

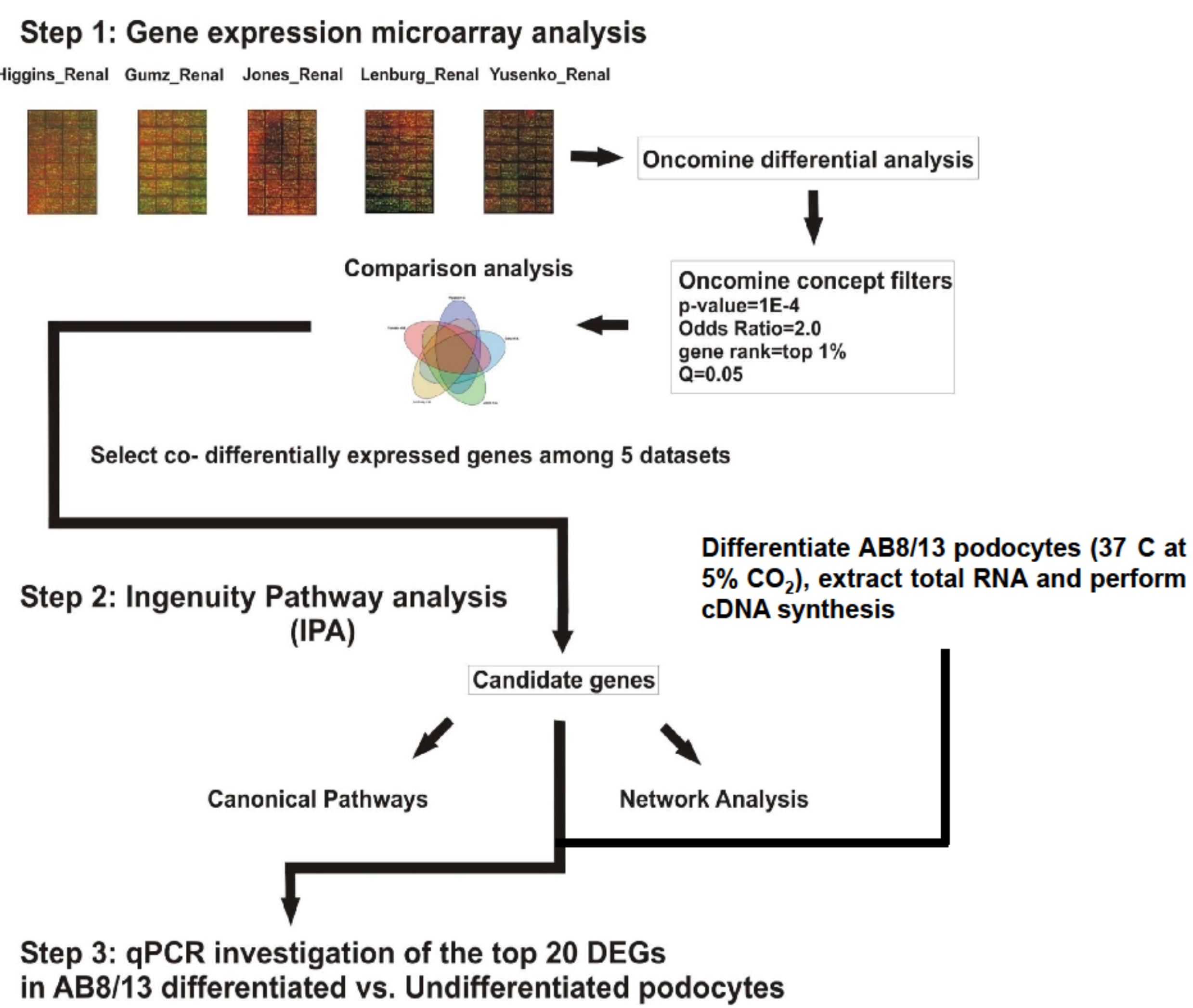
Background

Clear cell renal cell carcinoma (ccRCC) represents the most common subtype (83%) of RCC. It is one of the most therapy-resistant carcinomas, responding very poorly or not at all to radiotherapy, hormonal therapy, and chemotherapy. All these facts emphasize the importance of developing early diagnostic markers. Microarray gene expression profiling has been used in the past by various groups to identify novel markers of clear cell renal cell carcinoma (ccRCC).

