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Analysis of Expression Level and Prognostic Value of Ferroptosis Driver Genes in Clear Cell Renal Cell Carcinoma

Yinsong Hu[1,2,a], Haitao Jiao[3,b], Lei Liu[1,2,c,*], Jinyan Zhao[2,d,*]

[1]Bioinformatics Research Lab, Chengde Medical University, Chengde, Hebei, China
[2]College of Basic Medicine, Chengde Medical University, Chengde, Hebei, China
[3]Clinical Skills Teaching Center, Chengde Medical University, Chengde, Hebei, China
[a]595464156@qq.com, [b]840943878@qq.com, [c]homingreceptor@hotmail.com, [d]1219801366@qq.com
[*]Corresponding author

Abstract: *This study using bioinformatics methods to analyze the expression level and prognostic value of ferroptosis driver genes (FDGs) in clear cell renal cell cancer (ccRCC), and provide theoretical basis for the molecular regulatory mechanism and clinical application of FDGs in ccRCC. The Kidney Clear Cell Cancer dataset from the UCSC Xena database was selected, and differentially expressed genes (DEGs) were screened using R software. Functional enrichment analysis was performed on DEGs using the DAVID database. Filter the FDGs in DEGs based on the FDGs in the FerrDb database. Prognostic analysis was performed using Kaplan-Meier method with the survival information of ccRCC patients in UCSC Xena database. This study using TIMER2.0 database to analyze the expression level of FDGs in pan cancer. 252 DEGs were obtained through screening, mainly enriched in biological processes such as transmembrane transport of sodium/potassium/chloride ions, sodium potassium ion homeostasis, renal water homeostasis, and aldosterone regulated sodium reabsorption pathways. By intersecting with the FDGs, three genes were obtained: PVT1, HILPDA, and CDKN2A. They are upregulated in ccRCC tumor tissue. The analysis results of survival suggest that high expression of PVT1 and CDKN2A is relevant to poor prognosis in ccRCC patients. The analysis of pan cancer showed that PVT1 and CDKN2A were highly expressed in bladder cancer, breast cancer and other tumors. Patients of ccRCC with high expression level of PVT1 and CDKN2A have poor prognosis, and both have great importances in the formation and progression of ccRCC, which may become biomarkers for the prognosis of ccRCC.*

Keywords: *Bioinformatics; Clear cell renal cell cancer; PVT1; CDKN2A; Prognosis*

1. Introduction

The incidence and mortality of kidney cancer are in the front rank of all types of cancer[1].Renal cell carcinoma (RCC) is the most common one of kidney cancer. Clear cell renal cell carcinoma (ccRCC) accounts for approximately 85% of all RCC tumors[2]. With the increasing number of ccRCC tumors diagnosed, traditional prognostic prediction methods are unable to accurately predict the natural history of these tumors, and there is an urgent need to find alternative methods[3].

Ferroptosis is a new way of cell death. It typically caused by disturbances in intracellular iron metabolism and excessive accumulation of lipid peroxides[4]. Ferroptosis is involved in many diseases, including cancer[5]. Pharmacological regulation of ferroptosis through induction or inhibition has great potential in the treatment of cancer[6]. However, there is limited research on the prognostic evaluation of ferroptosis driver genes (FDGs) in ccRCC patients. Urgent need to explore prognostic evaluation biomarkers for ccRCC.

2. Materials and Methods

2.1. The acquisition and organization of ccRCC dataset

Download mRNA sequencing data of Kidney Clear Cell Cancer (KIRC) from UCSC Xena database (https://xena.ucsc.edu/). Filter the dataset to exclude genes with low expression. Download human

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genome annotation files from Ensembl database (https://www.ensembl.org/). Convert gene IDs in gene expression data into gene symbols and generate mRNA expression matrix for subsequent analysis.

2.2. Analyze differentially expressed genes

"DESeq2" package of v4.2.1 R software was used to analyze gene expression levels between normal and tumor samples in KIRC dataset. We screened for differentially expressed genes (DEGs) with the screening criteria being | Log2FC | > 4.5 and adjusted P-value < 0.05. The 'ggplot2' package is used to visualize the results of differential analysis and draw volcano maps.

2.3. Functional Enrichment Analysis of DEGs

DEGs are imported into DAVID database (https://david.ncifcrf.gov/) to perform gene ontology (GO) functional enrichment analysis and kyoto encyclopedia of genes and genomes (KEGG) signaling pathway enrichment analysis. GO analysis includes three parts: biological process (BP), cellular component (CC), and molecular function (MF).

