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Identification of key candidate genes and pathways in chromophobe renal cell carcinoma by bioinformatic analysis

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Abstract: **To investigate key candidate genes and potential pathways in Chromophobe renal cell carcinoma by bioinformatic analysis. Apply bioinformatic analysis to screen differentially expressed genes based on RNA sequencing data of chromophobe renal cell carcinoma in the Cancer Genome Atlas database.Cluster the candidate genes on the grounds of functions and signaling pathways. Identify 5663 differentially expressed genes based on bioinformatic analysis and performe 1136 gene ontology terms and 43 pathways. We have access to genetic changes and most of the corresponding genes are involved in ion channels, chemokines and G protein-coupled receptor signaling pathways.**

Keywords: Chromophobe renal cell carcinoma; Bioinformatical analysis; Differentially expressed genes

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1. Introduction

Renal cell carcinoma (RCC) is the most common tumor in the kidney[1]. And about 403,262 new cases of RCC are diagnosed annually, with approximately 175,098 deaths[2]. Chromophobe renal cell carcinoma (ChRCC) is a subtype of RCC, accounting for about 5% of RCC[3,4]. ChRCC usually are characterized by an inert local growth pattern and 10-year cancer-specific survival rate is over 90[5]. Prognosis of metastatic ChRCC patients is similar to those with clear cell type histology in terms of progression-free survival and overall survival[6]. The Cancer Genome Atlas (TCGA) is a large and comprehensive collection of clinical information and gene sequencing data. This paper uses TCGA for exploring differential expression genes and enriched signaling pathways in ChRCC.

2. Materials and Methods

2.1. Experimental sample information collection

RNA sequencing data (Level 3) of chromophobe renal cell carcinoma was collected from the TCGA database (https://cancergenome.nih.gov/), including 65 ChRCC tissues and 24 adjacent normal kidney tissues.

2.2. investigate the differential gene expressions of mRNAs in ChRCC

The file (genecode gencode.v22.annotation.gtf) was used to screen mRNA sequencing data, which covers a total of 20271 mRNA data. The R language package was then used for the calculation of aberrantly expressed mRNAs (fold change > 2 and P < 0.01). Exclude the mRNA whose 75% of expression quantity in the sample is less than 2.

2.3. Gene ontology analysis and enrichment pathway analysis

Gene ontology analysis and enrichment pathway analysis of differential genes were performed using the online database DAVID (https://david.ncifcrf.gov/). Gene ontology analysis and enrichment pathway analysis were performed with a P <0.05 as a critical criterion.

3. Results

3.1. Identification of differentially expressed genes in Colorectal Cancers

Bioinformatic analysis of differential expressed genes were performed on mRNA sequencing data of ChRCC specimens and adjacent normal tissues (Fold change > 2 and FDR corrected P <0.01), and 5663 differentially expressed genes were identified in the sample, with 2625 up-regulated and 3038 down-regulated differential expressed genes.

3.2. Ontology analysis in chromophobe renal cell carcinoma

The differential genes were analyzed using the online database DAVID (http://www.genome.jp/kegg) with a P <0.05 as a critical criterion. And they were classified into three functional groups: molecular function group, biological process group, and cellular component group.

