Bioinformatic gene analysis for potential biomarkers and therapeutic targets of kidney calculi-related renal cell carcinoma

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Abstract

Kidney calculi (KC) is considered to be a potential cause of renal cell carcinoma (RCC) due to urinary retention, hydronephrosis, pyelonephritis, and carcinoma of renal pelvis. We searched co-expressed genes in order to explore the relationships between kidney calculi (KC) and renal cell carcinoma (RCC) and reveal potential biomarkers and therapeutic targets of kidney calculi-related renal cell carcinoma. KC-related differentially expressed genes (DEGs) were identified via bioinformatic analysis Gene Expression Omnibus (GEO) datasets GSE73680 and GSE117518. Simultaneously, RCC-related DEGs were also identified via bioinformatic analysis GEO datasets GSE14994 and GSE40435. Subsequently, co-DEGs of KC-related RCC were found, and extensive target prediction and network analyses methods were used to assess protein–protein interaction (PPI) networks, Gene Ontology (GO) terms and pathway enrichment for DEGs, and co-expressed DEGs coupled with corresponding predicted miRNAs involved in KC and RCC were assessed as well. We identified 832 DEGs in KC and RCC samples. The co-DEGs of VIM–DCN–WNK1 and PXDN coupled with corresponding predicted miRNAs, especially miR-181c-5p and miR-181d-5p may be significantly associated with KC-related RCC. The Co-DEGs of VIM–DCN–WNK1 and PXDN link KC and RCC. Finally, the top 5 miRNAs for each Co-DEGs may be potential signaling pathways for KC-related RCC, especially miR-181c-5p and miR-181d-5p. Therefore, there is an association between KC and RCC and expression of VIM–DCN–WNK1 and PXDN genes may favor KC-related RCC.

Introduction

Kidney calculi (KC) is one of the most common urological condition. Data from National Health and Nutrition Examination Survey (NHANES) reported in 2012 noted the KC prevalence of 10.6% in men and 7.1% among women.¹ Globally, the incidence and prevalence of KC have increased over the years.^{1–3} Despite technological advances in the surgical management of KC have significantly reduced patient morbidity and recovery time, new stone formation and recurrence remain significant health issues. Recurrence rate after the first appearance of symptomatic stones is reported to be 30–50% within 10 years¹. Therefore, the research on the mechanism of the occurrence and development of KC emerges one after another.

Similar to the incidence of kidney stones, the incidence of renal cell carcinoma (RCC), the most common type of renal parenchymal tumor accounting for 90% of malignant neoplasms arising in the kidney in adults, still continues to keep growing^{4,5,8}. Although the best treatment for RCC is resection⁶, advanced patients can only be treated by chemoradiotherapy and other comprehensive methods⁷. If RCC could be predicted, it can be detected early and removed, which will improve the prognosis.

Although there are many risk factors for kidney cancer, such as smoking, obesity and hypertension^{8,a}, a Dutch team concluded that early diagnosis of KC (age < 40) was significantly associated with an increased risk of later diagnosis of kidney tumors (age ≥ 40)⁹. They found KC was associated with an increased RCC risk (HR:1.39, 95% CI 1.05–1.84)⁹. In fact, previous studies have assessed the relationship

between kidney stones and RCC and upper tract urothelial carcinoma (UTUC)^{10–12}. In addition, a meta-analysis, based on eight case-control studies and one retrospective cohort study, found an increased risk of RCC and both ureter and renal pelvis cancer in patients with KC¹³.

The association between kidney cancer and kidney stones is understandable, given the many risk factors they share, such as obesity, diabetes mellitus and several dietary factors. Since RCC in itself is a multigene-related tumor with extremely complex pathogenesis, we speculated that there may be some genetic association between KC and RCC. In this study, we identified co-expressed differentially expressed genes (co-DEGs) of KC and RCC and elucidated molecular mechanisms and pathology of KC-related DEGs (KC-DEGs) and RCC-related DEGs (RCC-DEGs). Finally, we provide a bioinformatic analysis of DEGs and predicted microRNAs (miRNAs) for KC patients prone to occurring RCC.

