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Original Article

Integrated bioinformatic analysis identifies COL4A3, COL4A4, and KCNJ1 as key biomarkers in Wilms tumor

Changgang Guo[1,3*], Xiling Jiang[2*], Junsheng Guo[1,3], Yanlong Wu[1], Guochang Bao[1,3]

[1]Department of Urology, Affiliated Hospital of Chifeng University, Chifeng 024000, China; [2]Department of Stomatology, Affiliated Hospital of Chifeng University, Chifeng 024000, China; [3]Urology Research Center, Chifeng University, Chifeng 024000, China. [*]Equal contributors.

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Abstract: Wilms tumor (WT) is one of the most common pediatric solid tumors, affecting 1 in 10,000 children, worldwide. A subset of WT patients has poor prognosis, which is associated with a high risk of advanced and/or recurrent disease. Therefore, candidate markers are urgently needed for the diagnosis and effective treatment of WT. We evaluated three mRNA microarray datasets to identify the differences between normal kidney tissue and WT tissue. Gene expression profiling revealed 130 differentially expressed genes (DEGs). Enrichment analysis and gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed for the DEGs. Subsequently, we established a protein-protein interaction (PPI) network to reveal the associations among the DEGs and selected 10 hub genes, all of which were downregulated in WT. The expression of *COL4A3, COL4A4, KCNJ1, MME,* and *SLC12A1* in WT tissues was significantly lower than that in normal renal tissues. Survival analyses using the Kaplan-Meier method showed that patients with WT and low expression of *COL4A3, COL4A4,* and *KCNJ1* exhibited remarkably poor overall survival. The correlations among *COL4A3, COL4A4,* and *KCNJ1* in WT were analyzed using cBioPortal; *COL4A3, COL4A4,* and *KCNJ1* were positively correlated with each other. Thus, these genes were considered clinically significant and might therefore play important roles in carcinogenesis and the development of WT.

Keywords: Wilms tumor, gene expression profiling, survival analysis, prognosis

Introduction

Wilms tumor (WT) is a type of kidney cancer that develops during childhood; it is usually diagnosed in children between 3 and 4 years of age [1]. WT accounts for more than 90% of renal tumors in children and is the most common solid renal malignant tumor worldwide, with 1 in every 10,000 children being affected by the disease [2, 3]. It is the fourth leading cause of malignant tumors in children, accounting for 5% of all cancers and 95% of all kidney cancers in children [4]. WT is prevalent on a global scale, although there are significant differences in the associated morbidity and prognosis [5]. The treatment regimen for WT usually includes surgery, chemotherapy, and radiotherapy [6] and the long-term survival outcomes for pediatric WT patients have gradually improved over the last several decades. However, specific subgroups of patients, such as those with relapse and anaplastic histology, have poor event-free survival and are at risk of developing significant late effects in response to therapy [7, 8].

The exact molecular mechanisms underlying the development of WT are still unclear; thus, it is necessary to investigate the potential biomarkers of WT as well as its biologic perturbations to prevent the occurrence of tumors and to develop effective therapeutic measures. In this respect, continued investigation of WT cell proliferation and WT recurrence, and carcinogenesis is necessary. Microarrays and RNA-sequencing have been widely utilized to explore the differentially expressed genes (DEGs) and gene expression profiling data are available for download from the Gene Expression Omnibus (GEO) database.

In this study, we downloaded and analyzed three gene expression profile datasets to iden-

Table 1. Statistics of the three microarray datasets

Dataset ID	Platforms	Country	NK	WT	Total
GSE19249	GPL570	USA	8	8	16
GSE6280	GPL96	Netherlands	6	2	8
GSE66405	GPL17077	Germany	4	28	32

