Prediction and analysis of novel key genes ITGAX, LAPTM5, SERPINE1 in clear cell renal cell carcinoma through bioinformatics analysis

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Background: Clear Cell Renal Cell Carcinoma (CCRCC) is the most aggressive subtype of Renal Cell Carcinoma (RCC) with high metastasis and recurrence rates. This study aims to find new potential key genes of CCRCC. Methods: Four gene expression profiles (GSE12606, GSE53000, GSE68417, and GSE66272) were downloaded from the Gene Expression Omnibus (GEO) database. The TCGA KIRC database was downloaded from The Cancer Genome Atlas (TCGA). Using GEO2R, the differentially expressed genes (DEG) in CCRCC tissues and normal samples were analyzed. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed in DAVID database. A protein-protein interaction (PPI) network was constructed and the hub gene was predicted by STRING and Cytoscape. GEPIA and Kaplan-Meier plotter databases were used for further screening of Key genes. Expression verification and survival analysis of key genes were performed using TCGA database, GEPIA database, and Kaplan-Meier plotter. Receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of key genes in CCRCC, which is plotted by R software based on TCGA database. UALCAN database was used to analyze the relationship between key genes and clinical pathology in CCRCC and the methylation level of the promoter of key genes in CCRCC.Results: A total of 289 up-regulated and 449 down-regulated genes were identified based on GSE12606, GSE53000, GSE68417, and GSE66272 profiles in CCRCC. The upregulated DEGs were mainly enriched with protein binding and PI3K-Akt signaling pathway, whereas down-regulated genes were enriched with the integral component of the membrane and metabolic pathways. Next, top 35 genes were screened out from the PPI network according to Degree, and three new key genes ITGAX, LAPTM5 and SERPINE1 were further screened out through survival and prognosis analysis. Further results showed that the ITGAX, LAPTM5, and SERPINE1 levels in CCRCC tumor tissues were significantly higher than those in normal tissues and were associated with poor prognosis. ROC curve shows that ITGAX, LAPTM5, and SERPINE1 have good diagnostic value with good specificity

and sensitivity. The promoter methylation levels of ITGAX, LAPTM5 and SERPINE1 in CCRCC tumor tissues were significantly lower than those in normal tissues. We also found that key genes were associated with clinical pathology in CCRCC. Conclusion: ITGAX, LAPTM5, and SERPINE1 were identified as novel key candidate genes that could be used as prognostic biomarkers and potential therapeutic targets for CCRCC.

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Abstract

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Methods: Four gene expression profiles（GSE12606, GSE53000, GSE68417, and GSE66272) were downloaded from the Gene Expression Omnibus (GEO) database. The TCGA KIRC database was downloaded from The Cancer Genome Atlas (TCGA). Using GEO2R, the differentially expressed genes (DEG) in CCRCC tissues and normal samples were analyzed. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed in DAVID database. A protein-protein interaction (PPI) network was constructed and the hub gene was predicted by STRING and Cytoscape. GEPIA and Kaplan-Meier plotter databases were used for further screening of Key genes. Expression verification and survival analysis of key genes were performed using TCGA database, GEPIA database, and Kaplan-Meier plotter. Receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of key genes in CCRCC, which is plotted by R software based on TCGA database. UALCAN database was used to analyze the relationship between key genes and clinical pathology in CCRCC and the methylation level of the promoter of key genes in CCRCC.

Results: A total of 289 up-regulated and 449 down-regulated genes were identified based on GSE12606, GSE53000, GSE68417, and GSE66272 profiles in CCRCC. The upregulated DEGs were mainly enriched with protein binding and PI3K-Akt signaling pathway, whereas down-regulated genes were enriched with the integral component of the membrane and metabolic pathways. Next, top 35 genes were screened out from the PPI network according to Degree, and three new key genes ITGAX, LAPTM5 and SERPINE1 were further screened out through survival and prognosis analysis. Further results showed that the ITGAX, LAPTM5, and SERPINE1 levels in CCRCC tumor tissues were significantly higher than those in normal tissues and were associated with poor prognosis. ROC curve shows that ITGAX, LAPTM5, and SERPINE1 have good diagnostic value with good specificity and sensitivity. The promoter methylation levels of ITGAX,

LAPTM5 and SERPINE1 in CCRCC tumor tissues were significantly lower than those in normal tissues. We also found that key genes were associated with clinical pathology in CCRCC.
Conclusion: ITGAX, LAPTM5, and SERPINE1 were identified as novel key candidate genes that could be used as prognostic biomarkers and potential therapeutic targets for CCRCC.
Keywords: protein-protein interaction; CCRCC; bioinformatical analysis; differentially expressed genes.

Introduction

Kidney cancer is a complex disease composed of a variety of cancers, showing different histology, clinical course, genetic changes, and response to treatment[1]. Renal Cell Carcinoma is the most common tumor in the kidney, Whose morbidity and mortality are rising worldwide. Renal cell carcinoma is divided into different subtypes, including clear cell renal cell carcinoma (CCRCC), chromogenic cell renal carcinoma (chRCC), and papillary renal cell carcinoma (pRCC)[2]. CCRCC is a metabolic disease[3], accounting for more than 80% of all renal cell carcinomas[4]. It is the most aggressive subtype of renal cell carcinoma with a high rate of metastasis and recurrence[5, 6]. Although some progress has been made in the treatment of CCRCC, the current treatments of CCRCC still focus on surgical treatment and traditional chemotherapy[7, 8]. At the same time, there is a lack of effective early diagnosis methods in the clinic, and some patients still have a relapse and targeted drug tolerance, leading to poor prognosis of radiotherapy and chemotherapy. Therefore, finding new targeted biomarkers relevant to the diagnosis and treatment of CCRCC remains of paramount importance.

In this study, bioinformatics methods were used to obtain CCRCC gene expression data from GEO database, and normal samples and CCRCC samples were selected for grouping processing. Next, 738 DEGs were screened, including 289 up-regulated genes and 449 down-regulated genes. And then, GO enrichment analysis and KEGG signal pathway analysis were performed by DAVID. The up-regulated DEGs are mainly concentrated on protein binding, plasma membrane, inflammation, signal transduction, and PI3K-Akt signaling pathways, while the down-regulated genes are mainly concentrated on the extracellular exosome, oxidation-reduction process, integral component of membrane, protein homodimerization activity, and metabolic pathways. Finally, the top 35 hub genes were screened by PPI network. Based on the novelty of ITGAX, LAPTM5 and SERPINE1 that have not been reported in CCRCC, the expression of these three genes is significantly associated with survival prognosis and all have high degrees. Therefore, ITGAX, LAPTM5, and SERPINE1 were finally selected as key genes. Further analysis showed that

ITGAX, LAPTM5, and SERPINE1 are highly expressed in CCRCC, which are significantly related to the survival prognosis of CCRCC. The methylation level of ITGAX, LAPTM5, SERPINE1 in CCRCC is reduced. Moreover，ITGAX, LAPTM5 and SERPINE1 are related to the Clinical pathology of CCRCC and have good diagnostic value for CCRCC. In conclusion, we provided a systematic and comprehensive analysis of CCRCC and is the first to suggest that ITGAX, LAPTM5, and SERPINE1 might be used as biomarkers for the new clinical diagnosis and treatment of CCRCC.

Materials & Methods

Data collection. Four gene expression profiles（GSE12606, GSE53000, GSE68417, and GSE66272) were downloaded from the GEO[9] database (http://www.ncbi.nlm. nih.gov/geo/). The TCGA KIRC database was downloaded from TCGA (https://www.cancer.gov/about-nci /organization /ccg/research/structural-genomics/tcga). The GSE12606[10] data sets was obtained by GPL570 platform, including three adjacent normal kidney specimens, and three CCRCC samples. The GSE53000[11] and GSE68417[12] data sets were based on GPL6244 Platforms. GSE53000 data sets was comprised of 60 samples including 6 adjacent normal kidney specimens and 54 CCRCC samples and GSE68417 data sets was comprised of 43 samples including 14 adjacent normal kidney specimens and 29 CCRCC samples. GSE66272[13, 14] data sets was obtained by GPL5029 platform and contained 27 CCRCC tumor samples and 26 normal kidney samples. The TCGA KIRC database contained 539 CCRCC tumor samples and 72 normal kidney samples.

Study design and data processing. In order to clarify our study, we designed a flow chart to demonstrate data collection, processing, analysis and verification (FIG 1). The online tool GEO2R （http://www.ncbi.nlm.nih.gCCRCC/geo/ geo2r）is an online analysis of GEO series based on the R programming language, which is used to screen DEGs between normal kidney and CCRCC samples from the GSE data sets. The data was standardized and filtered to select significant DEGs. P-value＜0.05, log Fold Change (|log FC|) ≥1 were chosen as the cutoff criteria. log Fold Change (| log FC |) ≥ 1 means that the multiple of change is greater than or equal to 2. It is generally considered that there is a difference between 2 times and more. Then, DEGs were further screened according to cutoff criteria: P-value＜0.05, log FC≥1 as up-regulated genes, P-value <0.05, log FC≤1 as down-regulated genes. Finally, importing all the up-regulated genes or down-regulated genes in the 4 datasets into Funrich 3.1.1 software, and taking the intersection of the up-regulated genes or down-regulated genes in the 4 datasets respectively. TCGA RNA-seq simple converter was used to standardize and log2 conversion of TCGA KIRC data. TCGA KIRC data were used for expression verification and ROC curve analysis of Key genes.