Methods

The datasets (GSE73680 and GSE117518) for KC were downloaded from GEO (<http://www.ncbi.nlm.nih.gov/geo/>)¹⁴. Expression profiling arrays of GSE73680 were generated using GPL17077 Agilent-039494 SurePrint G3 Human GE v2 8 × 60K Microarray 039381 (Probe Name version), while expression profiling arrays of GSE117518 were generated using GPL21827 Agilent-079487 Arraystar Human LncRNA microarray V4 (Probe Name version). Then, the GeneSpring GX v12.1 software standardized the quantile method and annotated the corresponding data. The data were screened for differential genes through the limma package of R language. Similarly, the datasets (GSE14994 and GSE40435) for RCC were downloaded from GEO. Expression profiling arrays of GSE14994 were generated using GPL3921 [ht_hg-u133a] Affymetrix HT Human Genome U133A Array, standardized by RMA method. While expression profiling arrays of GSE40435 were generated using GPL10558 Illumina humanht-12 V4.0 expression beadchip was used to obtain the corresponding data by quantile standardization method and annotation. The data were screened for differential genes through the limma package of R language. The criteria we chose were $p < 0.05$, $\text{foldchange} > 2$ or < 0.5 .

The GO Analysis method annotated the gene function of the differential genes based on the GO database to obtain all functions involved in the gene, and then calculated the significance level (p-value) and misjudgment rate (FDR) of each function by Fisher's precise test and multiple comparison test. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a database that systematically analyzes the relationships, gene functions and genome information of Genes (and their coding products). It is helpful for researchers to study Genes and expression information as a whole network. The integrated metabolic pathways provided by KEGG, including the metabolism of carbohydrates, nucleosides, amino acids, and biodegradation of organic compounds, not only provide all possible metabolic pathways, but also provide a comprehensive overview of the enzymes that catalyze each step. KEGG is a powerful tool for metabolic analysis and network research in vivo. Currently, KEGG Pathway is divided into eight categories, including the overall network, metabolic process, genetic information transmission, environmental information transmission, intracellular biological processes, biological systems, human diseases and drug development. Based on the KEGG database, Pathway analysis used Fisher's accurate test and chi square

test to analyze the significance of Pathway involving the target gene. The screening criteria for both were $P < 0.05$.

PPI networks of KC- and RCC-DEGs were analyzed using the search tool for the retrieval of interacting genes (STRING database, V11; <http://string-db.org/>) that predicted protein functional associations and protein–protein interactions. Subsequently, Cytoscape software (V3.5.1; <http://cytoscape.org/>) was applied to visualize and analyze biological networks and node degrees, after downloading analytic results of the STRING database with a combined_score ≥ 0.9 .

Finally, the CTD database was used to find the genes related to RCC or KC. Genes are scored indirectly through a database. Then we chose the top four scores. In addition, we applied online prediction tools utilizing microRNA Data Integration Portal (mirDIP) (<http://ophid.utoronto.ca/mirDIP>)¹⁵, and DIANA Tools (<http://diana.imis.athena-innovation.gr/DianaTools/>)¹⁶, to predict potential microRNA targeting. Subsequently, we used the mirDIP and DIANA Tools software to predict which of the selected miRNAs could target co-DEGs. We determined 5 top candidate miRNAs based on higher predicted.

Results

Identification of DEGs

We identified 15015 probes in GSE73680, of these, 10,468 probes were raised and 4,547 were lowered(Figure1a). Similarly, we identified 2105 probes in GSE117518, of these, 936 probes were raised and 1169 were lowered(Figure1b).The difference genes of the two data sets were intersected, and 343 intersection genes were obtained(Figure1c). In GSE14994, we identified 2332 probes, and 1430 probes were raised and 902 were lowered. While in GSE40435,1764 probes were identified, including 922 up-regulated probes and 842 down-regulated probes(Figure1d).The difference genes of the two data sets were intersected, and 832 intersection genes were obtained(Figure1e).The difference genes of the two data sets were intersected, and 832 intersection genes were obtained(Figure1f).