2.4. Screening for differentially expressed FDGs

We downloaded FDGs from FerrDb database (http://www.zhounan.org/ferrdb/current/), take the intersection of DEGs and FDGs, identify differentially expressed FDGs, and use the "VennDiagram" package to draw a Venn diagram.

2.5. Prognostic analysis of differentially expressed FDGs

We downloaded mRNA sequencing data and survival data of ccRCC patients from the UCSC Xena database, and performed prognostic analysis using the packages of "survival" and "survivminer". Patients were divided into two groups based on the median of gene expression level. Kaplan Meier method was used to plot survival curves. Log rank test was used to clarify the relationship between genes and overall survival (OS) of ccRCC patients.

2.6. The expression of differentially expressed FDGs in pan cancer

The TIMER2.0 database Gene_DE module was used to analyze the expression of differentially expressed FDGs obtained from the above screening between TCGA tumor tissue and normal tissue.

3. Results

3.1. Screening of differentially expressed genes

598 samples and 25477 genes were obtained from the KIRC dataset after data cleaning. From which 252 DEGs were obtained, including 146 significantly upregulated (red) and 106 significantly downregulated (blue). The results were visualized using a volcano plot (see Figure 1).

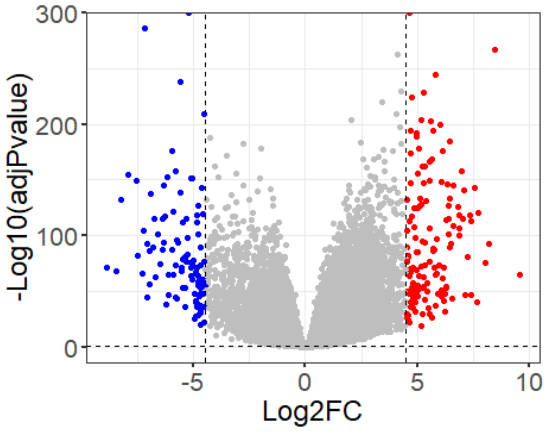

Figure 1: DEGs between normal and tumor tissues

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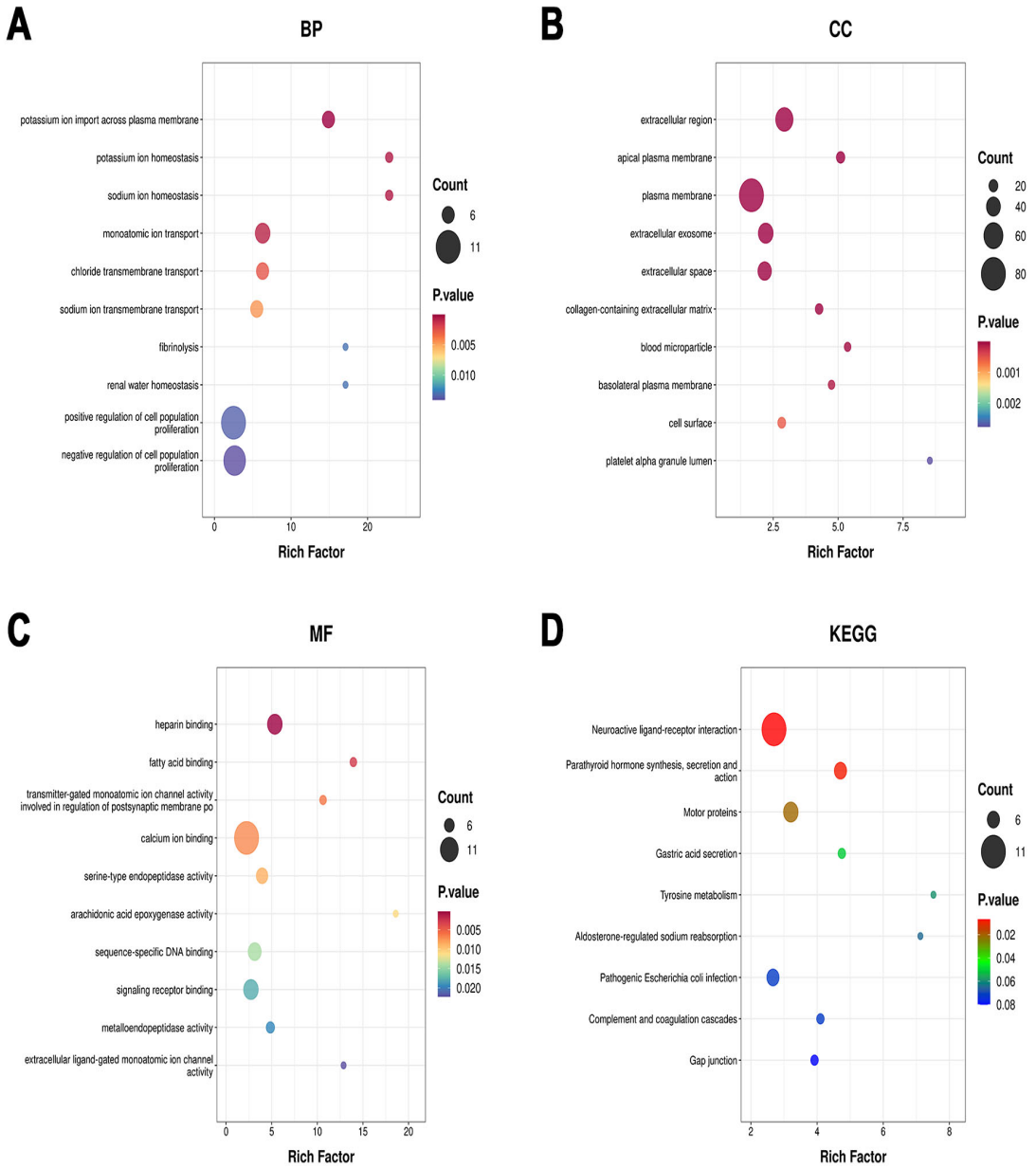
3.2. Analysis of DEGs functional enrichment