Enrichment analysis of DEGs. DAVID 6.8 (Database for Annotation, Visualization and Integrated Discovery, https://david.ncifcrf.gov/) [15, 16] database was used to analyze Gene Ontology (GO)[17]such as the biological process (BP)[18], cellular component (CC)[19]and molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG)[20]pathway enrichment analysis of

important DEGs, which promoted the visualization of gene and protein function[21]. Among them, the count value represents the number of genes enriched in the pathway. The cutoff value was P<0.05.

Protein-Protein Interaction (PPI) Network construction and hub gene screening. The online tool STRING [22] (http://string-db.org) was utilized for the analysis of protein-protein interactions . Importing all DEGs (including up-regulated genes and down-regulated genes) into the STRING database for analysis, and the confidence level≥0.4 is considered to be significant for PPI. STRING and Cytoscape software (version 3.6.1) were used to construct the PPI network, which is regarded as a network software platform for visualizing protein molecular interactions. Using the degree algorithm in the cytohubba plug-in of Cytoscape, the degree value could be calculated and thus the hub genes could also be screened from PPI network. The importance of genes is directly related to the degree of protein. According to the degree value (degree≥37), the first 35 genes were selected as hub genes. Three key genes ITGAX, LAPTM5 and SERPINE1with high degree were screened through survival and prognosis analysis as well as the novelty of genes.

Key genes analysis and verification. Three key genes were analyzed and verified comprehensively by using TCGA, GEPIA, Oncomine, Kaplan-Meier plotter and UALCAN databases. Similarly, TCGA database also applied to ROC curve analysis. ROC curve was plotted by R software, which could be used to analyze the diagnostic value of key genes in CCRCC. GEPIA (http://gepia.cancer-pku.cn/) analyzed the expression of key genes in CCRCC. Oncomine[23] (http://www.oncomine.org) was a database consisting of microarray data of various tumors, which verified the expression of key genes in CCRCC. Kaplan-Meier Plotter (http://kmplot.com/analysis/index) was used to analyze the survival of key genes in CCRCC. UALCAN[24] (http://ualcan.path.uab.edu/index.html) online database was used to analyze the relationship between key genes and Clinical pathology in CCRCC, and the promoter methylation level of key genes in CCRCC was analyzed.

Statistical analysis. Statistically significant differences between the normal tissues group and tumor tissues group were determined using Student's t tests. Also, Kaplan-Meier analysis was used to assess OS. ROC curve is a graphical plot [25] that reflects the sensitivity and specificity of continuous variables. $P < 0.05$ was considered to be statistically significant for all the tests. All the statistical analyses applied GraphPad prism 6.0 or R software.

Results

Volcano plots of the differentially expressed genes in four datasets. The data sets of CCRCC, which were GSE12606, GSE53000, GSE68417 and GSE66272, were downloaded from the GEO database (Table 1) and analyzed by GEO2R separately. A total of 9,560 DEGs were screened from the GSE12606 data sets, among which 1568 genes were up-regulated and 5436 genes were down-regulated instead. A total of 1,286 DEGs were screened from the GSE53000 data sets, among which 583 were up-regulated and 708 were down-regulated. A total of 1,890 DEGs were screened from the GSE68417 data sets among them 726 up-regulated and 1,164 down-regulated genes were selected. There were 3,627 up-regulated genes and 5,170 down-regulated genes among the 8,797 DEGs screened from the GSE66272 data sets. The screening criteria were P-value＜0.05, log Fold

Change (| log FC |) ⩾ 1. All of the DEGs from the four data sets were presented in Volcano plots (FIG 2A-D). Among them, red represents high-expressed genes, green represents low-expressed genes, and black represents genes whose expression levels are not significant in each data set. We gained the intersection of four independent data sets through Funrich 3.1.1 software and made a visualization analysis of Venn Diagram. After being overlapped, the common 738 genes (supplementary FIG.1) were identified, including 289 up-regulated and 449 down-regulated genes (FIG 2E-F).

GO and KEGG enrichment analysis of DEGs. To further explore the function of DEGs, enrichment analysis of up-regulated genes and down-regulated genes were displayed respectively. DAVID 6.8 was used to perform GO and KEGG analysis of DEGs in CCRCC (Table 2). In biological processes, up-regulated DEGs are mostly involved in cell adhesion, signal transduction, immune response, especially the regulation of inflammatory response (FIG 3A); while down-regulated DEGs are mostly involved in oxidation-reduction process proteolysis, ion transmembrane transport, response to drug, and ion transport (FIG 3E). In terms of cellular components, up-regulated DEGs are mainly distributed in the plasma membrane, integral component of the membrane, extracellular exosome, and membrane (FIG 3B); down-regulated DEGs are mainly distributed in integral component of membrane, plasma membrane, cellular exosomes, and integral component of plasma membrane (FIG 3F). In terms of molecular function, up-regulated DEGs generally have protein binding, identical protein bind, ATP binding capacity, and protein homodimerization activity (FIG 3C); down-regulated DEGs generally have identical protein homodimerization activity, calcium ion binding, sequence-specific DNA binding, and oxidoreductase activity (FIG 3G). In the KEGG signal pathway, the up-regulation of DEGs mostly involved in the PI3K-Akt signaling pathway, pathways in cancer, focal adhesion, and HIF-1 signaling pathway (FIG 3D); while the down-regulation of DEGs mainly involved in the metabolic pathway, Biosynthesis of antibiotics, Carbon metabolism, and Aldosterone-regulated sodium reabsorption (FIG 3H). CCRCC is a kind of metabolic disease. Metabolic reprogramming covers different processes including aerobic glycolysis, fatty acid metabolism and the utilization of tryptophan, glutamine as well as arginine[26], which has also been proved by the results of KEGG pathway enrichment analysis in the work. KEGG pathway results show that down-regulated DEGs are enriched in metabolic pathways, Glycolysis/Gluconeogenesis, Glycine, serine and threonine metabolism and so on.

In addition, GO and KEGG enrichment analyses were performed for all DEG (up-regulated and down-regulated genes) (supplementary FIG.2). Finally, 11 common enrichment results were found from the three analyses (including up-regulated DEG, down-regulated DEG and all DEG enrichment analyses). The common enrichment results are as follows: response to drug, plasma membrane, integral component of membrane, extracellular space, extracellular exosome, cell surface, cell surface, protein homodimerization activity, identical protein binding. The results of enrichment analysis were consistent with previous studies[27, 28]. It suggests that these 11 pathways may be important in CCRCC.

Construction of PPI network and screening of key genes. To identify the key genes, the STRING online database and Cytoscape software were used to analyze all DEGs (including up-regulated genes and down-regulated genes)and construct PPI network (FIG 4A). Based on the main role of proteins in biological functions, their interaction determines the molecular and cellular mechanisms that control the health and disease state of the organism[29]. Next, using the degree algorithm in the cytohubba plug-in of Cytoscape to screen the hub genes in PPI network. The gene whose degree$\geqslant$37 could be defined hub gene, therefore the first 35 genes (Table 3) in the PPI network were chosen to be hub genes (FIG 4D). Next, GO enrichment analysis was conducted for all DEGs and the first 35 DEGs in the PPI network. The result showed that all DEGs mainly enriched in signal transduction, oxidation-reduction process, cell adhesion, inflammatory response, plasma membrane, integral component of membrane, extracellular exosome, integral component of plasma membrane (FIG 4B-C). The top 35 DEGs were enriched in extracellular space, extracellular exosome, inflammatory response, an integral component of the plasma membrane, and cell surface (FIG 4E). By comparison, it turned out that the enrichment analysis results of all DEGs in PPI network contained the results of the top 35's. In the end, three new key genes with high degree which were ITGAX, LAPTM5 and SERPINE1, could be screened by using GEPIA and Kaplan-Meier plotter database.

The expression of key genes. Among the 35 genes, we focused on ITGAX, LAPTM5, and SERPINE1, which have not been reported to be related to the occurrence and development of CCRCC. Firstly, the expression levels of ITGAX, LAPTM5, and SERPINE1 in CCRCC tumor tissues are significantly higher than those in normal tissues adjacent to cancer according to GEPIA database (FIG 5A-C). Furthermore, the data of TCGA KIRC showed that compared with adjacent normal tissues, the mRNA expressions of ITGAX, LAPTM5, and SERPINE1 in 72 pairs of CCRCC tissues were significantly increased (FIG 5D-F). In the Oncomine Gumz renal database, the mRNA levels of ITGAX, LAPTM5 and SERPINE1 were also upregulated in CCRCC tissues when compared with adjacent normal kidney tissues (FIG 5H-G). According to the UALCAN database, the promoter methylation levels of ITGAX, LAPTM5 and SERPINE were decreased in CCRCC (FIG 5J-L). To sum up, according to the GEPIA database, Oncomine Gumz renal database and TCGA database, the expression levels of these three genes in CCRCC tumor tissues are significantly higher than those in normal tissues adjacent to cancer. It could be further speculated that the high expression of ITGAX, LAPTM5, and SERPINE1 in CCRCC tumor tissue might be related to the decrease of promoter methylation.

The association of key genes expression with clinical pathology in CCRCC.

Furthermore, the relationship between the mRNA expression of ITGAX, LAPTM5 and SERPINE1 and different clinical pathology grades were measured. The results showed that their mRNA expression was significantly related to pathological grades (FIG 6A-C). And the expression of ITGAX, LAPTM5, and SERPINE1 mRNA in CCRCC samples are also significantly correlated with severe clinical staging (FIG6 D-F). Among them, the expression levels of ITGAX, LAPTM5, and SERPINE1 were higher in stage 4 and grade 4. In conclusion, ITGAX, LAPTM5, and SERPINE1 are significantly associated with clinical pathology.