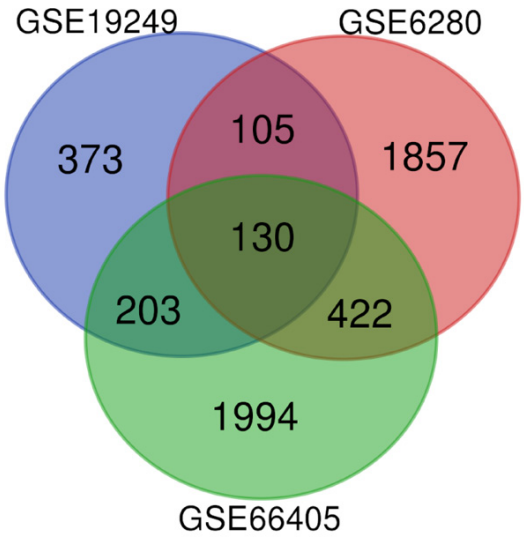


Figure 1. The Venn diagram of the DEGs. In total, 130 DEGs were remarkably differentially expressed in the three groups. DEGs, differentially expressed genes.

tify DEGs between normal renal tissue and WT tissue and to investigate the molecular functions and clinical significance of these genes in WT. In total, ten hub genes and 130 DEGs that may serve as potential biomarkers of WT were identified. These results provide new insights into the pathogenesis of disease. Importantly, some of the newly identified genes may serve as biomarkers for the diagnosis and prognosis of WT.

Materials and methods

Microarray data

GEO is a publicly-available genomics database that contains gene chips, microarrays, and high-throughput gene expression data (http://www.ncbi.nlm.nih.gov/geo) [9]. We downloaded three gene expression datasets (GSE19249, GSE6280, and GSE66405) from GEO [10-12], containing data corresponding to normal kidney samples and WT samples (8 vs. 8, 6 vs. 2, and 4 vs. 28, respectively) (**Table 1**).

DEG identification

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/), which allows interaction analysis, is a tool that can compare at least two datasets in GEO series to identify DEGs according to experimental conditions. Genes with values above the cutoff ($P<0.05$ and $|\log FC \text{ (fold change)}| \geq 1$) were regarded as significant DEGs between normal kidney samples and WT samples. DEGs with the same change in the dataset were combined using the web tool Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/).

KEGG and GO enrichment analysis of DEGs

Gene ontology (GO) is considered one of the main bioinformatic methods to analyze the biologic processes associated with the DEGs [13]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis is used to explore biologic systems and high-level functions based on a wide range of molecular datasets using high-throughput experimental technology methods [14]. The network of interactions between molecules was visualized using Cytoscape (version 3.6.1), an open source software [15]. ClueGO (version 2.5.4) is a plug-in app that allows the creation and visualization of functionally grouped networks of terms/pathways [16]. The GO annotation and KEGG pathway enrichment analysis were performed using ClueGO. $P<0.05$ was considered significant.

Construction of the PPI network

To construct a protein-protein interaction (PPI) network with a confidence score above 0.4 as the cut-off criterion, the Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org) online database was utilized [17]. Subsequently, Cytoscape was used to establish and visualize the PPI network for protein interactions.

Hub gene selection and analysis

The CytoHubba plugin in Cytoscape was used to explore maximal clique centrality (MCC) in each protein node. The top ten genes were identified as hub genes in our study. CytoHubba was used to analyze the network of hub genes and their co-expressed genes. Gene expression levels between normal kidney samples and WT samples were compared using the

Table 2. Significantly enriched GO terms and KEGG pathways of DEGs

Category	Term	Description	Count in gene set	*P*-value
BP term	GO: 0072001	renal system development	18.00	3.56E-11
BP term	GO: 0001822	kidney development	17.00	1.54E-10
BP term	GO: 0001655	urogenital system development	18.00	4.14E-10
BP term	GO: 0072006	nephron development	12.00	1.73E-09
BP term	GO: 0098739	import across plasma membrane	11.00	6.00E-09
BP term	GO: 0015698	inorganic anion transport	12.00	7.18E-08
BP term	GO: 0006821	chloride transport	10.00	1.07E-07
CC term	GO: 0005903	brush border	11.00	1.42E-09
CC term	GO: 0031526	brush border membrane	8.00	1.31E-08
CC term	GO: 0098862	cluster of actin-based cell projections	11.00	7.75E-08
CC term	GO: 0016323	basolateral plasma membrane	12.00	5.82E-07
MF term	GO: 0070851	growth factor receptor binding	8.00	1.15E-05
KEGG pathway	hsa: 04974	Protein digestion and absorption	8.00	2.71E-06
KEGG pathway	hsa: 04960	Aldosterone-regulated sodium reabsorption	5.00	2.95E-05
KEGG pathway	hsa: 04966	Collecting duct acid secretion	4.00	1.35E-04
KEGG pathway	hsa: 04512	ECM-receptor interaction	6.00	1.55E-04
KEGG pathway	hsa: 04514	Cell adhesion molecules (CAMs)	7.00	5.49E-04