Function enrichment in GO database

According to the analysis results, the significant function of differentially expressed genes, the number of genes contained and the enrichment degree in the database, the target graph of significance function can be made including biological process, molecular function and cellular component. Figure2a illustrates the significant function of KC-DEGs, and Heatmaps of KC-DEGs in relation to interleukin-6 secretion, negative regulation of chemokine production were conducted for genes expression and these data appear in Figure2b. Simultaneously, Figure2c and Figure2d has shown the genes expression value in relation to renal system development, small molecule catabolic process above the RCC-DEGs.

KEGG Pathway analysis

KEGG pathway analysis data appear in Figure3a-b. The results suggesting that the KC-DEGs were mainly enriched in pathways of AMPK signaling pathway (p-value: 4.32E-03), Neurotrophin signaling pathway (p-value: 1.59E-02), TGF-beta signaling pathway (p-value: 2.20E-02), C-type lectin receptor signaling pathway (p-value: 3.22E-02) and Adipocytokine signaling pathway (p-value: 2.98E-02). However, KEGG terms included Phagosome (p-value: 2.68E-13), Valine, leucine and isoleucine degradation (p-value: 2.84E-13), Carbon metabolism (p-value: 1.18E-08), Complement and coagulation cascades (p-value: 2.39E-08) and Glycolysis / Gluconeogenesis (p-value: 3.82E-08) were enriched in RCC-DEGs.

PPI network analysis

We identified 358 and 445 nodes from PPI network of KC-DEGs and RCC-DEGs, respectively and these data appear in Figure3c-d. Here, the hub nodes, including proteasome 26S subunit, non-ATPase 2 (*PSMD2*; degree = 16), proteasome 20S subunit alpha 8 (*PSMA8*; degree = 13), STIP1 homology and U-box containing protein 1 (*STUB1*; degree = 13), cell division cycle 23 (*CDC23*; degree = 12), integrin linked kinase (*ILK*; degree = 12) are considering as hub-genes in related to KC maintaining. However, the hub-genes, involved in kininogen 1 (*KNG1*; degree = 56), complement C3 (*C3*; degree = 51), quiescin sulfhydryl oxidase 1 (*QSOX1*; degree = 44), apolipoprotein B (*APOB*; degree = 42), and fcG (*FCG*; degree = 40) are demonstrated in RCC-DEGs with a relative higher degree.

Identification of functional and pathway enrichment among predicted miRNAs and Co-DEGs

The CTD database showed that Co-DEGs targeted KC and RCC. According to databases score genes indirectly, the inclusion criteria is score > 10. Thus, four genes were selected-Vimentin (*VIM*) Decorin (*DCN*) WNK lysine deficient protein kinase 1 (*WNK1*) peroxidasin (*PXDN*). The Figure4 showed the relationship between genes and diseases. In renal carcinoma, the disease most associated with *DCN* is RCC, while in calculi, the disease is KC (Figure4a). In addition, the same condition happens in *VIM*, *WNK1* and *PXDN* (Figure4b-d). Prediction analysis using mirDIP and DIANA bioinformatic tools identified the top 5 selected miRNAs targeting each Co-DEG involved in KC-related RCC and these data appear in Table1. These data enable us to understand how predicted miRNAs are related to KC-related RCC progress.

Discussion

With the high incidence of KC, its consequent harm should also be highly concerned, which also including the potential of progressing to be RCC. In clinical practice, early and comprehensive screening for KC patients is essential to prevent the occurrence of RCC. However the etiology of RCC is not clear, it is widely believed that RCC is closely related to KC, obesity, and hypertension. Although the concurrence of KC and RCC may be influenced by a series of confounding factors, there is still a close relationship between these two disease. Previous studies are suggested that KC can be used as a risk factor for postoperative recurrence in KC patients with RCC. Jeroen's group⁹ had reported that nearly half of all RCC cases in their study could be attributed to KC based on the population attributable fraction using a multivariable-adjusted HR of 3.08. Facing with such a high risk, the process of its occurrence and

development is worth exploring. KC patients usually have hydronephrosis, which can increase the risk of nephropathy and the disease process, and significantly affect the prognosis of disease. But at present there are few studies focused on the relationship between KC and RCC.