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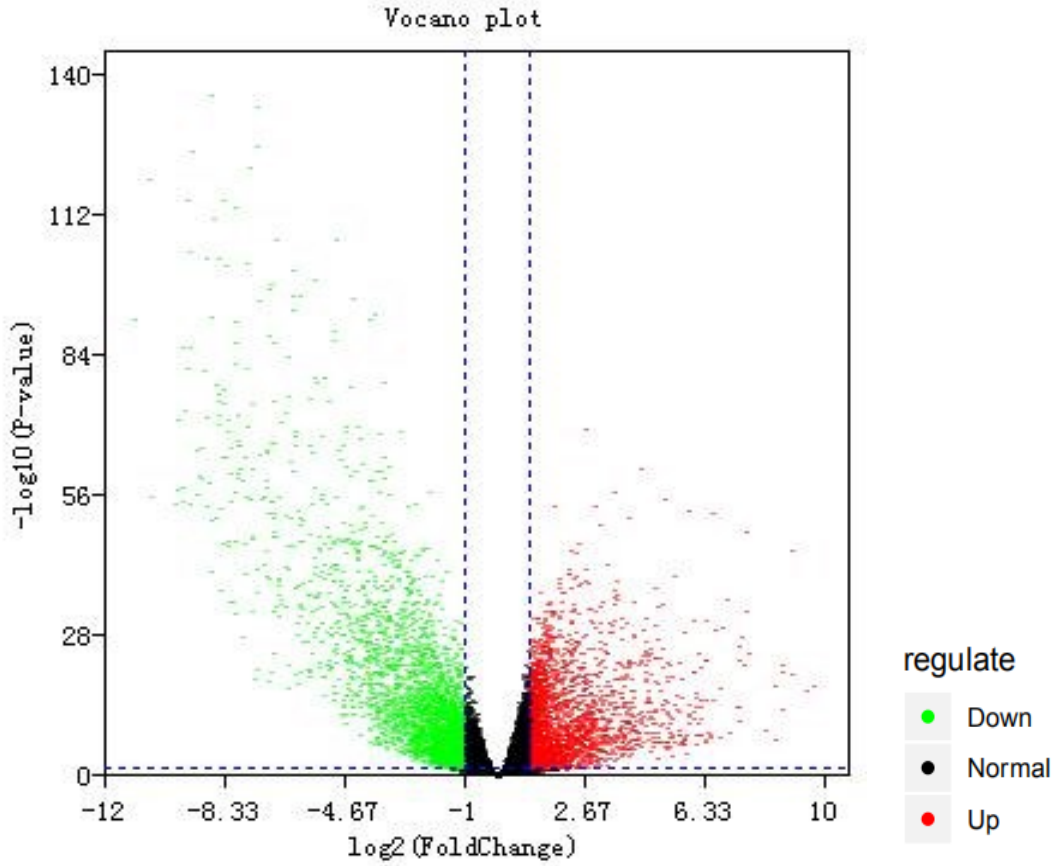


Figure 1. Volcano plot of the aberrantly expressed mRNAs between ChRCC and para-tumorous renal tissues.

Table 1. List of top 20 differentially expressed mRNAs in ChRCC

Downregulated in cancer		Upregulated in cancer	
Genes name	Log2 fold change	Genes name	Log2 fold change
AQP2	-11.09867174	NKX6-1	9.651564121
TMEM207	-10.65523315	TMPRSS11E	9.466177951
NPHS2	-10.57939947	LHX9	9.00454427
UGT1A9	-9.818185821	KLK4	9.00111518
SLC22A12	-9.749169183	TMPRSS11A	8.733797586
PRODH2	-9.739073486	SCG3	8.732447285
NAT8	-9.642001356	PIP	8.693238824
SLC22A6	-9.629186221	SIX6	8.483704586
PLG	-9.580058923	MOG	8.461381901
CYP4A11	-9.577135586	KCNU1	8.236636664
ACSM2B	-9.486056838	LRRTM1	7.724962588
UGT3A1	-9.467939177	GSDMC	7.637328182
SLC13A1	-9.427113334	KLK15	7.592093577
UGT2A3	-9.414919869	PSG9	7.561864119
ACSM2A	-9.407896656	SEZ6L	7.451799929
UNCX	-9.299622176	RTP2	7.424036678
SLC22A8	-9.204258083	ATP1A3	7.399269779
CLDN19	-9.169038635	KLK2	7.386607544
AFM	-9.121994253	HHATL	7.37769628
TM4SF5	-9.046707881	PRSS1	7.309334723