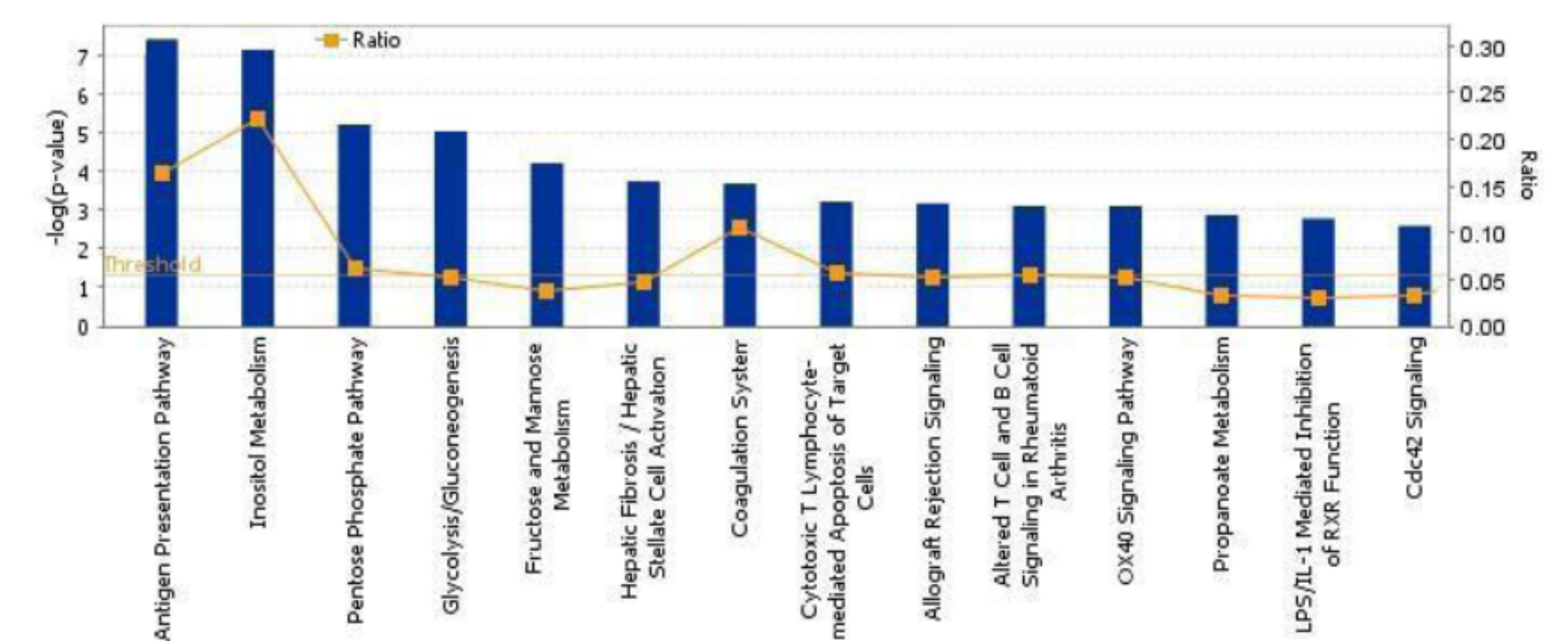
Purpose of the study

We hypothesized that a meta-analysis of publicly available gene expression datasets of ccRCC may identify a list of the common deregulated genes (DEGs), which should have higher potential to be used as novel tumor markers.

