years. However, in the past 20 years, the Warburg effect has received increasing attention for playing an essential role in the progression of cancer. This is being revealed in numerous recent reviews and comments. Nevertheless, "reprogramming of energy metabolism" proposed as an emerging hallmark of cancer by Hanahan and Weinberg (9), is perhaps the strongest evidence suggesting that metabolic changes in cancer cells have become the mainstreams of cancer research. A relatively recent publication has shown that the genes involved in aerobic glycolysis are over-expressed in at least 24 types of cancers in human, and that corresponds to approximately 70% of all cancers (10).

Vertebrate aldolases, also known as fructose-(bis) phosphate aldolase (ALDO), is a key enzyme participating in glycolysis and gluconeogenesis that catalyze fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (11). Human aldolase contains three isozymes: ALDOA, -B, and -C. ALDOA is expressed mainly in muscle and fetal liver, but those are not the only sites of expression. A series of literatures have revealed that high levels of ALDOA is closely related to lung carcinoma, advanced oral squamous cell carcinoma, and osteosarcoma (12-14). Some of these studies have reported that ALDOA can play a key role as an independent prognostic factor, as in cases of lung adenocarcinoma, breast cancer, and gastric cancer. ALDOC, the third kind of aldolase, is abundant in the brain (15). Recent studies have revealed abnormal expression of ALDOB is associated with neuronal damage and Alzheimer's disease (16,17). ALDOC suppresses oral squamous cell migration and negatively correlates to the clinical outcome. ALDOB is predominantly expressed in adult liver, so also known as liver-type aldolase (18),

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Highlight box

Key findings
- ALDOB was identified as the only one gene that impacts on the prognosis of RCC patients.

What is known and what is new?
- RCC is one of the ten most prevalent cancers in the world.
- Five genes were found to be overlappingly expressed in the top 20 greatest FC in two RCC human GEO datasets, however, the under-expressed of ALDOB in human RCC has a novel predictive value for clinical outcome.

What is the implication, and what should change now?
- ALDOB is of great value in the treatment and prognosis of RCC.

ALDOB has a similar sequence to ALDOA (66% identity) and ALDOC (68% identity) (19). Several genetic mutations and polymorphisms of the ALDOB gene lead to a deficiency in ALDOB and have been identified as the pathogenesis of hereditary fructose intolerance (20). Abnormal expression of ALDOB is associated with a variety of diseases, such as hepatitis, liver cirrhosis, and even cancer (21). Several documents have revealed that under-expression of ALDOB is related to hepatocellular carcinoma, gastric cancer (22). However, the role of ALDOB in RCC is yet to be reported.

Bioinformatics analysis of microarray data has been widely used to identify genetic variations in oncological research, and it is also essential in exploring novel prognostic biomarkers in tumor progression, as in the case of renal carcinoma (23). Recently, massive documental research was conducted to reveal the differentially expressed genes (DEGs) in clear cell RCC (ccRCC) and revealed their roles in biological processes, molecular functions and different pathways.

In the present study, data generated by gene expression microarray were investigated and the differential expression of ALDOB in tumor compared to adjacent non-tumor was found to be ranked with high level in multiple databases. Furthermore, the expression of ALDOB on the prediction of RCC prognosis and its mechanism behind, of which the interaction between ALDOB and molecules and its prognostic significance on RCC are also discussed here. We present the following article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2242/rc).

Methods

Microarray data

From the Gene Expression Omnibus (GEO) database (https://ncbi.nlm.nih.gov/geo/), two gene expression profiles, GSE15641 and GSE40435 were downloaded. The expression array for GSE15641 consisted of a messenger RNA (mRNA) expression profile of 49 RCC tumors, and 23 normal kidney samples. Transcriptional profiling with oligonucleotide microarrays (22,283 genes, GPL96) was performed on all samples. The expression microarray of GSE40435 included 101 pairs RCC tumors and adjacent non-tumor renal tissues. The data were obtained using lllumina HumanHT-12 v4 expression beadchips (GPL10558). GSE15641 were used to explore the expression of multiple RCC types.