Survival and diagnostic value of ITGAX, LAPTM5, and SERPINE1 in CCRCC. According to the Kaplan-Meier plotter databases, the overall survival of ITGAX, LAPTM5, and SERPINE1 genes was tested (FIG 7A-C). The results showed that the high expression of three key genes in CCRCC was negatively correlated with prognosis. Then, the ROC curve was used to evaluate the difference between CCRCC and the normal tissues in the TCGA datasets. The ROC curves (FIG 7D-E) of these three genes showed that their area under the curve (AUC) and the 95% confidence intervals (CI) are as follows: ITGAX (AUC=96.315, CI=0.9423-0.9840), LAPTM5, (AUC=94.808 CI=0.9243-0.9719), and SERPINE1(AUC=76.272, CI:0.7068 to 0.8186). Since ROC curves had good specificity and sensitivity, ITGAX, LAPTM5, and SERPINE1 had excellent diagnostic efficiency for distinguishing tumors and normal tissues.

Discussion

Clear Cell Renal Cell Carcinoma (CCRCC) is a metabolic disease whose morbidity is rising worldwide. The feature of kidney cancer is to participate in the target genes' mutation of metabolic pathways[26]. Recently, with the application of bioinformatics, the molecular characteristics of CCRCC have been greatly improved and the development of targeted therapy has been promoted. These advances have significantly improved the median survival of patients with advanced disease. However, about 30% of CCRCC local patients will still relapse or metastasize after surgical removal of the tumor[30]. Around 1/3 of the metastatic patients had poor prognosis and rare high drug resistance rate. Under the circumstance of different treatment, the identification of biomarkers was needed urgently so as to predict the drug's effects[31]. Therefore, identification of CCRCC key genes and prognostic judgment is still very crucial. In our research, some reported genes related to CCRCC, such as VEGFA[32], EGFR[33], were also screened out. Vascular Endothelial Growth Factor A (VEGFA) is a member of the PDGF/VEGF growth factor family. VEGFA has a potential role in the diagnosis and treatment of CCRCC. VEGFA can inhibit the proliferation of CCRCC 786-O cells, promote cell apoptosis, and inhibit cell migration and invasion [32]. Epidermal growth factor receptor (EGFR) is closely related to the progression of many epithelial malignancies and is an important therapeutic target[33]. EGFR is a cell surface protein, belonging to the ERBB family. EGFR binds to epidermal growth factor to induce receptor dimerization and tyrosine self phosphorylation, which eventually leads to cell proliferation[34]. EGFR can activate a variety of signaling pathways, mainly MAPK / ERK and PI3K / AKT pathways[35]. Compared with the former 4 studies, this study found three new key genes which were ITGAX, LAPTM5 and SERPINE1, and applied several methods to do functional analysis and systematic research to key genes such as methylation level analysis, survival analysis, ROC curve analysis and so on. In previous studies, GSE12606 mainly focused on the functional analysis of HLA ligand in CCRCC, while GSE5300 mainly focused on the structure and revolution of CCRCC genome, and GSE66272 mainly focused on the role of miRNA in CCRCC and GSE68417 mainly focused on the analysis to the gene expression profile of CCRCC. On the contrary, this study put emphasis on screening the biomarkers used to do early diagnosis in CCRCC and analyzing the intersection of DEGs in four datasets.

Besides, three new potential marker genes ITGAX, LAPTM5, SERPINE1 were also screened out and proved. Integrin alpha x (ITGAX) is a member of the integrin family, commonly function as a receptor for extracellular matrix. It is reported that ITGAX is involved in the angiogenesis of dendritic cells and tumor angiogenesis[36]. In addition, ITGAX is identified as a new type of aggressive prostate cancer susceptibility gene[37]. Lysosomal protein transmembrane 5 (LAPTM5), known as E3 protein, may play a role in hematopoiesis and prevent excessive activation of lymphocytes[38]. It is reported that LAPTM5 can regulate the proliferation and viability of bladder cancer cells, leading to cell cycle arrest in the G0/G1 phase[39]. LAPTM5 is also associated with the spontaneous regression of neuroblastoma[40]. Studies have shown that Serpin family E member 1 (SERPINE1） is a regulator of Glioblastoma (GBM) cell proliferation, and is related to poor prognosis of patients and mesenchymal GBM. Down-regulation of SERPINE1 in primary GBM cells inhibited the growth and invasiveness of tumors in the brain, and SERPINE1 plays a key role in the spread of GBM[41].

Conclusions

To sum up, the expression levels of ITGAX, LAPTM5, SERPINE1 in CCRCC tumor tissues are significantly higher than those in normal tissues adjacent to cancer and are related to the tumor stage and tumor grade. ITGAX, LAPTM5, and SERPINE1 have high diagnostic efficiency for tumors and normal tissues, and their expressions are associated with poor prognosis of CCRCC. The decrease of promoter methylation of ITGAX, LAPTM5 and SERPINE1 in CCRCC tumor tissues indicates that the high expression of key genes in CCRCC might be relevant to the low methylation level. The limitation of this study lied in that the internal molecular mechanisms where key genes played a role remained unclear, which need a further research. Further studies are needed to explore the detailed mechanisms of these key genes in CCRCC. In conclusion, we identified ITGAX, LAPTM5, and SERPINE1 as potential marker genes of CCRCC by bioinformatics methods, providing insights for future therapeutic design. Meanwhile, we conducted a relatively systematic and comprehensive analysis on CCRCC data, thereby providing a theoretical basis for identifying therapeutic targets of CCRCC, promoting early detection, and monitoring tumor progression.

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References

1. Linehan, W.M. *et al.* The Metabolic Basis of Kidney Cancer. *Cancer discovery* **9**, 1006-1021 (2019).
2. Linehan, W.M. & Ricketts, C.J. The Cancer Genome Atlas of renal cell carcinoma: findings and clinical implications. *Nature reviews. Urology* **16**, 539-552 (2019).
3. Wettersten, H.I., Aboud, O.A., Lara, P.N., Jr. & Weiss, R.H. Metabolic reprogramming in clear cell renal cell carcinoma. *Nature reviews. Nephrology* **13**, 410-419 (2017).
4. Makhov, P. *et al.* Resistance to Systemic Therapies in Clear Cell Renal Cell Carcinoma: Mechanisms and Management Strategies. *Molecular cancer therapeutics* **17**, 1355-1364 (2018).
5. Jiang, Y. *et al.* FOXM1-Activated LINC01094 Promotes Clear Cell Renal Cell Carcinoma Development via MicroRNA 224-5p/CHSY1. *Molecular and cellular biology* **40** (2020).
6. Yuan, L. *et al.* Identification of key genes and pathways in human clear cell renal cell carcinoma (ccRCC)

by co-expression analysis. *International journal of biological sciences* **14**, 266-279 (2018).
7. Loo, V., Salgia, M., Bergerot, P., Philip, E.J. & Pal, S.K. First-Line Systemic Therapy for Metastatic Clear-Cell Renal Cell Carcinoma: Critical Appraisal of Emerging Options. *Targeted oncology* **14**, 639-645 (2019).
8. Bex, A. *et al.* Comparison of Immediate vs Deferred Cytoreductive Nephrectomy in Patients With Synchronous Metastatic Renal Cell Carcinoma Receiving Sunitinib: The SURTIME Randomized Clinical Trial. *JAMA oncology* **5**, 164-170 (2019).
9. Barrett, T. *et al.* NCBI GEO: archive for functional genomics data sets--update. *Nucleic acids research* **41**, D991-995 (2013).
10. Stickel, J.S. *et al.* HLA ligand profiles of primary renal cell carcinoma maintained in metastases. *Cancer immunology, immunotherapy : CII* **58**, 1407-1417 (2009).
11. Gerlinger, M. *et al.* Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. *Nature genetics* **46**, 225-233 (2014).
12. Thibodeau, B.J. *et al.* Characterization of clear cell renal cell carcinoma by gene expression profiling. *Urologic oncology* **34**, 168.e161-169 (2016).
13. Wotschofsky, Z. *et al.* Integrated microRNA and mRNA Signature Associated with the Transition from the Locally Confined to the Metastasized Clear Cell Renal Cell Carcinoma Exemplified by miR-146-5p. *PloS one* **11**, e0148746 (2016).
14. Liep, J. *et al.* Cooperative Effect of miR-141-3p and miR-145-5p in the Regulation of Targets in Clear Cell Renal Cell Carcinoma. *PloS one* **11**, e0157801 (2016).
15. Huang da, W., Sherman, B.T. & Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols* **4**, 44-57 (2009).
16. Huang da, W., Sherman, B.T. & Lempicki, R.A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic acids research* **37**, 1-13 (2009).
17. Expansion of the Gene Ontology knowledgebase and resources. *Nucleic acids research* **45**, D331-d338 (2017).
18. Berchtold, E., Csaba, G. & Zimmer, R. RelExplain-integrating data and networks to explain biological processes. *Bioinformatics (Oxford, England)* **33**, 1837-1844 (2017).
19. Borg, T.K. & Baudino, T.A. Dynamic interactions between the cellular components of the heart and the extracellular matrix. *Pflugers Archiv : European journal of physiology* **462**, 69-74 (2011).
20. Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* **28**, 27-30 (2000).
21. Dennis, G., Jr. *et al.* DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome biology* **4**, P3 (2003).
22. von Mering, C. *et al.* STRING: a database of predicted functional associations between proteins. *Nucleic acids research* **31**, 258-261 (2003).
23. Rhodes, D.R. *et al.* Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia (New York, N.Y.)* **9**, 166-180 (2007).
24. Chandrashekar, D.S. *et al.* UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia (New York, N.Y.)* **19**, 649-658 (2017).
25. Cao, R. & López-de-Ullibarri, I. ROC Curves for the Statistical Analysis of Microarray Data. *Methods in molecular biology (Clifton, N.J.)* **1986**, 245-253 (2019).
26. Lucarelli, G. *et al.* Metabolomic insights into pathophysiological mechanisms and biomarker discovery in clear cell renal cell carcinoma. *Expert review of molecular diagnostics* **19**, 397-407 (2019).
27. Wang, S., Yu, Z.H. & Chai, K.Q. Identification of EGFR as a Novel Key Gene in Clear Cell Renal Cell Carcinoma (ccRCC) through Bioinformatics Analysis and Meta-Analysis. *BioMed research international* **2019**, 6480865 (2019).
28. Tian, Z.H., Yuan, C., Yang, K. & Gao, X.L. Systematic identification of key genes and pathways in clear cell renal cell carcinoma on bioinformatics analysis. *Annals of translational medicine* **7**, 89 (2019).
29. Safari-Alighiarloo, N., Taghizadeh, M., Rezaei-Tavirani, M., Goliaei, B. & Peyvandi, A.A. Protein-protein interaction networks (PPI) and complex diseases. *Gastroenterology and hepatology from bed to bench* **7**, 17-31 (2014).
30. Li, Q.K., Pavlovich, C.P., Zhang, H., Kinsinger, C.R. & Chan, D.W. Challenges and opportunities in the proteomic characterization of clear cell renal cell carcinoma (ccRCC): A critical step towards the personalized care of renal cancers. *Seminars in cancer biology* **55**, 8-15 (2019).
31. Deleuze, A. *et al.* Immunotherapy in Renal Cell Carcinoma: The Future Is Now. *International journal of*