Oncomine database (http://www.oncomine.com) [18, 19]. Survival analysis for the hub genes was performed using a Kaplan-Meier curve and *P*-values were calculated using the UCSC Xena Functional Genomics Explorer (https://xenabrowser.net/) [20], with data retrieved from the TARGET database (ocg.cancer.gov/programs/target). Correlations between gene expression levels were reported in cBioPortal (http://www.cbioportal.org) [21, 22].

Statistical analysis

Student's *t*-test for independent samples was performed to assess the significant differences between fetal kidney tissues and WT tissues. The Kaplan-Meier method was used to generate survival curves, and the log-rank test was used to assess significant differences in overall survival time between the high and low expression level groups. Correlations were separately evaluated using the pairwise correlation among genes according to the Pearson chi-square test. A value of $P<0.05$ was considered significant.

Results

Identification of DEGs in WT

DEGs (811 in GSE19249, 2514 in GSE6280, and 2,749 in GSE66405) were determined after standardizing the three gene expression profiles. We performed Venn diagram analysis to visualize the intersection of the DEG profiles (**Figure 1**). Ultimately, 130 genes were differentially expressed in the three groups; among these, 11 were upregulated and 119 were downregulated.

KEGG and GO enrichment analysis of DEGs

The results (**Table 2**) of GO annotation analysis showed that the DEGs were enriched in terms associated with the following biologic processes (BP): "renal system development", "kidney development", and "urogenital system development". DEGs were enriched in the following cellular component (CC) terms: "basolateral plasma membrane", "cluster of actin-based cell projections", and "brush border". Additionally, DEGs were enriched in the following molecular function (MF)-associated term: "growth factor-receptor binding". Furthermore, KEGG pathway analysis showed that the DEGs were mostly associated with "protein digestion and absorption", "aldosterone-regulated sodium reabsorption", and "collecting duct acid secretion" (**Table 2**).

Construction of the PPI network and hub gene selection

We established a PPI network related to the DEGs using STRING tools and Cytoscape. In