Identification of DEGs

GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) is an interactive online tool to identify DEGs by comparing samples from GEO series. GEO2R was used to screen for DEGs between RCC and normal tissue samples. Student's *t*-test was used to test the statistical significance of differential expression of genes. The Bejamini-Hochberg method was used to calculate the false discovery rate (FDR). The cut-off criteria were an adjusted P value of <0.05, P<0.05 and logFC ≥2 or <–2 for DEGs.

Cell transfection and quantitative real-time polymerase chain reaction (qRT-PCR)

The following lentivirus vectors were purchased from Shanghai GeneChem Co.: expression for short hairpin RNA (shRNA)-MACROD2 and negative control (hU6-MCS-Ubiquitin-EGFP-IRES-puromycin). The target sequence for ALDOB shRNA construction: GTGGGAATCAAGTTAGACCAA. Transduction was performed according to the instructions of the manufacturer. qRT-PCR was detected by using a SYBR PrimeScript RT-PCR Kit (Takara Bio, Shiga, Japan), per the manufacturer's instructions. Primers sequences are as follows: GAPDH, forward (F): 5'-GGTATGACAACGAATTTGGC-3', reverse (R): 5'-GAGCACAGGGTACTTTATTG-3'; PFKP, F: 5'-TGACGTGGACATCCGCAAAG-3', R: 5'-CTGGAAGGTGGACAGCGAGG-3'; PFKM, F: 5'-GACCCGTGGTTCTCGTCTC-3', R: 5'-AAAGGCTGATGGCGTCCC-3'; PKLR, F: 5'-TGGGAAAACTGGGTGGGATGGATG-3', R: 5'-GAAGGAAGCAGCCGGGGATTTGAC-3'; FBP1, F: 5'-CTACGCCAGGGACTTTGACC-3', R: 5'-GGCCCCATAAGGAGCTGAAT-3'.

Statistical analysis

Protein-protein interaction (PPI) network and module analysis

The Search Tool for the Retrieval of Interacting Genes (STRING) database (https://cn.string-db.org/) is an online software that provides comprehensive interactions of lists of proteins and genes (24).

Survival analysis of genes in RCC

OncoLnc (http://www.oncolnc.org/) is an online software for interactively analyzing survival relationship, and

enabling the download of clinical data and gene expression data for mRNAs, microRNAs (miRNAs), or long noncoding RNAs (lncRNAs). OncoLnc includes nearly 9,000 patient survival data from 21 pan-cancer studies accomplished by The Cancer Genome Atlas (TCGA). The total survival analysis of ccRCC was performed by a Kaplan-Meier plot. The ccRCC patients were divided into two groups with a 50% threshold on the basis of high or low expression for kininogen 1 (KNG1), aldolase, fructose-bisphosphate B (ALDOB), complement C1q B chain (C1QB), neuronal pentraxin 2 (NPTX2), triosephosphate isomerase 1 (TPI1), phosphofructokinase, liver type (PFKL), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphofructokinase, platelet (PFKP), phosphofructokinase, muscle (PFKM), GAPDH, spermatogenic (GAPDHS), pyruvate kinase L/R (PKLR), fructose-bisphosphatase 1 (FBP1), fructose-bisphosphatase 2 (FBP2), and pyruvate kinase M1/2 (PKM).

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

Identification of DEGs in two RCC database

The analysis of the GSE15641 dataset identified 625 DEGs with the threshold of P value of <0.01 and a logFC value of ≥2, including 415 increased and 210 decreased genes. While the genes in the GSE40435 dataset were analyzed and 343 DEGs with 101 upregulated and 242 downregulated genes were identified. The top 20 genes with the greatest fold change (FC) in each dataset were selected and the heat map was made, respectively (*Figure 1A,1B*). In order to find a more accurate target gene, we took the intersection of two databases and got KNG1, ALDOB, C1QB, NPTX2, and uromodulin (UMOD) for next investigation by Venn diagram (*Figure 1C*).