In our study, major Co-DEGs included VIM, DCN, WNK1 and PXDN, which are associated with both KC and RCC. The VIM bring a high influence score in RCC and KC, especially in RCC. The result is consistent with previous researches considering the invasion and metastasis of RCC via altering the miR490-3p/vimentin signals¹⁷. VIM plays an important role in a variety of tumors, not only in RCC, but also in such as breast carcinoma¹⁸, nasopharyngeal carcinoma^{19,20}, Glioblastoma²¹ and gastric cancer²². In addition, Yu' group found extracellular vimentin modulates human dendritic cell activation, in order to prevent tissue-damage from contributing to the development of autoimmunity²³. Similarly, Santos et al suggests that VIM may be a key regulator of the NLRP3 inflammasome²⁴. Both of them indicated that VIM is closely associated with inflammation. It may be an implication that KC suffers may have a risk of developing RCC via altering the VIM.

As for DCN, in our results, it's mainly associated with kidney neoplasms, RCC, KC, renal colic and chronic kidney disease-mineral and bone disorder. According to Xu's research, they illustrated that the loci of DCN deficiency is significantly associated with RCC growth and metastasis²⁵. Similarly, it's also related to inflammation²⁶. Indeed, Merline et al considered DCN could control inflammation and cancer²⁷. Additionally, WNK1 could promote renal tumor progression according to Kim's study²⁸. Some researchers also deem it's related to immunity and inflammation³⁰. More interestingly, PXDN is considered to be associated with renal fibrosis in the murine unilateral ureteral obstruction model²⁹. Thus, there may be a relationship between KC and RCC that they may arise from loci mutations or gene variants.

Although our study may reveal some potential relationships between KC and RCC, it can not consider the biological mechanism must relate KC to RCC. In general, previous studies considered that chronic irritation and infection recruit inflammatory cells, which secrete cytokines and chemokines. In turn, free radical species from oxygen and nitrogen are produced, facilitating the onset of cancer through, among others, increasing cell proliferation³¹⁻³³. However, the Co-DEGs in our study are compatible with the supposition.

Finally, the results of KEEG and GO pathway analysis about Co-DEGs illustrate some possible pathway to investigate the effect of KC to RCC. We found that miR-181d-5p, miR-181c-5p were Co-DEGs and may be potential signaling pathway of KC-related RCC. In previous studies, miR-181c-5p had been reported it could promote an inflammatory response leading to diseases progression³⁴⁻³⁵. While miR-181d-5p had been considered to induce inhibition of oxidative stress response³⁶. Obviously, it coincides with the previous supposition again.

Conclusion

The Co-DEGs of VIM,DCN-WNK1 and PXDN link KC and RCC. Finally, the top 5 miRNAs for each Co-DEGs may be potential signaling pathways for KC-related RCC, especially miR-181c-5p and miR-181d-5p. Therefore, there is an association between KC and RCC, and expression of VIM-WNK1 and PXDN genes may favor KC-related RCC.

Limitation

This study is a microarray analysis that all the results based on gene expression value. Therefore, validation should be carried out both in vitro, in vivo and clinical trials. The genes and potential pathway we found do not appear to be present in previous studies about KC-related RCC. Thus, our study may provide some directions for further research.

Declarations

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Author contributions:

Yunfei Xu: Concept/design, critical revision of the article, approval of the article.

Jun Lu: Concept/design, data analysis/interpretation, drafting of the article, approval of the article.

Qunlong Liu: Data collection, data analysis/interpretation, make the figure, approval of the article.

Huan Liu: Concept/design, revision of the article, approval of the article.

Conflict of Interest Statement

The authors of this manuscript have no conflict of interest.