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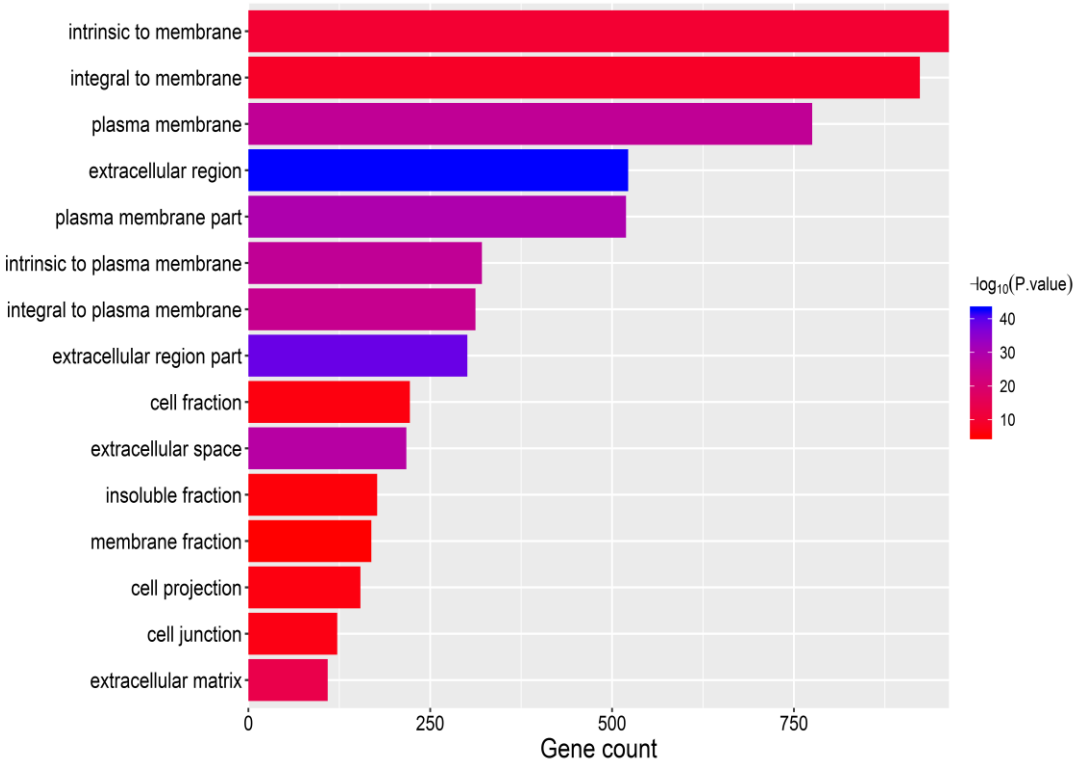


Figure 2. Significant enriched gene ontology terms (top15) of biological process group in down-regulated genes.