molecular sciences **21** (2020).
32. Zeng, F.C. *et al.* Downregulation of VEGFA inhibits proliferation, promotes apoptosis, and suppresses migration and invasion of renal clear cell carcinoma. *OncoTargets and therapy* **9**, 2131-2141 (2016).
33. Cossu-Rocca, P. *et al.* EGFR kinase-dependent and kinase-independent roles in clear cell renal cell carcinoma. *American journal of cancer research* **6**, 71-83 (2016).
34. Mitsudomi, T. & Yatabe, Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. *The FEBS journal* **277**, 301-308 (2010).
35. Yarden, Y. & Sliwkowski, M.X. Untangling the ErbB signalling network. *Nature reviews. Molecular cell biology* **2**, 127-137 (2001).
36. Wang, J., Yang, L., Liang, F., Chen, Y. & Yang, G. Integrin alpha x stimulates cancer angiogenesis through PI3K/Akt signaling-mediated VEGFR2/VEGF-A overexpression in blood vessel endothelial cells. *Journal of cellular biochemistry* **120**, 1807-1818 (2019).
37. Williams, K.A. *et al.* A systems genetics approach identifies CXCL14, ITGAX, and LPCAT2 as novel aggressive prostate cancer susceptibility genes. *PLoS genetics* **10**, e1004809 (2014).
38. Cai, X. *et al.* Expression and Polymorphisms of Lysosome-Associated Protein Transmembrane 5 (LAPTM5) in Patients with Systemic Lupus Erythematosus in a Chinese Population. *Biochemical genetics* **53**, 200-210 (2015).
39. Chen, L. *et al.* Downregulation of LAPTM5 suppresses cell proliferation and viability inducing cell cycle arrest at G0/G1 phase of bladder cancer cells. *International journal of oncology* **50**, 263-271 (2017).
40. Inoue, J. *et al.* Lysosomal-associated protein multispanning transmembrane 5 gene (LAPTM5) is associated with spontaneous regression of neuroblastomas. *PloS one* **4**, e7099 (2009).
41. Seker, F. *et al.* Identification of SERPINE1 as a Regulator of Glioblastoma Cell Dispersal with Transcriptome Profiling. *Cancers* **11** (2019).

Table 1(on next page)

Summary of TCGA and GEO Clear Cell Renal Cell Carcinoma datasets.

1

Author, year	Sample	GEO no.	Platform	Normal	Tumor	（Refs.)
Stickel JS et al, 2009	CCRCC	GSE12606	GPL570	3	3	**[10]**
Gerlinger M et al, 2014	CCRCC	GSE53000	GPL6244	6	54	**[11]**
Thibodeau BJ et al, 2016	CCRCC	GSE68417	GPL6244	29	14	**[12]**
Wotschofsky Z et al, 2016	CCRCC	GSE66272	GPL5029	27	26	**[13]**
TCGA	CCRCC	-	-	72	539	

2 TCGA, The Cancer Genome Atlas. GEO, Gene Expression Omnibus

3

Table 2(on next page)

GO and KEGG analysis of differentially expressed DEG associated with CCRCC.

1

2 **Table 2 GO and KEGG analysis of up-and down-regulated DEGs associated with CCRCC**

Expression	Category	Term	Count	P-value
Upregulated	GO_BP	GO:0006954~inflammatory response	30	1.30E-12
	GO_BP	GO:0007165~signal transduction	29	1.31E-02
	GO_BP	GO:0007155~cell adhesion	26	5.33E-08
	GO_BP	GO:0006955~immune response	24	1.76E-07
	GO_BP	GO:0042493~response to drug	21	5.92E-08
	GO_BP	GO:0045087~innate immune response	19	1.36E-04
	GO_BP	GO:0030198~extracellular matrix organization	18	1.08E-08
	GO_BP	GO:0050900~leukocyte migration	17	6.55E-11
	GO_BP	GO:0001525~angiogenesis	17	4.06E-07
	GO_BP	GO:0008284~positive regulation of cell proliferation	17	2.57E-03
	GO_CC	GO:0005886~plasma membrane	108	1.13E-09
	GO_CC	GO:0016021~integral component of membrane	107	2.03E-04
	GO_CC	GO:0070062~extracellular exosome	66	2.68E-04
	GO_CC	GO:0016020~membrane	64	4.11E-07
	GO_CC	GO:0005887~integral component of plasma membrane	62	6.07E-14
	GO_CC	GO:0005576~extracellular region	50	1.98E-06
	GO_CC	GO:0005615~extracellular space	49	2.48E-08

GO_CC	GO:0009986~cell surface	31	1.08E-09
GO_CC	GO:0005783~endoplasmic reticulum	20	4.86E-02
GO_CC	GO:0000139~Golgi membrane	17	1.94E-02
GO_MF	GO:0005515~protein binding	154	6.05E-03
GO_MF	GO:0005524~ATP binding	36	6.15E-03
GO_MF	GO:0042803~protein homodimerization activity	21	7.66E-03
GO_MF	GO:0042802~identical protein binding	20	1.92E-02
GO_MF	GO:0005102~receptor binding	19	7.41E-06
GO_MF	GO:0004872~receptor activity	14	2.70E-05
GO_MF	GO:0004672~protein kinase activity	12	2.09E-02
GO_MF	GO:0005215~transporter activity	8	3.41E-02
GO_MF	GO:0004888~transmembrane signaling receptor activity	8	4.43E-02
GO_MF	GO:0005201~extracellular matrix structural constituent	7	5.17E-04
KEGG	hsa04151: PI3K-Akt signaling pathway	19	2.01E-04
KEGG	hsa04510: Focal adhesion	17	3.76E-06
KEGG	hsa05200: Pathways in cancer	17	5.96E-03
KEGG	hsa04066: HIF-1 signaling pathway	12	3.37E-06
KEGG	hsa04015: Rap1 signaling pathway	12	3.56E-03
KEGG	hsa04145: Phagosome	10	3.39E-03

	KEGG	hsa05205: Proteoglycans in cancer	10	2.05E-02
	KEGG	hsa05150: Staphylococcus aureus infection	9	1.14E-05
	KEGG	hsa04611: Platelet activation	9	4.86E-03
	KEGG	hsa05133: Pertussis	8	7.79E-04
Downregulated	GO_BP	GO:0055114~oxidation-reduction process	33	3.33E-05
	GO_BP	GO:0034220~ion transmembrane transport	19	5.29E-06
	GO_BP	GO:0042493~response to drug	18	1.62E-03
	GO_BP	GO:0006811~ion transport	14	1.66E-05
	GO_BP	GO:0055085~transmembrane transport	14	8.16E-03
	GO_BP	GO:0007588~excretion	13	4.97E-11
	GO_BP	GO:0010628~positive regulation of gene expression	13	3.13E-02
	GO_BP	GO:0006814~sodium ion transport	12	4.94E-06
	GO_BP	GO:0001822~kidney development	12	8.92E-06
	GO_BP	GO:0008152~metabolic process	12	3.22E-03
	GO_CC	GO:0016021~integral component of membrane	171	4.63E-07
	GO_CC	GO:0070062~extracellular exosome	170	1.01E-33
	GO_CC	GO:0005886~plasma membrane	162	2.99E-12
	GO_CC	GO:0005887~integral component of plasma membrane	76	3.16E-11
	GO_CC	GO:0005615~extracellular space	50	1.87E-03

GO_CC	GO:0016324~apical plasma membrane	44	4.39E-22
GO_CC	GO:0016323~basolateral plasma membrane	28	2.05E-14
GO_CC	GO:0009986~cell surface	24	5.53E-03
GO_CC	GO:0005759~mitochondrial matrix	19	8.74E-04
GO_CC	GO:0043025~neuronal cell body	15	1.88E-02
GO_MF	GO:0042803~protein homodimerization activity	33	7.43E-04
GO_MF	GO:0005509~calcium ion binding	29	7.77E-03
GO_MF	GO:0043565~sequence-specific DNA binding	20	4.43E-02
GO_MF	GO:0016491~oxidoreductase activity	13	3.24E-03
GO_MF	GO:0046983~protein dimerization activity	11	3.31E-03
GO_MF	GO:0008201~heparin binding	11	5.22E-03
GO_MF	GO:0005088~Ras guanyl-nucleotide exchange factor activity	10	1.78E-03
GO_MF	GO:0003824~catalytic activity	10	3.74E-02
GO_MF	GO:0016787~hydrolase activity	10	4.07E-02
GO_MF	GO:0046934~phosphatidylinositol-4,5-bisphosphate 3-kinase activity	9	1.10E-04
KEGG	hsa01100: Metabolic pathways	68	8.56E-08
KEGG	hsa01130: Biosynthesis of antibiotics	19	5.17E-05
KEGG	hsa01200: Carbon metabolism	11	1.83E-03
KEGG	hsa04960: Aldosterone-regulated sodium reabsorption	9	1.45E-05

	KEGG	hsa04978: Mineral absorption	9	3.69E-05
	KEGG	hsa00010: Glycolysis / Gluconeogenesis	9	7.57E-04
	KEGG	hsa04610: Complement and coagulation cascades	8	4.14E-03
	KEGG	hsa04966: Collecting duct acid secretion	7	1.11E-04
	KEGG	hsa00260: Glycine, serine and threonine metabolism	7	9.10E-04
	KEGG	hsa00280: Valine, leucine and isoleucine degradation	7	2.46E-03

Table 3(on next page)

The top 35 DEGs identified with the degree in PPI network.