The GO analysis results showed that the involvement of DEGs in BP mainly includes transmembrane transport of sodium/potassium/chloride ions, sodium potassium ion homeostasis, renal water homeostasis, and regulation of cell proliferation (see Figure 2A); CC mainly consists of plasma membrane, extracellular vesicles, and extracellular matrix containing collagen (see Figure 2B); MF mainly includes heparin binding, calcium ion binding, sequence specific DNA binding, signal receptor binding, etc. (see Figure 2C). The KEGG results showed that DEGs were significantly enriched in pathways such as neuroactive ligand receptor interactions, synthesis and secretion of parathyroid hormone, motor proteins, tyrosine metabolism, and aldosterone regulated sodium reabsorption (see Figure 2D).



A. Biological Process; B. Cellular Component; C. Molecular Function; D. KEGG

Figure 2: GO and KEGG enrichment analysis of DEGs

3.3. Screening of differentially expressed FDGs

264 FDGs were obtained from the FerrDb database and intersected with DEGs to obtain three genes

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(see Figure 3), namely PVT1, HILPDA, and CDKN2A, all of which were upregulated in ccRCC tumor tissues.

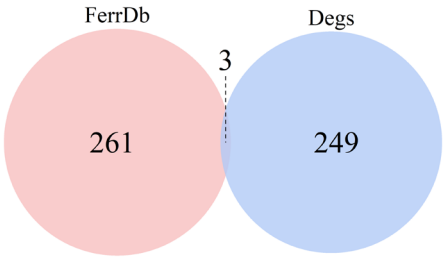

Figure 3: Intersection of DEGs and FDGs

3.4. Prognostic analysis of PVT1, HILPDA, and CDKN2A

The mRNA sequencing data and clinical survival data were sorted and intersected to obtain 522 cases of survival data. According to the median expression levels of PVT1, HILPDA, and CDKN2A in ccRCC tumor tissue, patients were divided into two groups by expression level, 261 higher cases and 261 lower cases. The Kaplan Meier survival curve results showed that the OS of patients with low expression of PVT1 (P=0.00012) and CDKN2A (P=0.043) was markedly higher than that of patients with high expression, HILPDA (P=0.33) was not correlated with the OS of patients (see Figure 4), indicating that PVT1 and CDKN2A are significantly correlated with prognosis and may become prognostic markers for ccRCC patients.