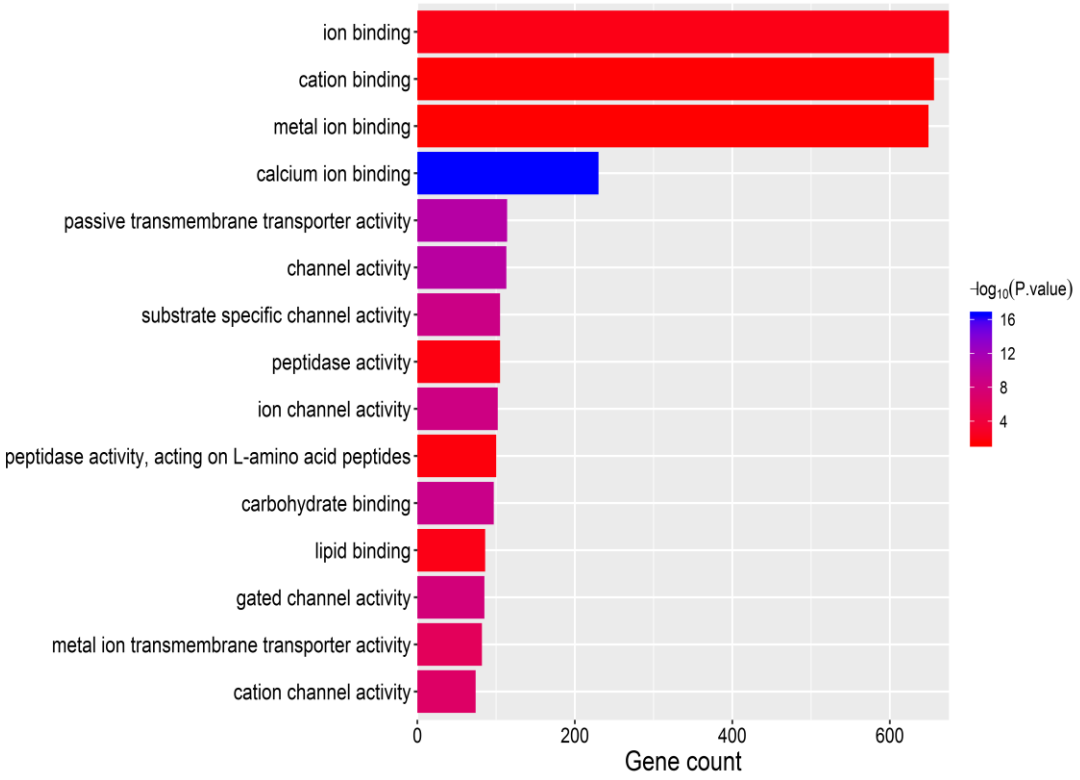


Figure 3. Significant enriched gene ontology terms (top15) of cellular component group in down-regulated genes.

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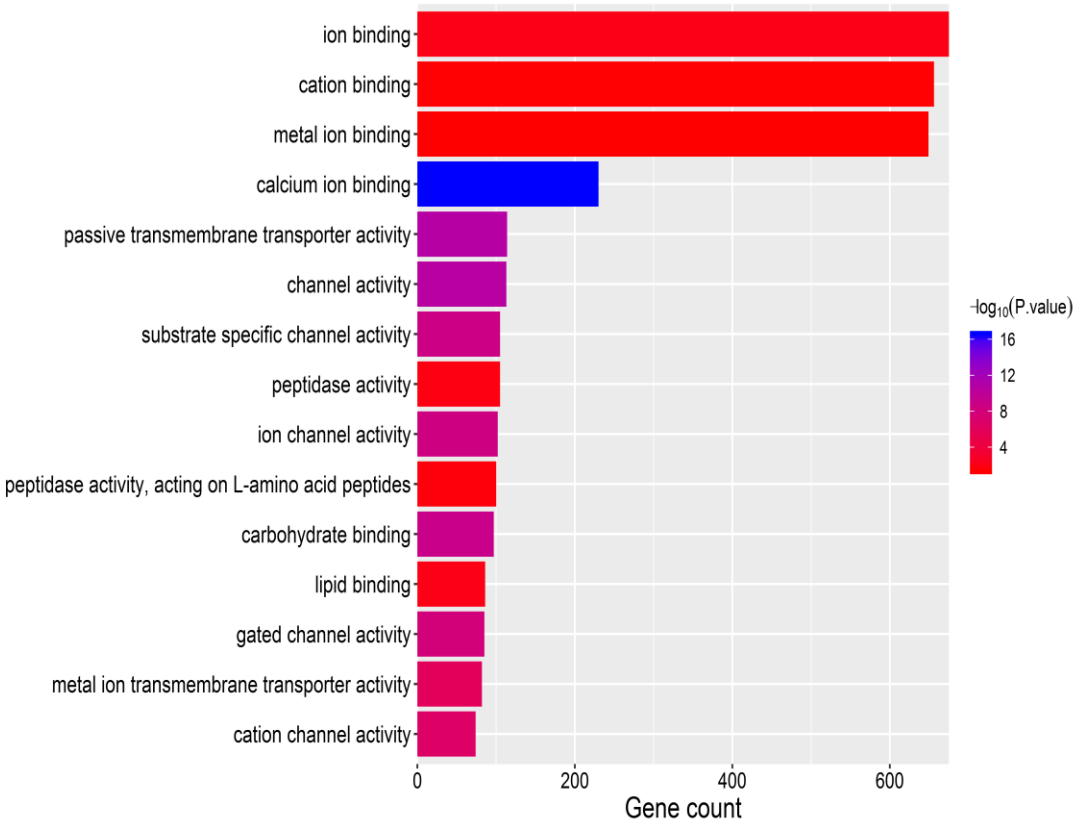