Table 3. The top 35 DEGs identified with the degree in PPI network. (Degree≥37)

Expression	Genes	Degree	Genes	Degree
Upregulated	EGFR	110	C3AR1	52
	VEGFA	100	CYBB	51
	PTPRC	93	LCP2	50
	FN1	90	TLR7	49
	ITGB2	70	C3	45
	TLR2	67	CCL5	44
	MMP9	66	TIMP1	43
	CD86	64	HCK	43
	CXCR4	62	FCGR2A	42
	ICAM1	62	CD53	41
	CCND1	61	CTSS	41
	TYROBP	59	LAPTM5	40
	CSF1R	58	LOX	38
	PLEK	55	SERPINE1	37
	ITGAX	53	CAV1	37
Downregulated	ALB	134	KNG1	52
	EGF	80	KIT	44
	ERBB2	67		

Figure 1

Flow chart of data collection, processing, analysis and verification in this study.

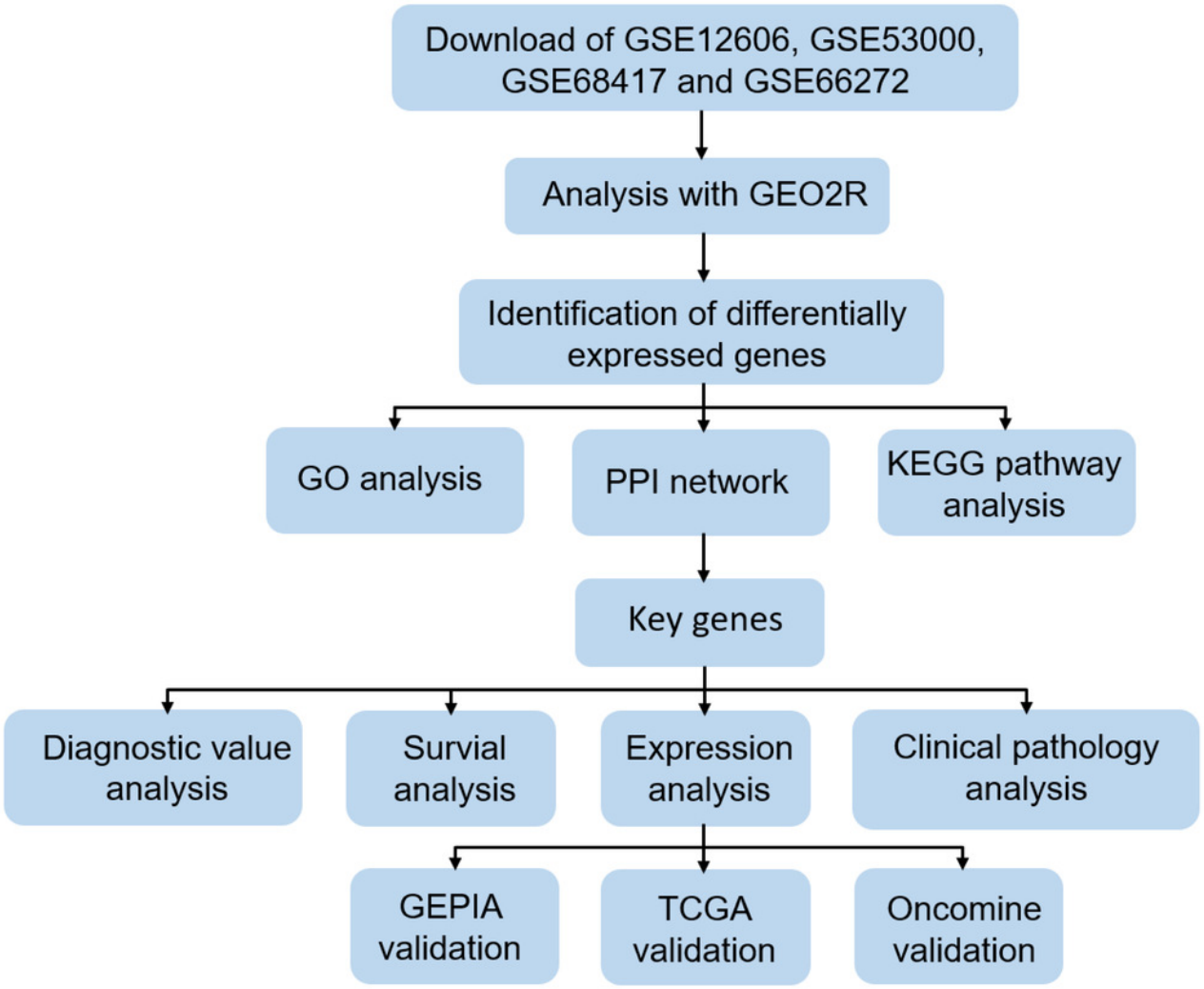
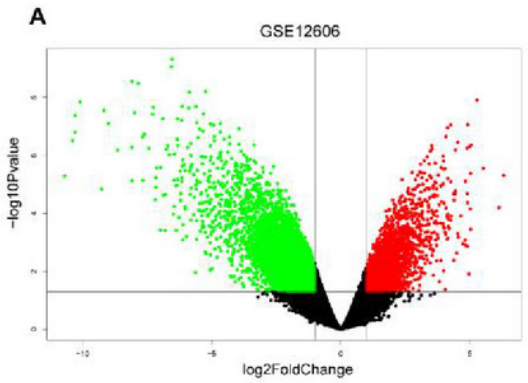
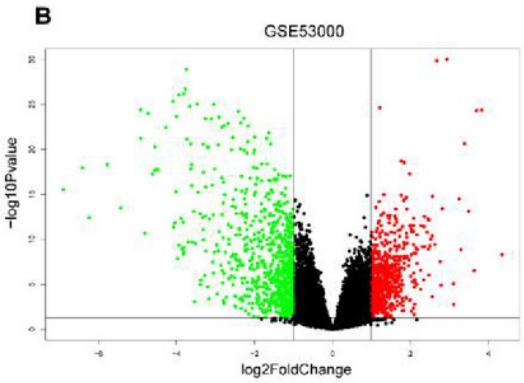


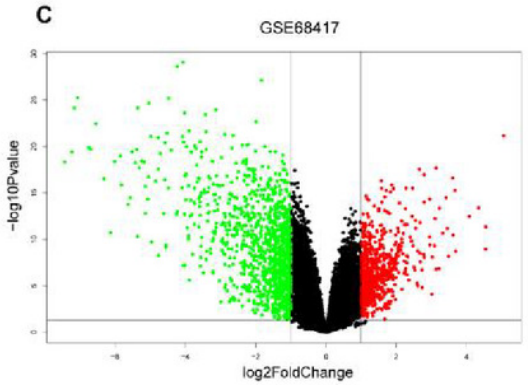
Figure 2

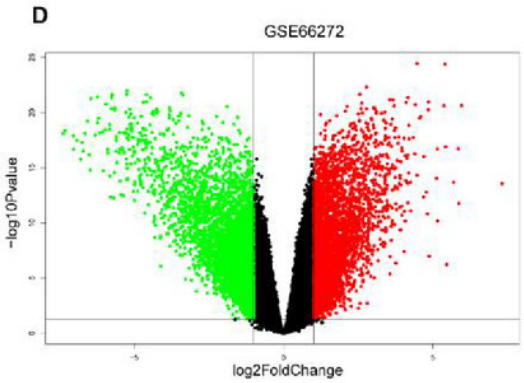
Screening of differentially expressed genes.

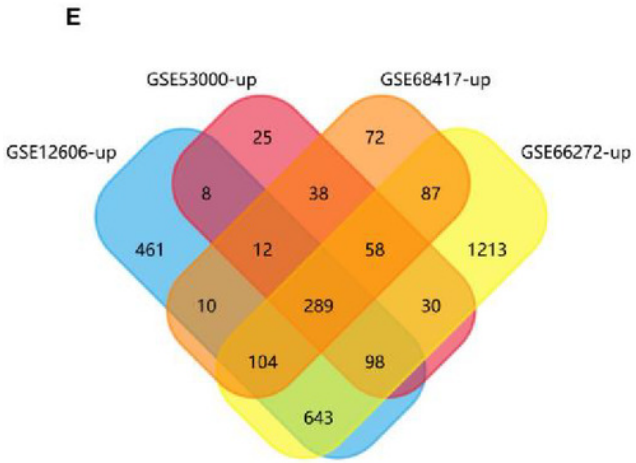
(A-D) The volcano plot of all DEGs respectively in GSE12606, GSE53000, GSE68417, and GSE66272 datasets. Red and green nodes represent up-regulated genes and down-regulated genes, respectively. (E-F)738 DEGs were identified in four profile datasets (GSE12606, GSE53000, GSE68417, and GSE66272), 289 upregulated genes, 449 downregulated genes.