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Table

Table I The Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment among predicted miRNAs and Co-DEGs

Genes	Predicted miRNAs	Category	Function	P-value
VIM	hsa-miR-30e-5p	KEGG pathway	Axon guidance	7.71E-13
	hsa-miR-124-3p		Long-term depression	2.47E-12
	hsa-miR-320a		Amphetamine addiction	8.13E-09
	hsa-miR-320b		Gap junction	1.04E-08
	hsa-miR-30d-5p		Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3.14E-08
		GO terms	cell-cell signaling	7.25E-06
			cytoskeleton organization	1.62E-05
			immune system process	3.47E-05
			commissural neuron axon guidance	8.84E-05
			regulation of cellular response to growth factor stimulus	0.00035515
DCN	hsa-miR-590-3p	KEGG pathway	Amphetamine addiction	1.11E-16
	hsa-miR-181d-5p		Phosphatidylinositol signaling system	1.73E-10
	hsa-miR-181c-5p		Glioma	6.30E-10
	hsa-miR-181a-5p		Long-term potentiation	3.19E-05
	hsa-miR-181b-5p		post-translational protein modification	8.88E-16
		GO terms	epidermal growth factor receptor signaling pathway	3.77E-15
			nucleobase-containing compound catabolic process	1.03E-14
			membrane organization	4.40E-12
			fibroblast growth factor receptor signaling pathway	9.96E-12
			inositol phosphate metabolic process	6.04E-11
			regulation of nitric-oxide synthase activity	2.72E-10
			nitric oxide metabolic process	4.92E-10
			phosphatidylinositol-mediated signaling	1.15E-09
			phospholipid metabolic process	3.59E-09
WNK1	hsa-miR-124-3p	KEGG pathway	Estrogen signaling pathway	2.71E-11

	hsa-miR-128-3p		Bacterial invasion of epithelial cells	0.00015868
	hsa-miR-181d-5p	GO terms	phospholipid metabolic process	2.44E-08
	hsa-miR-181c-5p		nucleobase-containing compound catabolic process	2.77E-08
	hsa-miR-454-3p		platelet activation	4.72E-08
			transcription initiation from RNA polymerase II promoter	1.55E-07
			glycerophospholipid biosynthetic process	1.75E-07
PXDN	hsa-miR-9-5p	KEGG pathway	Thyroid hormone signaling pathway	6.94E-08
	hsa-miR-29c-3p	GO terms	response to stress	5.23E-08
	hsa-miR-29a-3p		cellular protein metabolic process	6.94E-08
	hsa-miR-29b-3p		nitric oxide metabolic process	1.43E-07
	hsa-miR-202-3p		chondroitin sulfate metabolic process	2.06E-06
			regulation of nitric-oxide synthase activity	1.41E-05
			negative regulation of translation	4.94E-05

VIM:Vimentin DCN:Decorin WNK1:WNK lysine deficient protein kinase 1 PXDN:peroxidasin.

Figures

Figure 1

We identified 15015 probes in GSE73680, of these, 10,468 probes were raised and 4,547 were lowered(Figure1a). Similarly, we identified 2105 probes in GSE117518, of these, 936 probes were raised and 1169 were lowered(Figure1b).The difference genes of the two data sets were intersected, and 343 intersection genes were obtained(Figure1c). In GSE14994, we identified 2332 probes, and 1430 probes were raised and 902 were lowered. While in GSE40435,1764 probes were identified, including 922 up-regulated probes and 842 down-regulated probes(Figure1d).The difference genes of the two data sets were intersected, and 832 intersection genes were obtained(Figure1e).The difference genes of the two data sets were intersected, and 832 intersection genes were obtained(Figure1f).

Figure 2

Figure2a illustrates the significant function of KC-DEGs, and Heatmaps of KC-DEGs in relation to interleukin-6 secretion, negative regulation of chemokine production were conducted for genes expression and these data appear in Figure2b. Simultaneously, Figure2c and Figure2d has shown the genes expression value in relation to renal system development, small molecule catabolic process above the RCC-DEGs.

Figure 3

KEGG pathway analysis data appear in Figure3a-b. We identified 358 and 445 nodes from PPI network of KC- DEGs and RCC-DEGs, respectively and these data appear in Figure3c-d.

Figure 4

The Figure4 showed the relationship between genes and diseases. In renal carcinoma, the disease most associated with DCN is RCC, while in calculi, the disease is KC(Figure4a). In addition, the same condition happens in VIM, WNK1 and PXDN(Figure4b-d).