Figure 4. Significant enriched gene ontology terms (top15) of molecular function group in down-regulated genes.

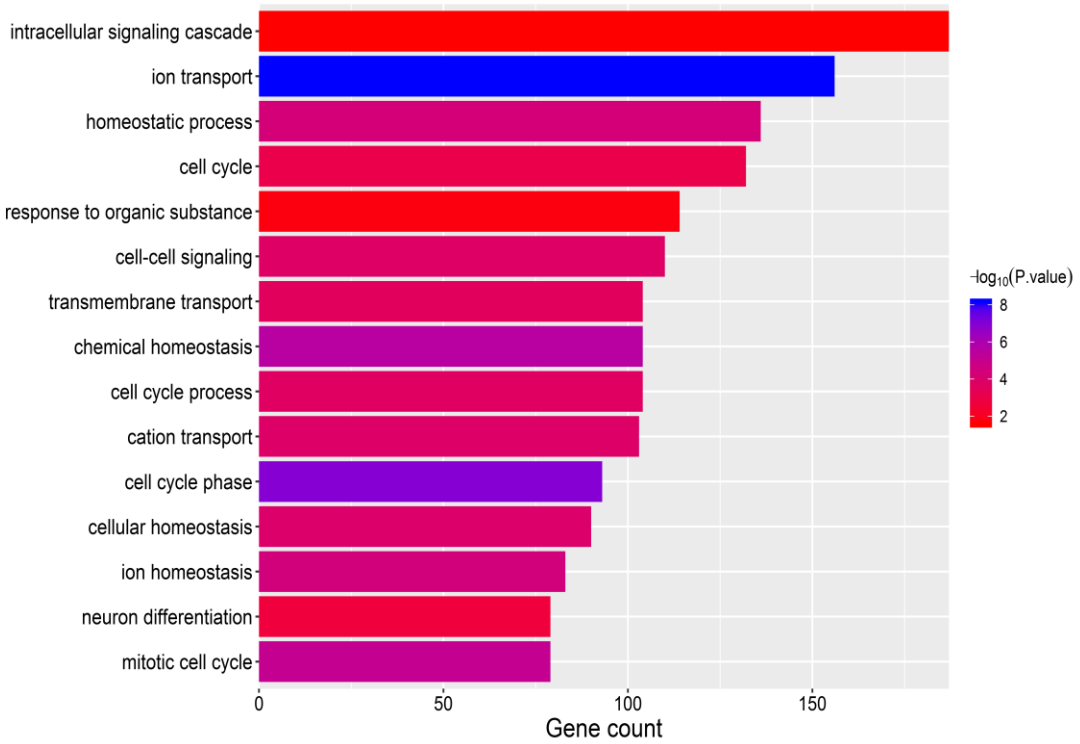


Figure 5. Significant enriched gene ontology terms (top15) of biological process group in up-regulated genes.