A
GSE12606
−log10Pvalue
log2FoldChange


B
GSE53000
−log10Pvalue
log2FoldChange


C
GSE68417
−log10Pvalue
log2FoldChange


D
GSE66272
−log10Pvalue
log2FoldChange


E
GSE53000-up
GSE68417-up
GSE12606-up
GSE66272-up
25
72
8
38
87
461
12
58
1213
10
289
30
104
98
643

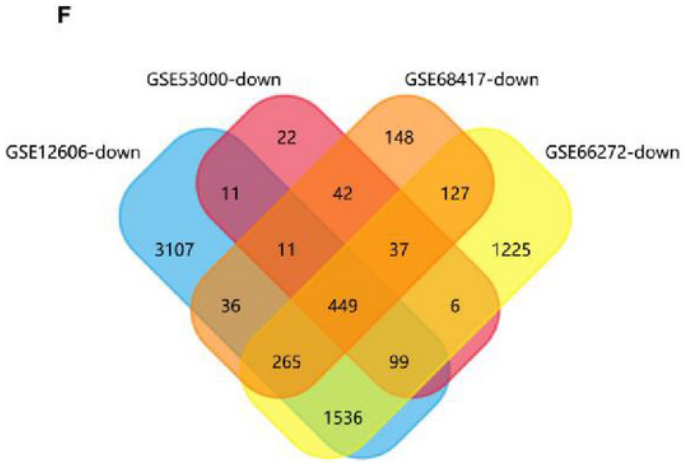

F
GSE53000-down
GSE68417-down
GSE12606-down
GSE66272-down
22
148
11
42
127
3107
11
37
1225
36
449
6
265
99
1536

Figure 3

Enrichment analysis of GO and KEGG with up-regulated DEG.

(A) The biological process of GO analysis showed that the up-regulation of DEGs was mainly related to cell adhesion, inflammatory response, signal transduction, and immune response. (B) The enrichment analysis of up-regulated DEGs cell components is mainly related to the cellular exosomes, integral component of membrane, plasma membrane, and integral component of plasma membrane. (C) The molecular function of GO analysis showed that the up-regulation of DEGs was mainly related to protein binding, identical protein bind, ATP binding capacity, and protein homodimerization activity. (D) The KEGG pathways related to the up-regulation of DEGs expression mainly include the PI3K-Akt signaling pathway, focal adhesion, pathways in cancer, and HIF-1 signaling pathway. (E) The biological process of GO analysis showed that the downregulation of DEGs was mainly related to oxidation-reduction process proteolysis, ion transmembrane transport, response to the drug, and ion transport. (F) The enrichment analysis of down-regulated DEGs cell components is mainly related to integral components of membrane, plasma membrane, cellular exosomes, and integral component of plasma membrane. (G) The molecular function of GO analysis showed that the downregulation of DEGs was mainly related to protein homodimerization activity, calcium ion binding, oxidoreductase activity, and sequence-specific DNA binding. (H) The KEGG pathways related to the down-regulation of DEGs expression mainly include metabolic pathway, Biosynthesis of antibiotics, Carbon metabolism, and Aldosterone-regulated sodium reabsorption.

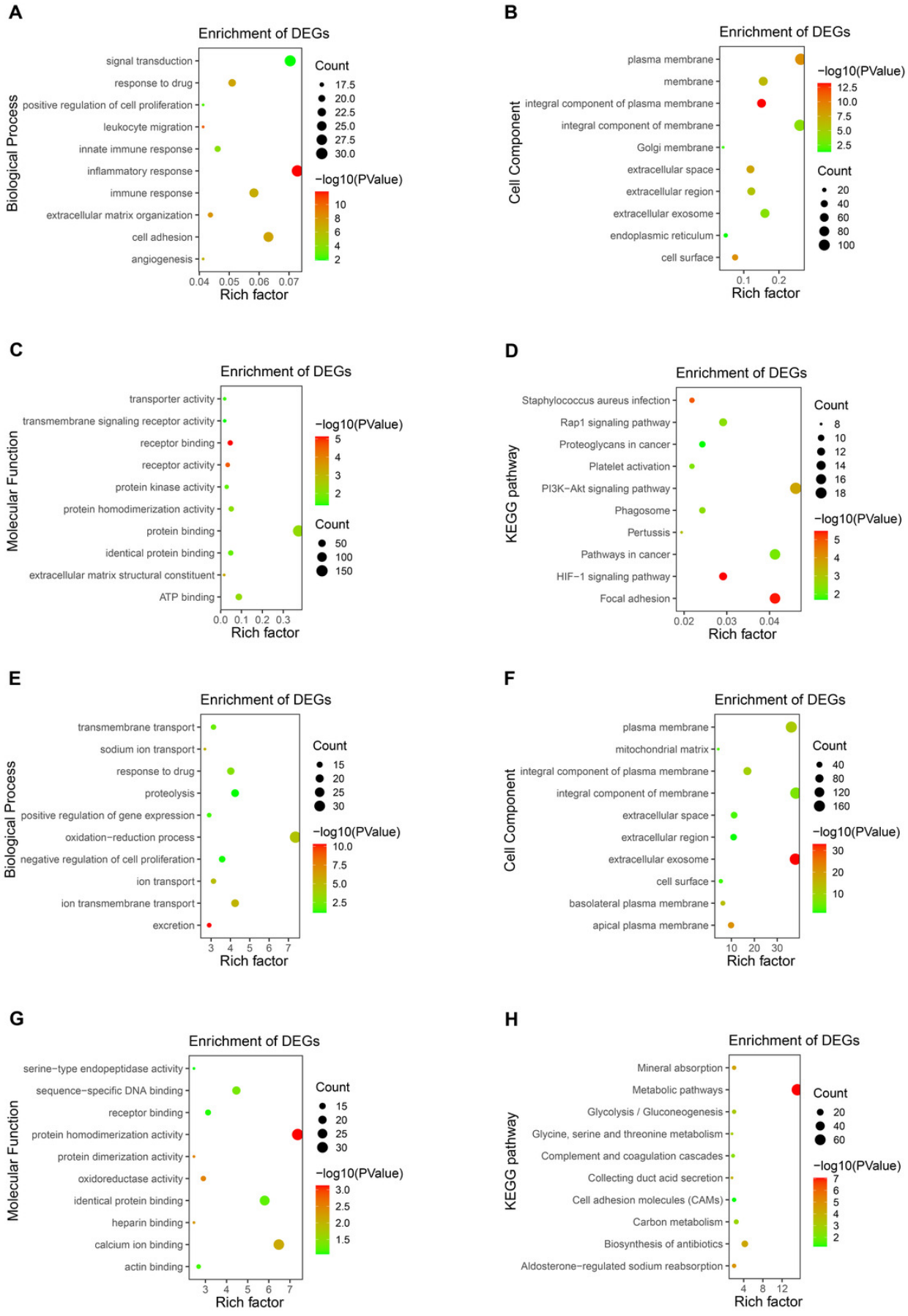
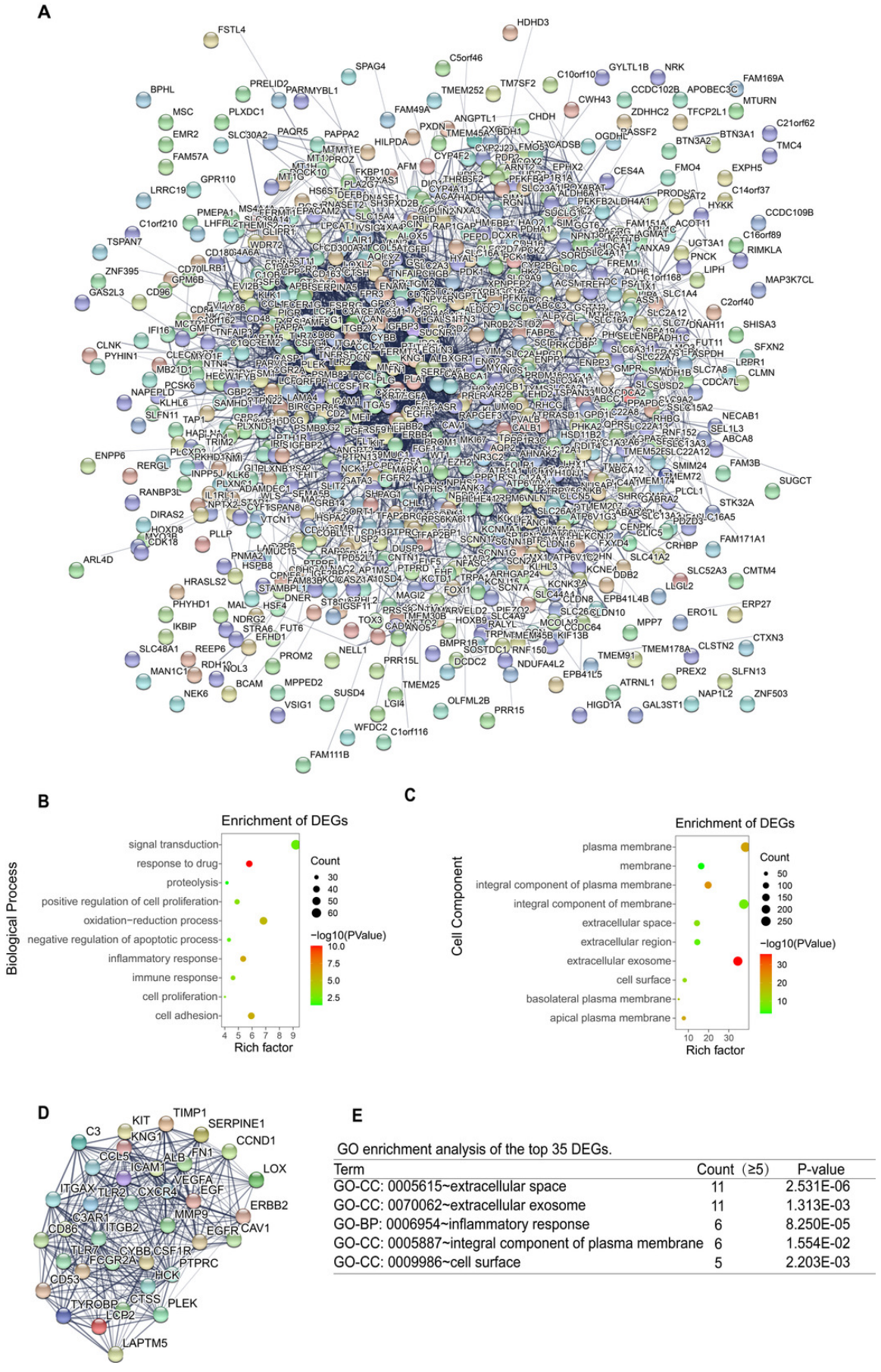