Materials and methods

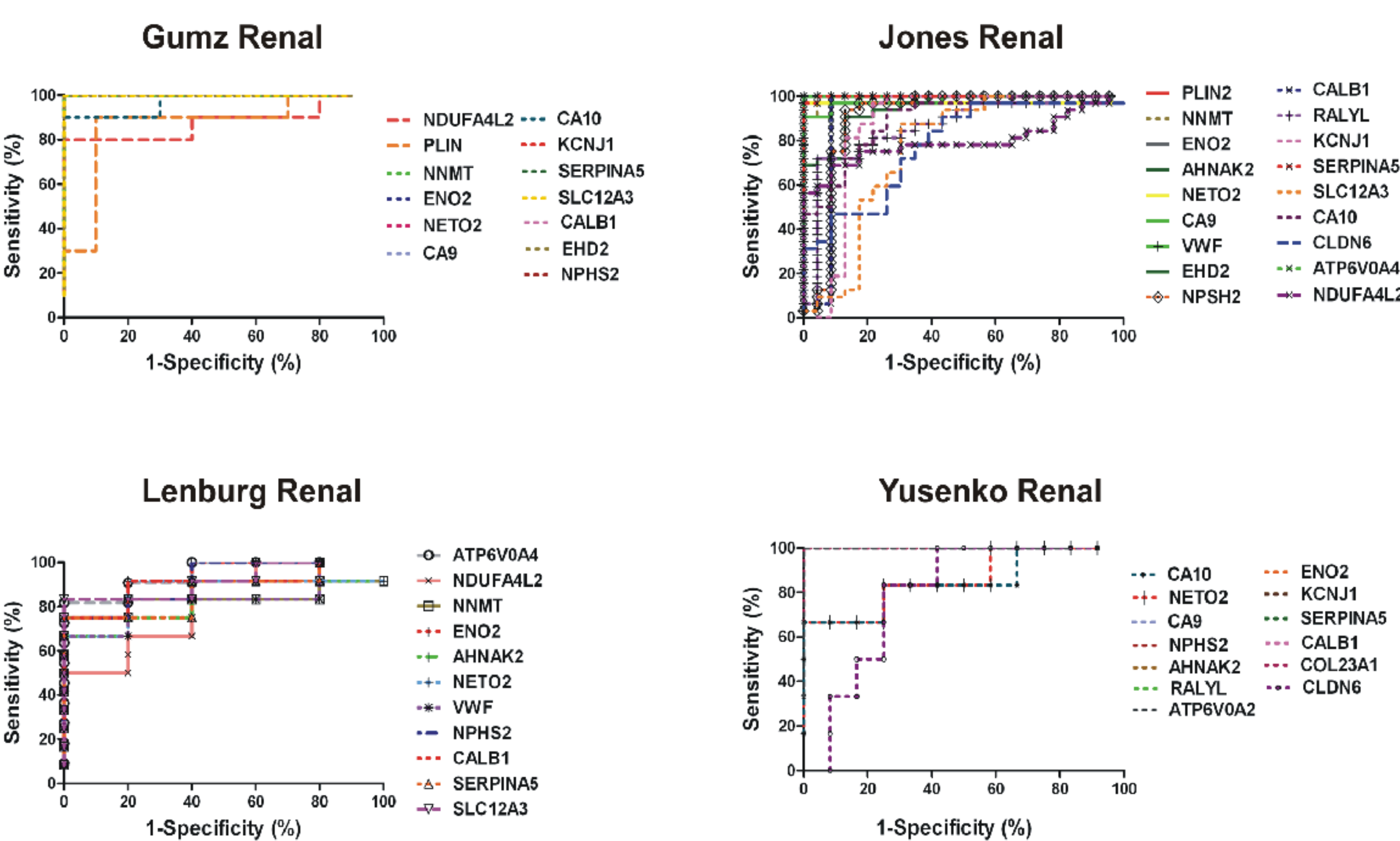
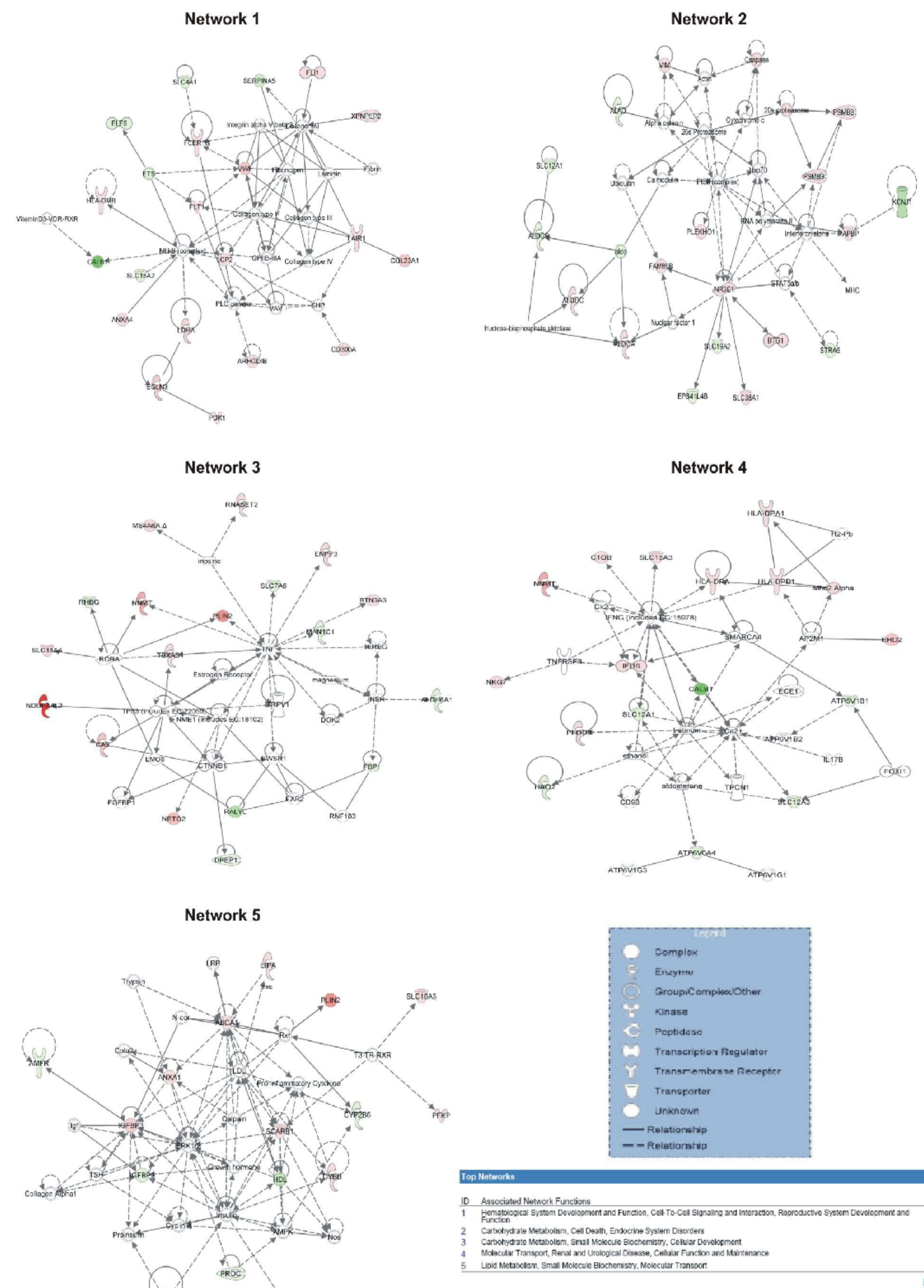