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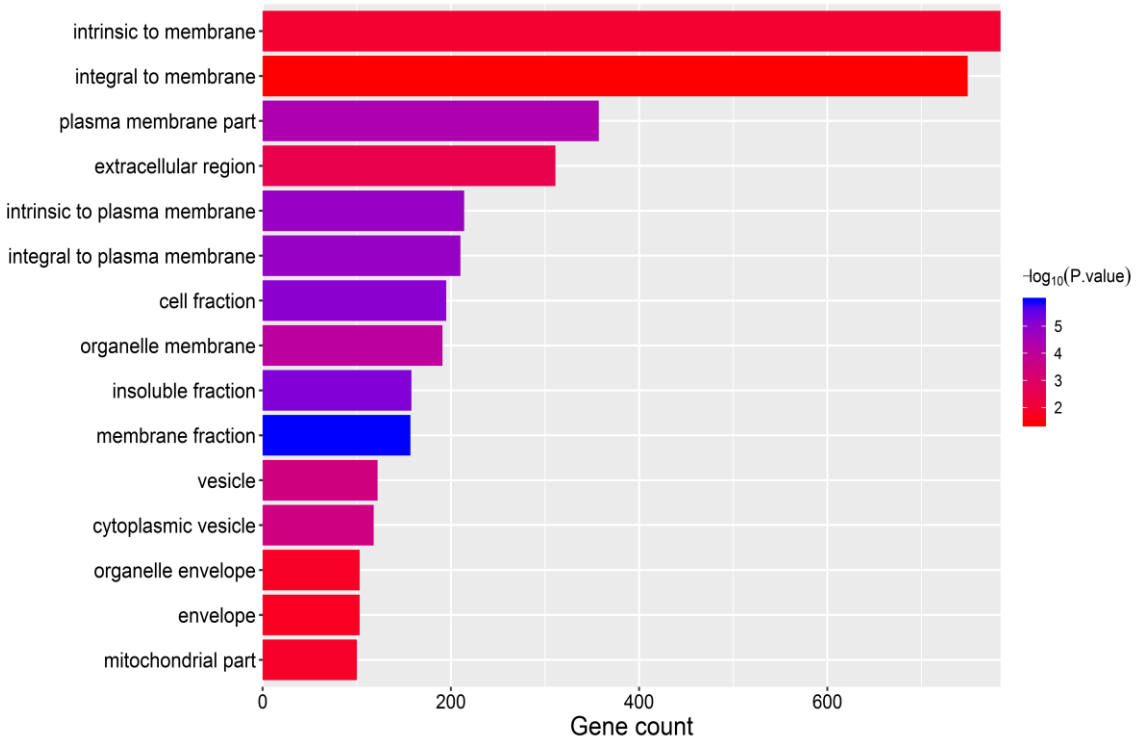


Figure 6. Significant enriched gene ontology terms (top15) of cellular component group in up-regulated genes.