Association between KNG1, ALDOB, C1QB, NPTX2, and UMOD expression with prognosis of ccRCC patients

To better understanding the prognostic impact of the aforesaid differential expressed candidates (KNG1, ALDOB, C1QB, NPTX2, and UMOD) on the overall survival of ccRCC patients, survival information of 522 ccRCC patients from TCGA dataset through OncoLnc data

portal was extracted and analyzed. Kaplan-Meier survival analyses were conducted with the high and low expression (threshold: 50%) groups on the basis of these genes and clinical outcome using OncoLnc. As shown in *Figure 1D*, the survival rate in the ccRCC patients who conferred high expression of ALDOB was significantly increased compared to patients with low expression of ALDOB by log-rank test (P=0.00000327). While the levels of KNG1, C1QB, and NPTX2 showed no statistically significant correlation between clinical prognosis rate of ccRCC patients (P=0.42, P=0.0707, and P=0.171, respectively). UMOD did not meet the expression cutoff for the analysis, so there was no information about UMOD in the OncoLnc. Then the expression of UMOD in the kidney renal papillary cell carcinoma was examined, another subtype of kidney cancer. However, the expression levels of UMOD conferred no significant association with the prognosis of renal papillary cell carcinoma (Figure S1). In addition, to determine the biological effects of ALDOB expression in ccRCC cells, we used shRNA to knock down ALDOB in Caki-1 cell. We detected the proliferation ability of ALDOB in Caki-1 cell by cell counting kit-8 (CCK-8), which showed that the capacity of proliferation was increased in the ALDOB knockdown cells (Figure S2). To sum up, these findings prompted us to study the mechanisms of ALDOB's progress to ccRCC.

### PPI network construction and analysis of modules

In order to investigate the intrinsic interaction of ALDOB in ccRCC progression, we constructed the PPI network by online tool STRING. In total, 55 nodes were mapped in the PPI network of ALDOB. The 10 highest-scoring direct interaction with ALDOB nodes were screened as hub genes (*Figure 2A*): *TPI1, PFKL, GAPDH, PFKP, PFKM, GAPDHS, PKLR, FBP1, FBP2, PKM*.

Identification of ALDOB interactors and their association with prognostic of ccRCC patients

To further examine the association of the expression levels of TPI1, PFKL, GAPDH, PFKP, PFKM, GAPDHS, PKLR, FBP1, FBP2, and PKM with the prognosis of ccRCC patients, Kaplan-Meier survival analyses were conducted on the 522 ccRCC patients with the high and low expression (threshold: 50%) groups on the basis of these genes and clinical outcome using OncoLnc. As showed in *Figure 2B*, the survival rate in the ccRCC patients who