A
Enrichment of DEGs
Biological Process
signal transduction
response to drug
positive regulation of cell proliferation
leukocyte migration
innate immune response
inflammatory response
immune response
extracellular matrix organization
cell adhesion
angiogenesis
0.04 0.05 0.06 0.07
Rich factor
Count
17.5
20.0
22.5
25.0
27.5
30.0
−log10(PValue)
10
8
6
4
2
B
Enrichment of DEGs
Cell Component
plasma membrane
membrane
integral component of plasma membrane
integral component of membrane
Golgi membrane
extracellular space
extracellular region
extracellular exosome
endoplasmic reticulum
cell surface
0.1 0.2
Rich factor
−log10(PValue)
12.5
10.0
7.5
5.0
2.5
Count
20
40
60
80
100
C
Enrichment of DEGs
Molecular Function
transporter activity
transmembrane signaling receptor activity
receptor binding
receptor activity
protein kinase activity
protein homodimerization activity
protein binding
identical protein binding
extracellular matrix structural constituent
ATP binding
0.0 0.1 0.2 0.3
Rich factor
−log10(PValue)
5
4
3
2
Count
50
100
150
D
Enrichment of DEGs
KEGG pathway
Staphylococcus aureus infection
Rap1 signaling pathway
Proteoglycans in cancer
Platelet activation
PI3K−Akt signaling pathway
Phagosome
Pertussis
Pathways in cancer
HIF−1 signaling pathway
Focal adhesion
0.02 0.03 0.04
Rich factor
Count
8
10
12
14
16
18
−log10(PValue)
5
4
3
2
E
Enrichment of DEGs
Biological Process
transmembrane transport
sodium ion transport
response to drug
proteolysis
positive regulation of gene expression
oxidation−reduction process
negative regulation of cell proliferation
ion transport
ion transmembrane transport
excretion
3 4 5 6 7
Rich factor
Count
15
20
25
30
−log10(PValue)
10.0
7.5
5.0
2.5
F
Enrichment of DEGs
Cell Component
plasma membrane
mitochondrial matrix
integral component of plasma membrane
integral component of membrane
extracellular space
extracellular region
extracellular exosome
cell surface
basolateral plasma membrane
apical plasma membrane
10 20 30
Rich factor
Count
40
80
120
160
−log10(PValue)
30
20
10
G
Enrichment of DEGs
Molecular Function
serine−type endopeptidase activity
sequence−specific DNA binding
receptor binding
protein homodimerization activity
protein dimerization activity
oxidoreductase activity
identical protein binding
heparin binding
calcium ion binding
actin binding
3 4 5 6 7
Rich factor
Count
15
20
25
30
−log10(PValue)
3.0
2.5
2.0
1.5
H
Enrichment of DEGs
KEGG pathway
Mineral absorption
Metabolic pathways
Glycolysis / Gluconeogenesis
Glycine, serine and threonine metabolism
Complement and coagulation cascades
Collecting duct acid secretion
Cell adhesion molecules (CAMs)
Carbon metabolism
Biosynthesis of antibiotics
Aldosterone−regulated sodium reabsorption
4 8 12
Rich factor
Count
20
40
60
−log10(PValue)
7
6
5
4
3
2

Figure 4

PPI network and GO enrichment analysis of key genes.

(A) The PPI network is constructed, including up-regulated genes and down-regulated genes, and considers that the confidence level≥0.4 is significant for PPI. (B-C) GO enrichment analysis of all DEGs in PPI network. (D) PPI network of the top 35 DEGs according to the degree (degree≥37). (E) GO enrichment analysis of the top 35 DEGs.



Term	Count (≥5)	P-value
GO-CC: 0005615~extracellular space	11	2.531E-06
GO-CC: 0070062~extracellular exosome	11	1.313E-03
GO-BP: 0006954~inflammatory response	6	8.250E-05
GO-CC: 0005887~integral component of plasma membrane	6	1.554E-02
GO-CC: 0009986~cell surface	5	2.203E-03

Figure 5

Key gene expression between normal kidney and CCRCC tissues.

(A-C) Box plot showing the expression of ITGAX, LAPTM5, SERPINE1 in GEPIA database. These three genes are highly expressed in CCRCC. (D-F) In the TCGA database, compared with adjacent normal tissues, the expression of ITGAX, LAPTM5, SERPINE1 mRNA in 72 pairs of CCRCC tissues increased significantly. (G-I) The expression of ITGAX, LAPTM5, SERPINE1 are significantly increased in Oncomine Gumz renal database. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (J-L)The box plot showing the promoter methylation levels of ITGAX, LAPTM5 and SERPINE1 in the UALCAN database. $P < 0.01$.

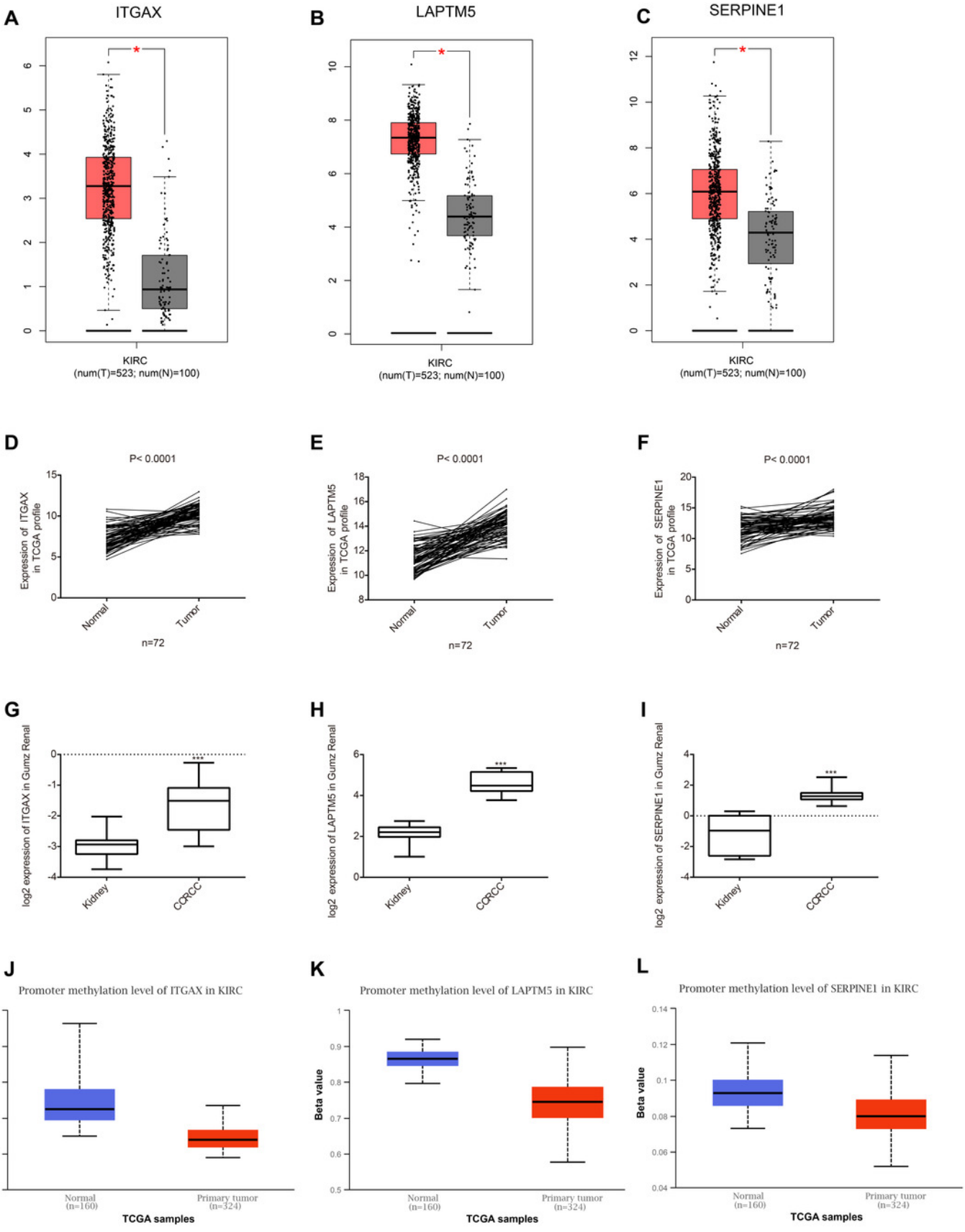

A
ITGAX
KIRC
(num(T)=523; num(N)=100)
B
LAPTM5
KIRC
(num(T)=523; num(N)=100)
C
SERPINE1
KIRC
(num(T)=523; num(N)=100)
D
P< 0.0001
Expression of ITGAX in TCGA profile
Normal
Tumor
n=72
E
P< 0.0001
Expression of LAPTM5 in TCGA profile
Normal
Tumor
n=72
F
P< 0.0001
Expression of SERPINE1 in TCGA profile
Normal
Tumor
n=72
G
log2 expression of ITGAX in Gumz Renal
Kidney
CCRCC
H
log2 expression of LAPTM5 in Gumz Renal
Kidney
CCRCC
I
log2 expression of SERPINE1 in Gumz Renal
Kidney
CCRCC
J
Promoter methylation level of ITGAX in KIRC
Beta value
Normal (n=160)
Primary tumor (n=324)
TCGA samples
K
Promoter methylation level of LAPTM5 in KIRC
Beta value
Normal (n=160)
Primary tumor (n=324)
TCGA samples
L
Promoter methylation level of SERPINE1 in KIRC
Beta value
Normal (n=160)
Primary tumor (n=324)
TCGA samples