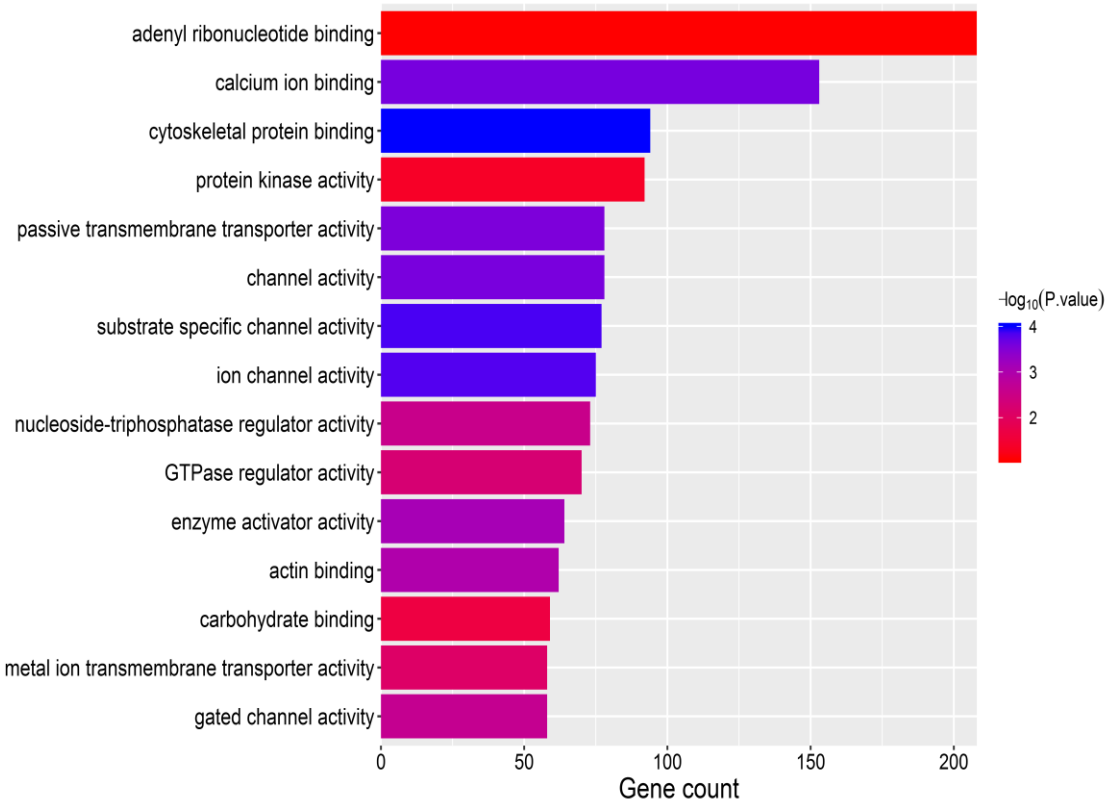


Figure 7. Significant enriched gene ontology terms (top15) of molecular function group in up-regulated genes.

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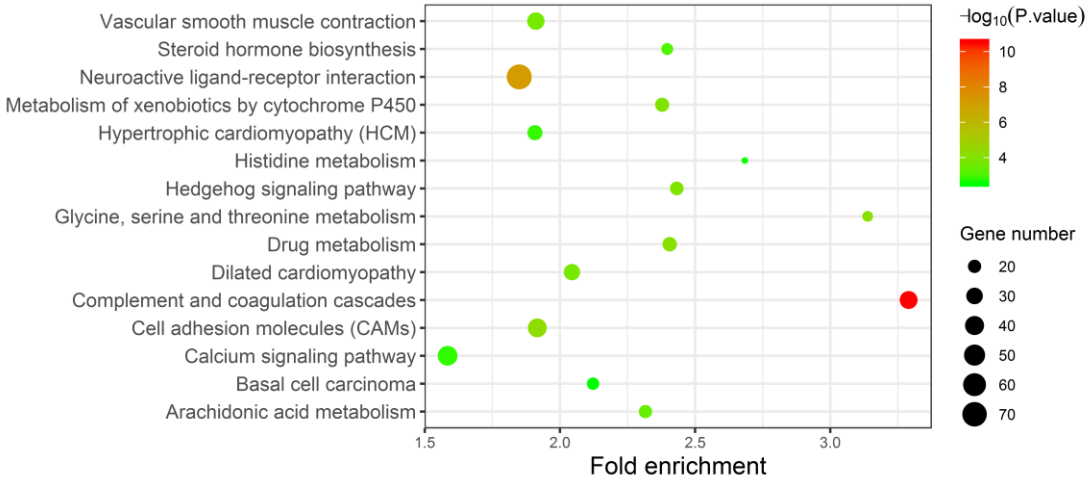


Figure 8. Significantly enriched pathway (top 15) terms of down-regulated genes.