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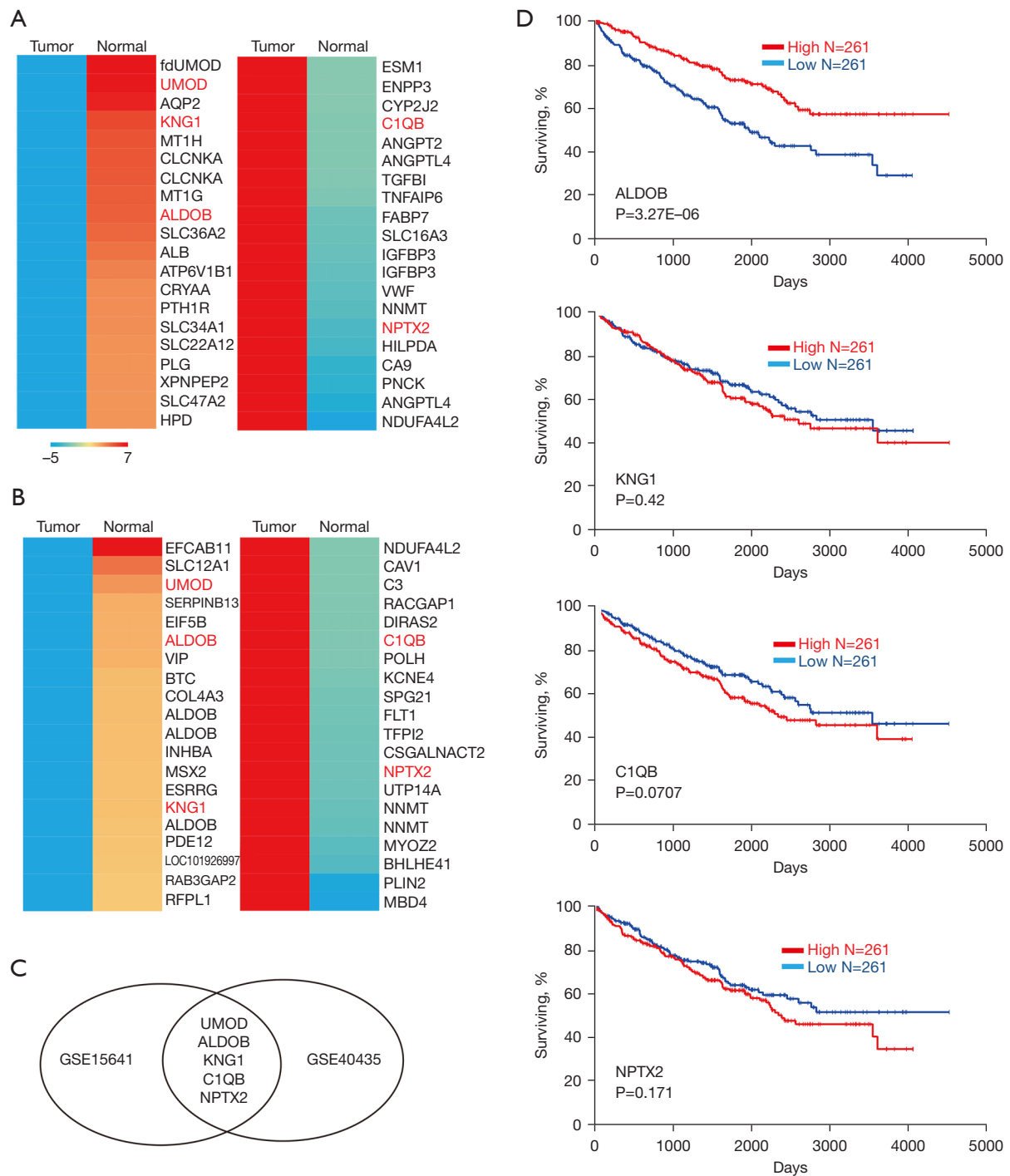
Figure 1 Heatmap of top 20 upregulated genes and top 20 downregulated genes in two databases [(A) GSE15641; (B) GSE40435]. (C) Five candidate genes were identified by Venn diagram. (D) Survival plots of KNG1, ALDOB, C1QB, and NPTX2 were obtained by OncoLnc. UMOD, uromodulin; KNG1, kininogen 1; ALDOB, aldolase, fructose-bisphosphate B; C1QB, complement C1q B chain; NPTX2, neuronal pentraxin 2.
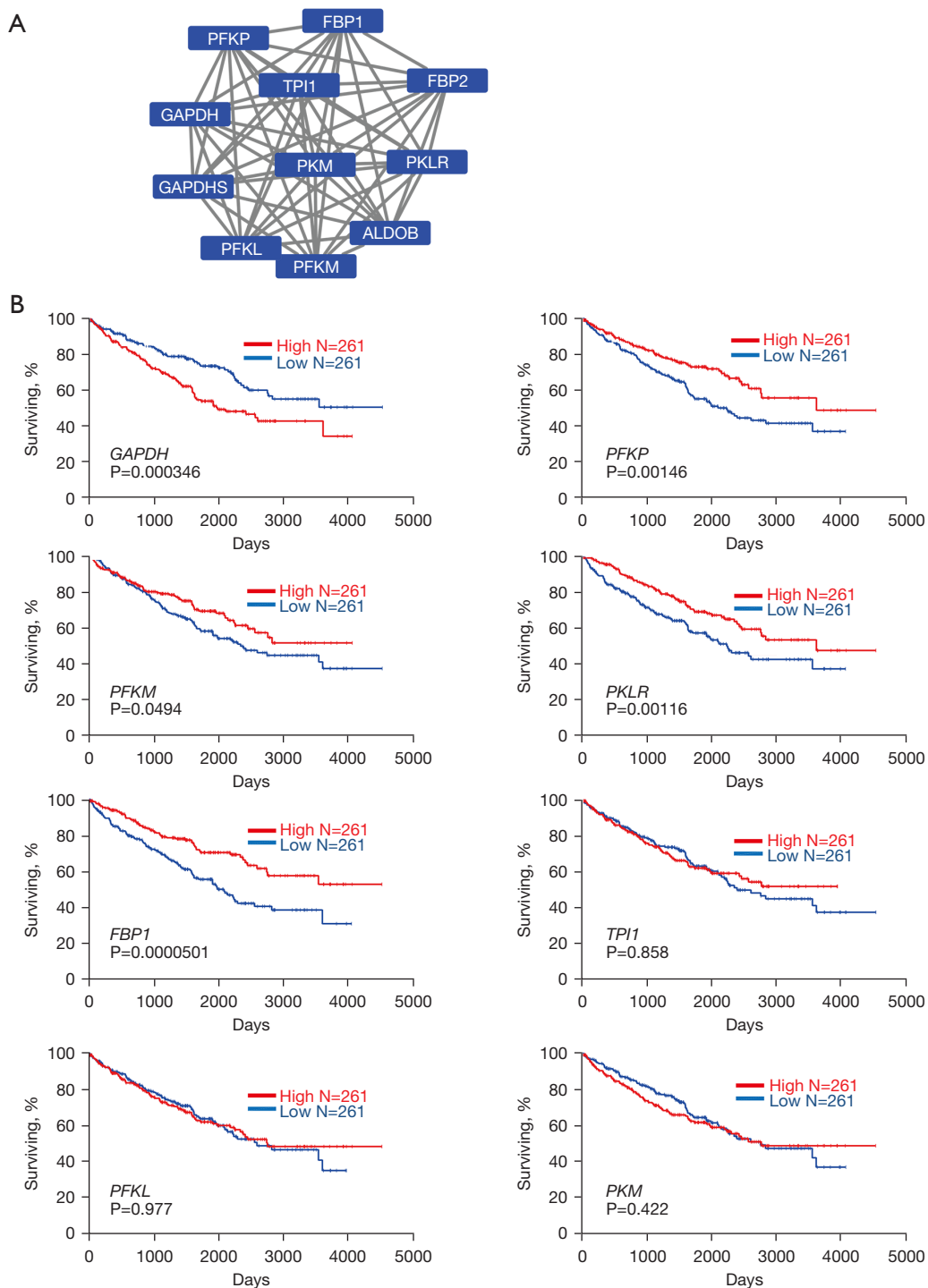
Figure 2 The 10 highest-scoring genes interacted with ALDOB. (A) PPI network were constructed by the STRING online database. (B) Survival analysis of genes (*GAPDH*, *PFKP*, *PFKM*, *PKLR*, *FBP1*, *TPI1*, *PFKL*, and *PKM*) through OncoLnc. FBP1, fructose-bisphosphatase 1; PFKP, phosphofructokinase, platelet; TPI1, triosephosphate isomerase 1; FBP2, fructose-bisphosphatase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PKM, pyruvate kinase M1/2; PKLR, pyruvate kinase L/R; GAPDHS, GAPDH, spermatogenic; ALDOB, aldolase, fructose-bisphosphate B; PFKL, phosphofructokinase, liver type; PFKM, phosphofructokinase, muscle; PPI, protein-protein interaction; STRING, Search Tool for the retrieval of Interacting Genes.