Figure 6

The relationship between ITGAX, LAPTM5, and SERPINE1 expression and clinical pathology of CCRCC.

(A-C) Boxplot showing that ITGAX, LAPTM5, and SERPINE1 mRNA expression were significantly related to pathological grades, and patients in grade 4 have the highest expression in CCRCC according to UALCAN databases. (D-F) Boxplot showing that the expression of ITGAX, LAPTM5, and SERPINE1 mRNA in CCRCC samples are significantly correlated with severe clinical staging and the mRNA expression of ITGAX, LAPTM5, and SERPINE1 were higher in patients with stage 4 according to UALCAN databases. *P < 0.05, **P < 0.01, ***P < 0.001.

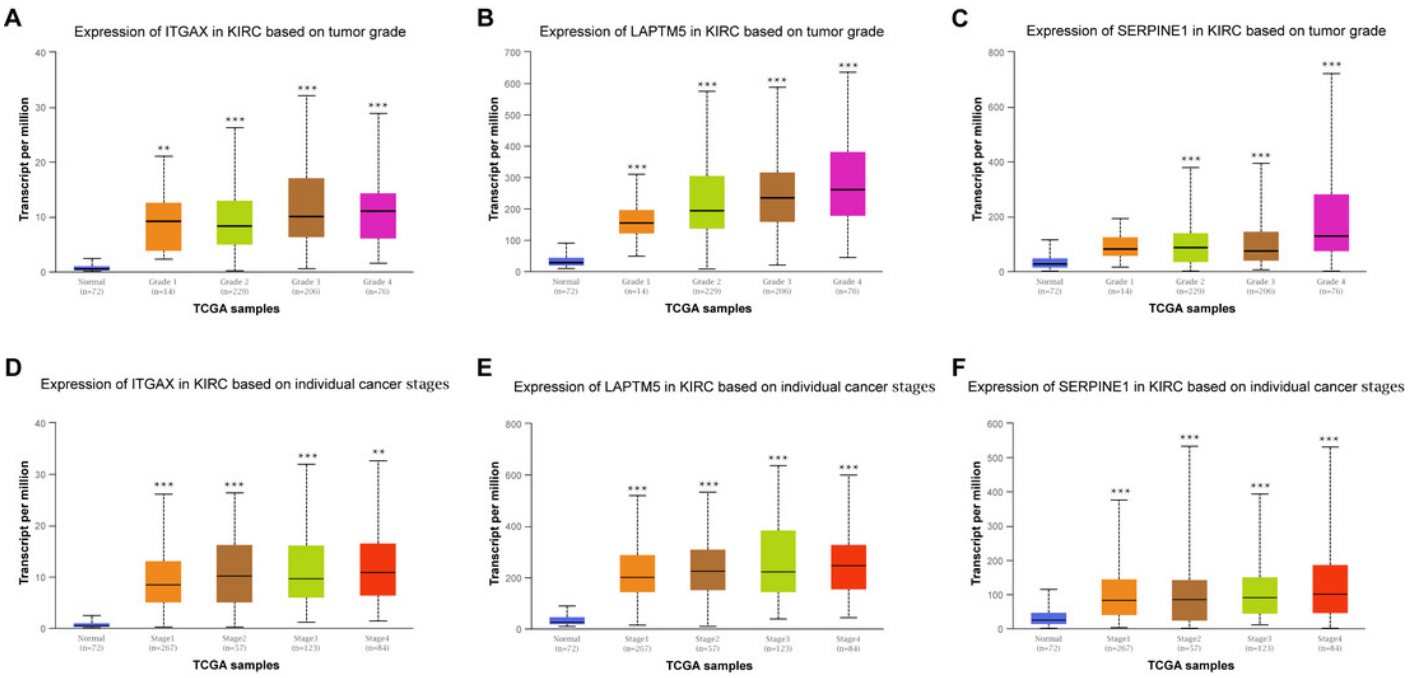
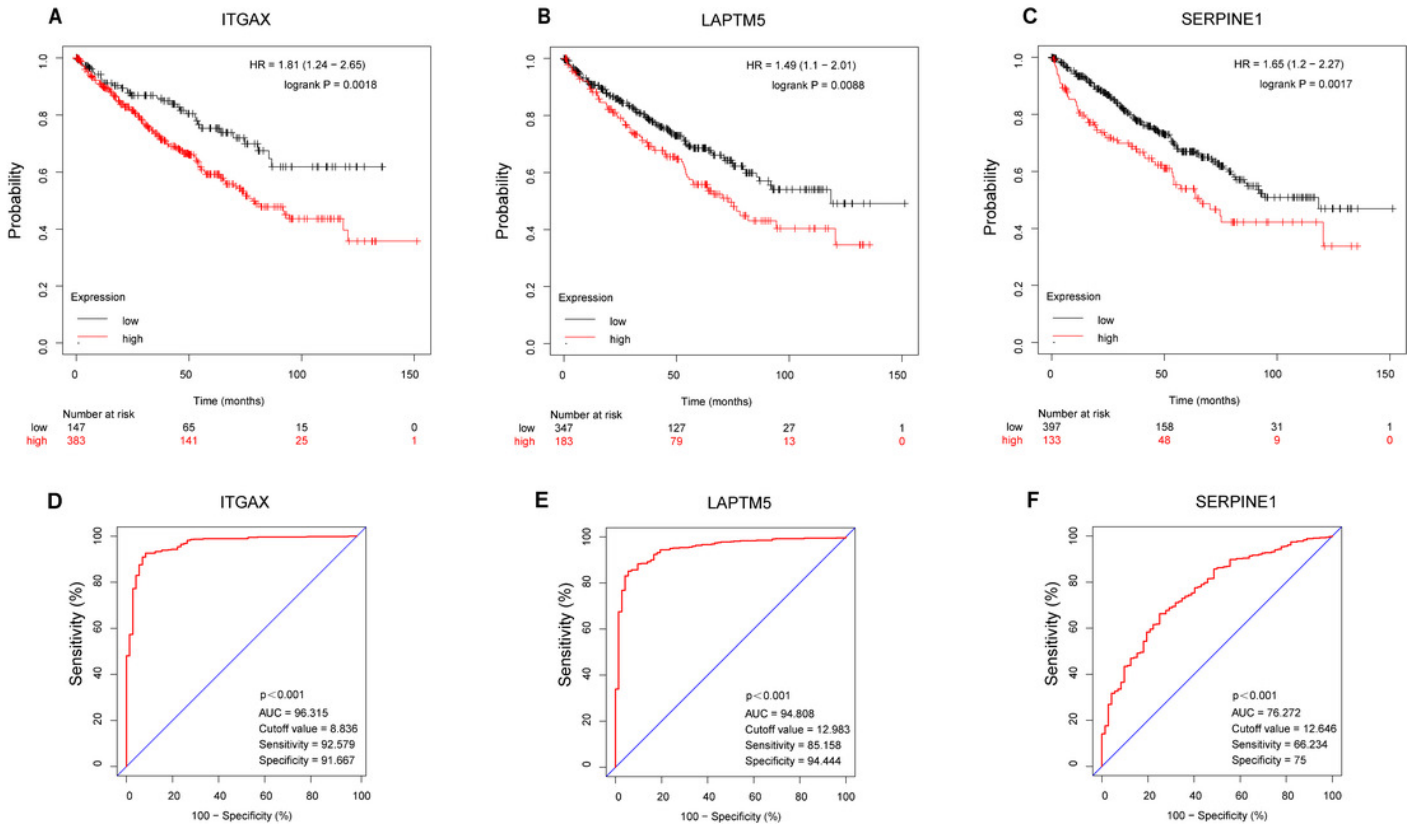


Figure 7

Survival and diagnostic value of ITGAX, LAPTM5, and SERPINE1 in CCRCC

(A-C) The overall survival of ITGAX, LAPTM5, and SERPINE1.The results showed that the high expression of three key genes in CCRCC was negatively correlated with prognosis. (D) ROC curve of ITGAX (AUC=96.315, cutoff value=8.836, Sensitivity=92.579 Specificity=91.667). (E) ROC curve of LAPTM5, (AUC=94.808, cutoff value=12.983 Sensitivity=85.158 Specificity=94.444). (F) ROC curve of SERPINE1, AUC=76.272, cutoff value=12,646, Sensitivity=66.234 Specificity=75).