Results



We included in 93 commonly up-regulated and 76 commonly down-regulated genes in ccRCC vs. normal tissue. The top 10 up-regulated molecules were NDUFA4L2, PLIN2, NNMT, ENO2, AHNK2, NETO2, CA9, VWF, COL23A1 and EHD2, and the top 10 down-regulated molecules were NPHS2, CALB1, RALYL, KCNJ1, KNG1, SERPINA5, CLDN8, SLC12A3, CA10 and ATP6V0A4. Their expression levels were validated with qRT-PCR in the Caki-2 and ACHN ccRCC cell lines, compared to the AB8/13 undifferentiated podocyte and HEK293 cells. The top canonical pathways included the antigen presentation pathway, the inositol metabolism and the pentose phosphate pathway among others. The common DEGs participate in biological functions such as cancer, inflammatory response, renal and urological disease, reproductive system disease and respiratory disease. The top molecular and cellular functions were cell-to-cell signaling and interaction, cellular function and maintenance, molecular transport, cellular growth and proliferation and carbohydrate metabolism.

In the Oncomine platform, genes deregulated in ccRCC relative to the corresponding normal tissue were filtered by a corrected Q value cut-off and concept filters. The identified genes that were common among 5 different microarray datasets were chosen as the candidate markers and their performance to discriminate between cancer and normal tissue was measured by ROC test. Their networks (score>25) were analyzed by Ingenuity Pathway Analysis (IPA). Further Gene Ontology (GO) enrichment was performed for the most common DEGs. The Caki-2 and ACHN ccRCC cell lines were used in order to validate the expression of the most common DEGs. The AB8/13 undifferentiated podocyte and HEK293 cells were used as controls.



We performed a ROC test for the top 20 DEGs in ccRCC using the MAS5-calculated signal intensity that was extracted from the GEO repository for each dataset. Specifically, in the "Gumz Renal" dataset, NDUFA4, NETO2, COL23A1, SLC12A3 and CA10 exhibited very good discrimination between ccRCC and normal tissue. Similarly, in the "Jones Renal" dataset, PLIN2, NNMT, ENO2, AHNK2, NETO2, CA9, VWF, EHD2, NPHS2, CALB1, RALYL, KCNJ1, SERPINA5, SLC12A3, CA10, CLDN6 and ATP6V0A4 had median AUC values of 0.969 (p<0.001) and in the "Lenburg Renal" dataset, NDUFA4, NNMT, ENO2, AHNK2, NETO2, VWF, NPHS2, CALB1, SERPINA5, SLC12A3 and ATP6V0A4 exhibited median AUC values of 0.900 (p<0.001). In the Yusenko dataset, ENO2, AHNK2, NETO2, CA9, COL23A1, NPHS2, CALB, RALYL, KCNJ1, SERPINA5, CLDN6 and ATP6V0A4 had median AUC values of 1.000 (p<0.01).

Conclusions

The present study highlights the most responsible genes for the formation of ccRCC and their associated networks. These genes could be used as predictive markers for the disease.

The top functions of the most important gene networks (score>25) were: Hematological system development and function, cell-to-cell signaling and interaction, reproductive system development and function (network 1); carbohydrate metabolism, cell death, endocrine system disorders (network 2); carbohydrate metabolism, small molecule biochemistry, cellular development (network 3); molecular transport, renal and urological disease, cellular function and maintenance (network 4); lipid metabolism, small molecule biochemistry, molecular transport (network 5).