conferred high expression of PFKP, PFKM, PKLR, and FBP1 was significantly increased compared to patients with low expression of these genes by log-rank test (P=0.00146, P=0.0494, P=0.0116, P=0.0000501, respectively), while high expression of GAPDH showed poor prognosis (P=0.000346) and the levels of TPI1, PFKL and PKM showed no statistically significant correlation between clinical prognosis rate of ccRCC patients, respectively (P=0.858, P=0.977, P=0.422, respectively). Furthermore, we performed the qRT-PCR experiments to investigate the downstream targets of ALDOB. The results showed that the expression of GAPDH were upregulated and the level of PFKP, PFKM, PKLR, and FBP1 were downregulated in the ALDOB knockdown cell lines (Figure S3).

Discussion

Cancer has traditionally been regard as a disease of uncontrolled cell growth mediated by multiple oncogenes, such as mutations that activate growth factor receptors (25). Although glycolysis provides less energy than the Krebs cycle and less energy is detrimental to cell growth, glycolysis is more common in tumors. This paradox led to a series of discoveries suggesting that many metabolic processes in tumors are not directly linked to proliferation (26). Several classical metabolic pathways are upregulated, depressed or bypassed entirely in cancer cells and many of these metabolic changes, especially in kidney cancers (27). This study has led to changes concept in metabolic concepts, so-known as metabolic reprogramming, is "successful" for kidney cancer. The reprogramming allows cancer cells to proliferate rapidly to satisfy their basic needs for cell growth components, including nucleic acid and protein constituents, as well as high levels of molecules that adjust the enhanced tumor energy situation.

As one of the most studied cancer, kidney cancer perhaps is a typical representative malignant tumor characterized by metabolic reprogramming (28). Genes that are dysregulated in kidney cancer are involved in many different cellular pathways that modulate various aspects of cell metabolism, such as oxygen sensing, the TCA cycle, glutamine metabolism, and tumor energy metabolism. As a result, kidney cancer has been labelled a metabolic disease (29). The most common type of kidney cancer is ccRCC, an aggressive cancer that derives from the proximal renal tubular epithelium and is highly lethal due to its metastatic nature (30). Metabolic reprogramming, a hallmark of malignancy involving changes in glycolytic

enzymes, is becoming widely recognized (31). ALDO, which includes four catalytic tetramers, is a key enzyme in glycolysis. Evidence has shown that ALDO not only takes part in glycometabolism but is also involved in cell proliferation, signal transduction, and transcription. Therefore, the aberration of ALDO could be responsible for malignant transformation (32). In recent years, studies of the underlying roles of ALDO in cancer have become popular, and have revealed that the abnormal expression of ALDO has a close relationship with many kinds of cancers. ALDOB, which contains a large number of ALDOB-specific amino acid residues in its isozyme-specific region 2, is mainly responsible for cleaving fructose-1-phosphate into dihydroxyacetone phosphate and glyceraldehyde. Previous literature revealed that ALDOB were involved in the ccRCC development (33), the findings in this current study.

At the initial screening, we identified five DEGs through two databases. KNG1 is a precursor of kallikrein system associated with cardiovascular and renal function, blood pressure regulation and physiological and pathological processes (34). KNG1 could exhibit anti-angiogenic function and inhibit the proliferation of endothelial cells (35). Recently, KNG1 has been shown to have a role on carcinogenesis. The cancer patients who have a low expression of KNG1 can promote the survival of the cancer cells (36). Through mining data from TCGA, KNG1 was identified as the core factor associated with the prognosis of glioma patients (37). We also obtained the differential expression of KNG1 through database analysis, but it was not related to patient prognosis.

Functionally, C1QB is one of the subunits of complement subcomponent (38), which is related to immune responses in the human body. The expression of C1QB in the serum was associated with the ccRCC tumor stage and grade, but the clinical outcome has not been reported (39). However, we found that the levels of C1QB was much higher than adjacent non-tumor tissues, but had no relationship with prognosis. In this study, the levels of C1QB was differentially expressed, and patients with high expression tended to have shorter survival, but the P value did not reach the statistical difference.