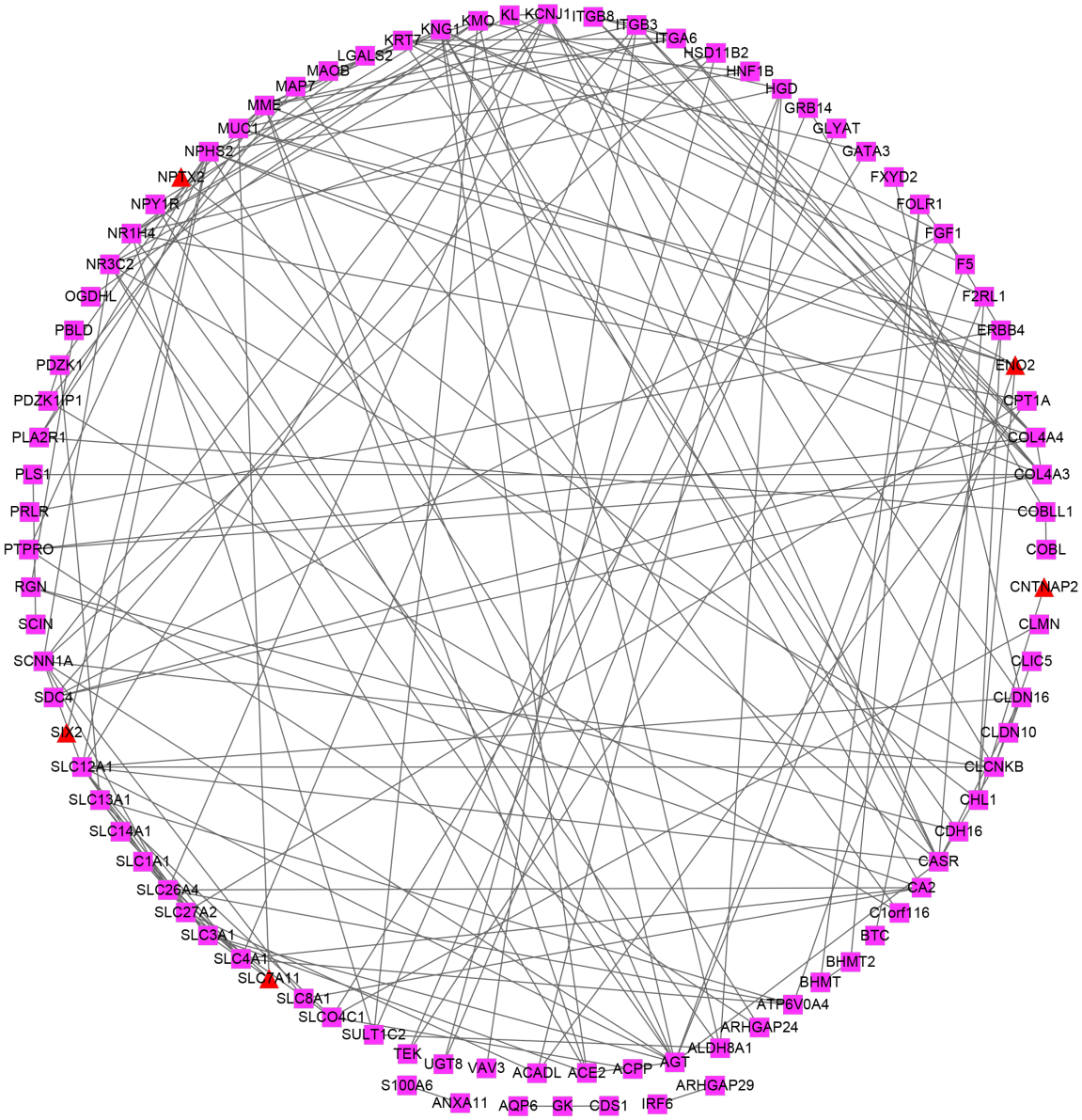


Figure 2. The PPI network was established according to DEGs. Upregulated genes were red nodes, and downregulated genes were purple nodes. PPI, protein-protein interaction; DEGs, differentially expressed genes.

total, 169 edges and 92 nodes were present in the PPI network (**Figure 2**). In the PPI network, the top ten hub genes were recognized using CytoHubba (**Table 3**). The expression of potassium inwardly rectifying channel subfamily J member 1 (*KCNJ1*) was the most significantly altered. All hub genes were underexpressed in WT samples.

Expression analysis of hub genes

A network of hub genes and their co-expressed genes was generated using CytoHubba (**Figure 3**). In the Cutcliffe Renal dataset [18], the expression levels of *COL4A3, COL4A4, KCNJ1, MME*, and *SLC12A1* in WT patient samples were considerably lower than those in normal kidney samples (**Figure 4**). In the Yusenko Renal dataset [19], the gene expression levels in WT patient samples were significantly comparable to those in the normal kidney samples (**Figure 5**).

Survival analysis

Using data from the TARGET database (https://ocg.cancer.gov/programs/target), the correlation between the overall survival rates of

Table 3. Top ten hub genes with higher MCC of connectivity

No.	Gene symbol	Full name	Alias	MCC
1	*KCNJ1*	potassium inwardly rectifying channel subfamily J member 1	*KIR1.1, ROMK, ROMK1*	46
2	*SLC12A1*	solute carrier family 12 member 1	*BSC1, NKCC2*	45
3	*CASR*	calcium sensing receptor	*CAR, EIG8, FHH, FIH, GPRC2A, HHC, HHC1, HYPOC1, NSHPT, PCAR1, hCasR*	40
4	*CLCNKB*	chloride voltage-gated channel Kb	*CLCKB, ClC-K2, ClC-Kb*	37
5	*COL4A3*	collagen type IV alpha 3 chain	*ATS2, ATS3*	37
6	*COL4A4*	collagen type IV alpha 4 chain	*ATS2, BFH, CA44*	36
7	*AGT*	angiotensinogen	*ANHU, SERPINA8, hFLT1*	36
8	*ITGB3*	integrin subunit beta 3	*BDPLT16, BDPLT2, CD61, GP3A, GPIIIa, GT*	34
9	*ITGA6*	integrin subunit alpha 6	*CD49fB, VLA-6, ITGA6*	32
10	*MME*	membrane metalloendopeptidase	*CALLA, CD10, CMT2T, NEP, SCA43, SFE*	27