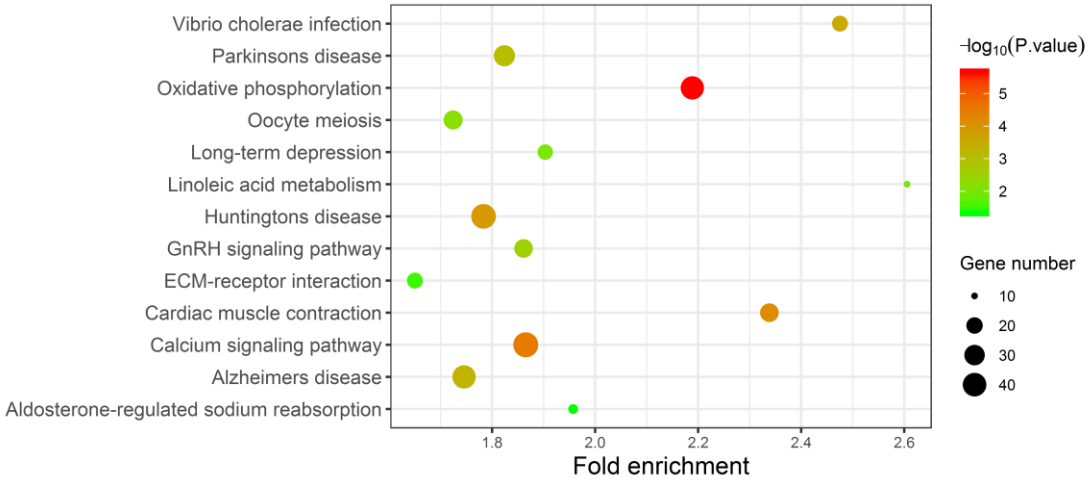


Figure 9. Significantly enriched pathway terms of up-regulated genes.

3.3. Signaling pathway enrichment analysis

The online websites of KEGG PATHWAY was used to enrich the function and signaling pathways of differential genes. The results are Figure 8 and Figure 9.

4. Discussion

In the past few decades, a large number of basic and clinical studies have been conducted to reveal the causes and underlying mechanisms of ChRCC formation and progression, but the mortality of ChRCC are still high in the world. We made a deeply analysis on genetic level basis on bioinformatics methods. We observed the changes of receptor tyrosine kinase and inflammatory factors. And TP53 was frequently mutated in ChRCC[5]. These results were consistent with the findings of the previous study. CD117 is a type III receptor tyrosine kinase that plays a role in intracellular signal transduction of several cell types. It regulates apoptosis, cell differentiation, proliferation, and cell adhesion. In normal tissues of the kidney, CD117 shows weak immunoreactivity only in the cytoplasm of the distal tubules. While the CD117 gene product is overexpressed in the ChRCC[7]. Likewise, several chemokines and chemokine receptors promote RCC growth and metastasis. Stromal cell-derived factor 1 isoform γ (SDF1-γ) levels were significantly higher in ChRCC compared to normal kidneys, and the levels of median transcripts of IL-8 and SDF1-γ in ChRCC were significantly higher[8]. And what's more, recurrent chRCC patients showed differential gene expression of specific biochemical pathways[9].

G protein-coupled receptors are a general term for a large class of membrane protein receptors which participated in many cellular signal transduction processes.G-protein coupled estrogen receptor (GPER) was highly detected in various RCC cell lines and plays an important role in cancer progression [10]. Research indicates that Gα that is a subunit of G protein promotes tumors in RCC and may act through

a PKA-dependent pathway[11]. And protease-activated receptors (PARs), a new family of G-protein coupled receptors, also play a crucial role in vascular development and tumor progression through a variety of mechanisms[12].

Our research shows that development of ChRCC accompany angiogenesis or the formation of new blood vessels. Microvessel density (MVD) is an indicator of angiogenesis and has been proposed to predict the prognosis of patients with renal cell carcinoma[13]. Tumor formation and growth ultimately require neovascularization and potential mechanisms for acquired drug resistance include activation of alternative or compensatory angiogenesis pathways and increased tumor invasiveness[14].

5. Conclusion

Taken above, using integrated bioinformatical analysis, we have identified 5663 differentially expressed genes based on bioinformatic analysis and performed 1136 gene ontology terms and 43 pathways. These findings could significantly improve our awareness of the cause and underlying molecular events in ChRCC, these candidate genes and pathways could be therapeutic targets for ChRCC.

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