NPTX2 is homologous to NPTX1 (NP1) and neuronal pentraxin receptor (NPTXR, NPR), both of which have been characterized in nervous system tissues (40). NPTX2 has a broader expression pattern, than can observed in many tissues, such as nerve, pancreas, skeletal muscle, heart, and liver (41). NTPX1 and NTPX2 bind to NTPXR to form homopolymers or heteropolymers, binding pentraxins to

cell membrane (42). Previous studies have identified the role of pentraxin in neuronal growth and synaptic plasticity of neuronal cells (43,44). Furthermore, NPTX2 is highly expressed in ccRCC primary tumors and metastases, and contributes to the cell viability and promotes cell migration (45). In this study, the levels of NTPX2 were significantly differentially expressed, and patients with high expression tended to have shorter survival, but the P value did not reach the statistically significance.

Phosphofructose kinase (PFK), catalyzes the production of fructose 1,6-bisphosphate and adenosine diphosphate (ADP) from fructose 6-phosphate and ATP, is a key enzyme of the glycolytic pathway. Mammalian PFK includes three L, M, and P isoforms (46). In normal tissues, PFKL is predominately expressed in liver and kidney while platelets are abundant with PFKM and PFKP, respectively. A recent study illustrated that PFKP promotes aerobic glycolysis as well as suppresses p53 to maintain cellular proliferation (47). We also observed that the expression of FPKP or PFKM were overexpressed in RCC compared to normal tissues. Furthermore, we validated the patients with high levels of PFK or PFKM conferred a bad prognosis.

PKLR, as an isozyme of PKL, is the only enzyme for glycolytic pyruvate generation and regulation of biosynthetic pathways, is of great importance for liver cells. Mineralocorticoid receptor inhibits hepatocellular carcinoma progression and the Warburg effect through regulating the miR-338-3p-PKLR axis (48). PKLR facilitates liver colonization by assisting glutathione synthesis in colorectal cancer (49). The levels of PKLR were up-regulated in RCC compared to normal tissues. Notably, we validated the patients with high levels of PFK or PFKM conferred a bad prognosis.

GAPDH is also a glycolytic key enzyme. GAPDH is involved in many cellular functions. For example, GAPDH contributes to nuclear tRNA export, DNA replication and repair, cellular metabolism, carcinogenesis, and cell death (50). Although GAPDH is widely used as an internal control, its expression status is different in different human cell lines (51). GAPDH levels have upregulated significantly and usually associated with reduced survival (52), while GAPDH is considered as a pro-apoptotic agent (53). Therefore, these dysregulations of GAPDH in cancers indicate the dual roles of this enzyme in cell fata. Indeed, as a glycolysis-related factors, GAPDH is essential for cancer cells. Here, we revealed that the patients who had high levels of GAPDH were correlated with reduced survival.

There are many uncertainties about the pathogenesis of RCC, and study of the biomarkers and mechanisms of RCC remains a global prevalence as the disease is on the rise. In this study, total 968 DEGs were filtrated combined with two kind of dataset containing the largest number of clinical specimens, comprising 516 increased regulated genes and 442 decreased regulated genes.

Conclusions

We found five genes that were overlapped in top 20 with the greatest FC in the two large human GEO dataset. ALDOB was identified as the only one gene that impacts on the prognosis of RCC patients. The under-expressed of ALDOB in human RCC has a novel predictive value for clinical outcome. Furthermore, several genes (GAPDH, PFKP, PFKM, PKLR, and FBP1) interacted with ALDOB also exhibited the prognostic value in patients.