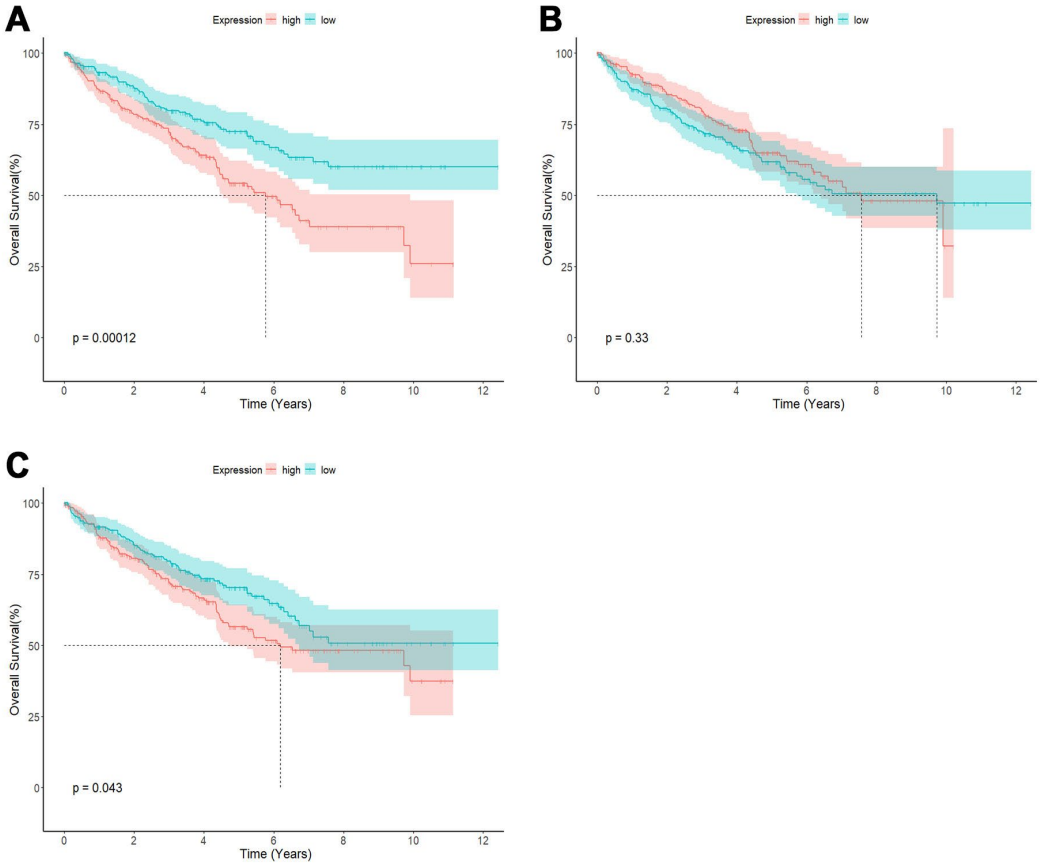

Figure 4: Kaplan Meier survival curve of genes. A. PVT1; B. HILPDA;C. CDKN2A

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3.5. Analysis of the expression of PVT1 and CDKN2A in pan cancer

The analysis results of TIMER2.0 database showed that the expression levels of PVT1 and CDKN2A in ccRCC tumor tissues were markedly higher than those in normal ones. In addition, they are also significantly up-regulated in bladder cancer, breast cancer, cholangiocarcinoma, colon cancer, renal papillary cell carcinoma, liver cancer, lung cancer and other tumor tissues (see Figure 5).

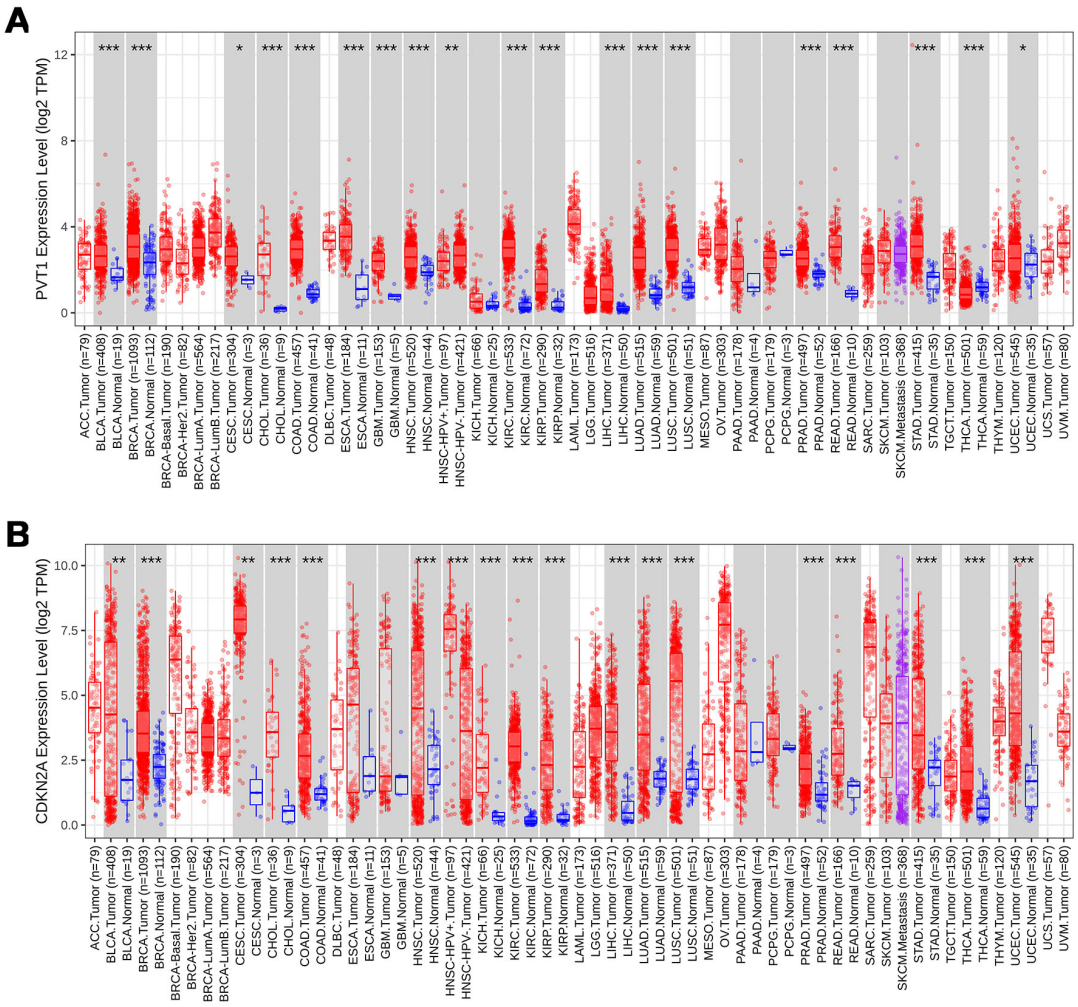

Figure 5: The expression of genes in pan cancer. A. PVT1;B. CDKN2A

4. Discussion

This study is based on the KIRC dataset in the UCSC Xena database, and 252 differentially expressed genes were screened through differential analysis. The functional enrichment analysis results showed that DEGs were chiefly enriched in biological processes such as transmembrane transport of sodium/potassium/chloride ions, sodium potassium ion homeostasis, renal water homeostasis, and regulation of cell proliferation; The KEGG results showed that DEGs were markedly enriched in pathways such as neuroactive ligand receptor interactions, synthesis and secretion of parathyroid hormone, motor proteins, tyrosine metabolism, and aldosterone regulated sodium reabsorption.

Regulating the expression of ferroptosis related genes can regulate the migration and invasion of ccRCC cancer cells[7]. It also can evaluate the prognosis of patients[8]. This study obtained three FDGs from DEGs, namely PVT1, HILPDA, and CDKN2A. Using mRNA expression data and related clinical survival data from ccRCC patients in the UCSC database, Kaplan Meier was applied to analyze the prognostic value of three genes in ccRCC. The results showed that ccRCC patients with high expression of PVT1 and CDKN2A exhibited poor prognosis. Which is a danger factor for poor prognosis in ccRCC patients. It is expected to become an important biomarker for evaluating the

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prognosis of ccRCC patients.

PVT1 lncRNA a large gene locus which located on chromosome 8q24.21. It frequently amplifies in human cancers. It participate in the progression of tumors through multiple mechanisms[9]. CDKN2A is a cyclin dependent kinase inhibitor gene located on chromosome 9p21.3[10] and can serve as a biomarker for the diagnosis and treatment of tumors[11-13]. The expression analysis of PVT1 and CDKN2A in pan cancerous tissues showed that the expression of PVT1 and CDKN2A in bladder cancer, breast cancer and other tumor tissues was markedly higher than that in normal ones. Indicating that PVT1 and CDKN2A have great importances in the formation and progression of various tumors.

5. Conclusions

This study used bioinformatics methods to screen two FDGs PVT1 and CDKN2A which were associated with ccRCC. ccRCC patients with high expression level of PVT1 and CDKN2A had poor prognosis. This suggests that both have great importances in the formation and progression of ccRCC, providing direction for prognosis evaluation of ccRCC patients.

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