Acknowledgments

Footnote

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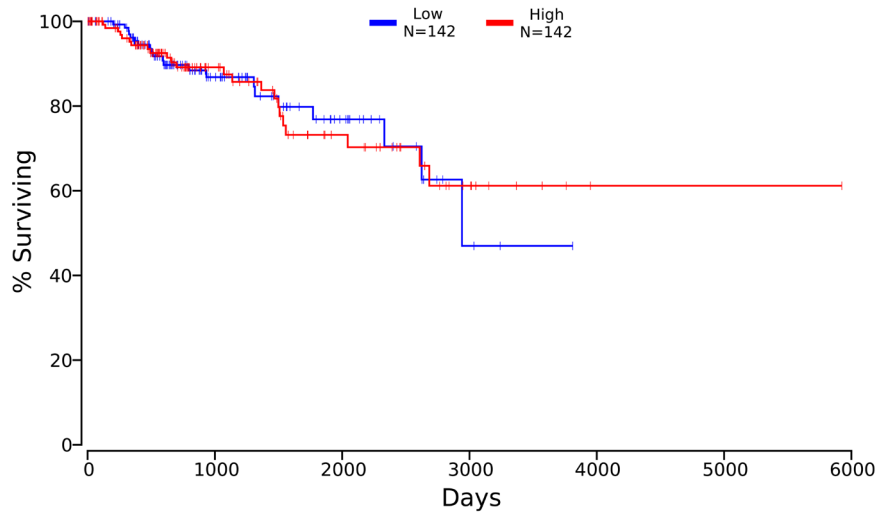
Figure S1 Kaplan-Meier analysis results of UMOD in renal papillary cell carcinoma. UMOD, uromodulin.
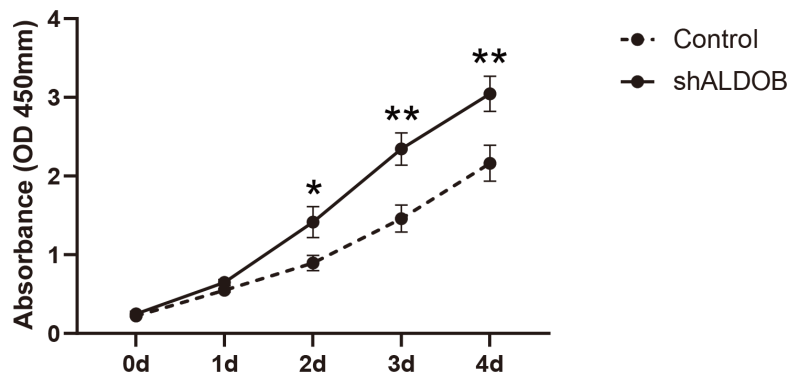
Figure S2 ALDOB inhibited proliferation of ccRCC cells *in vitro*. CCK-8 assay was used to detect cell proliferation. *P<0.05; **P<0.01. OD, optical density; sh, short hairpin; ALDOB, aldolase, fructose-bisphosphate B; ccRCC, clear cell renal cell carcinoma; CCK-8, cell counting kit-8.
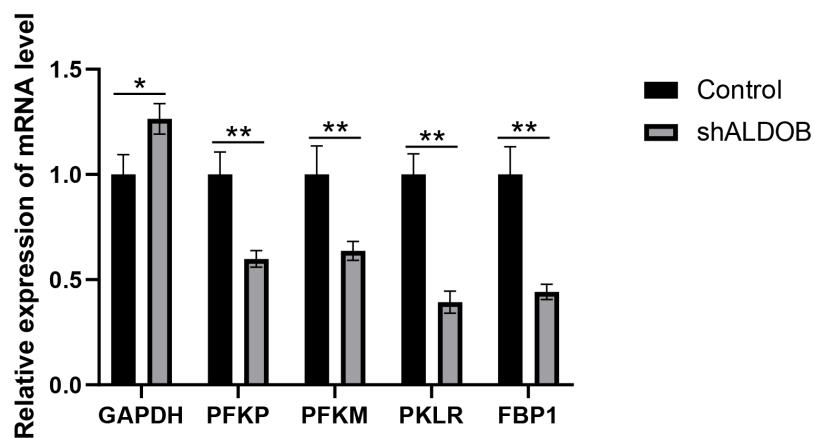
Figure S3 qRT-PCR experiments confirmed to investigate the downstream targets of ALDOB. GAPDH, PFKP, PFKM, PKLR, FBP1 expression was confirmed using qRT-PCR analysis after the knockdown of ALDOB. *P<0.05; **P<0.01. mRNA, messenger RNA; sh, short hairpin; ALDOB, aldolase, fructose-bisphosphate B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PFKP, phosphofructokinase, platelet; PFKM, phosphofructokinase, muscle; PKLR, pyruvate kinase L/R; FBP1, fructose-bisphosphatase 1; qRT-PCR, quantitative real-time polymerase chain reaction.