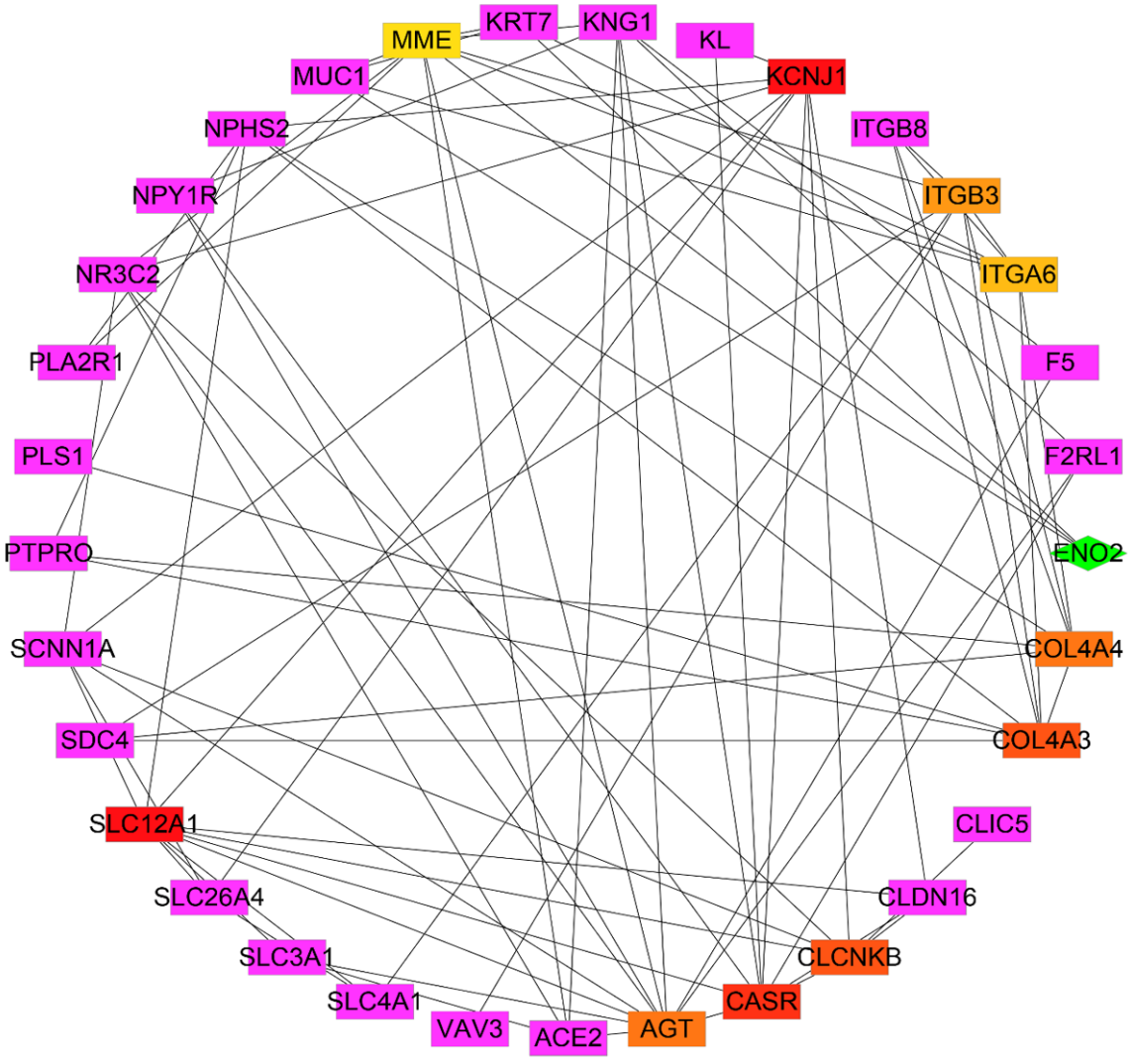
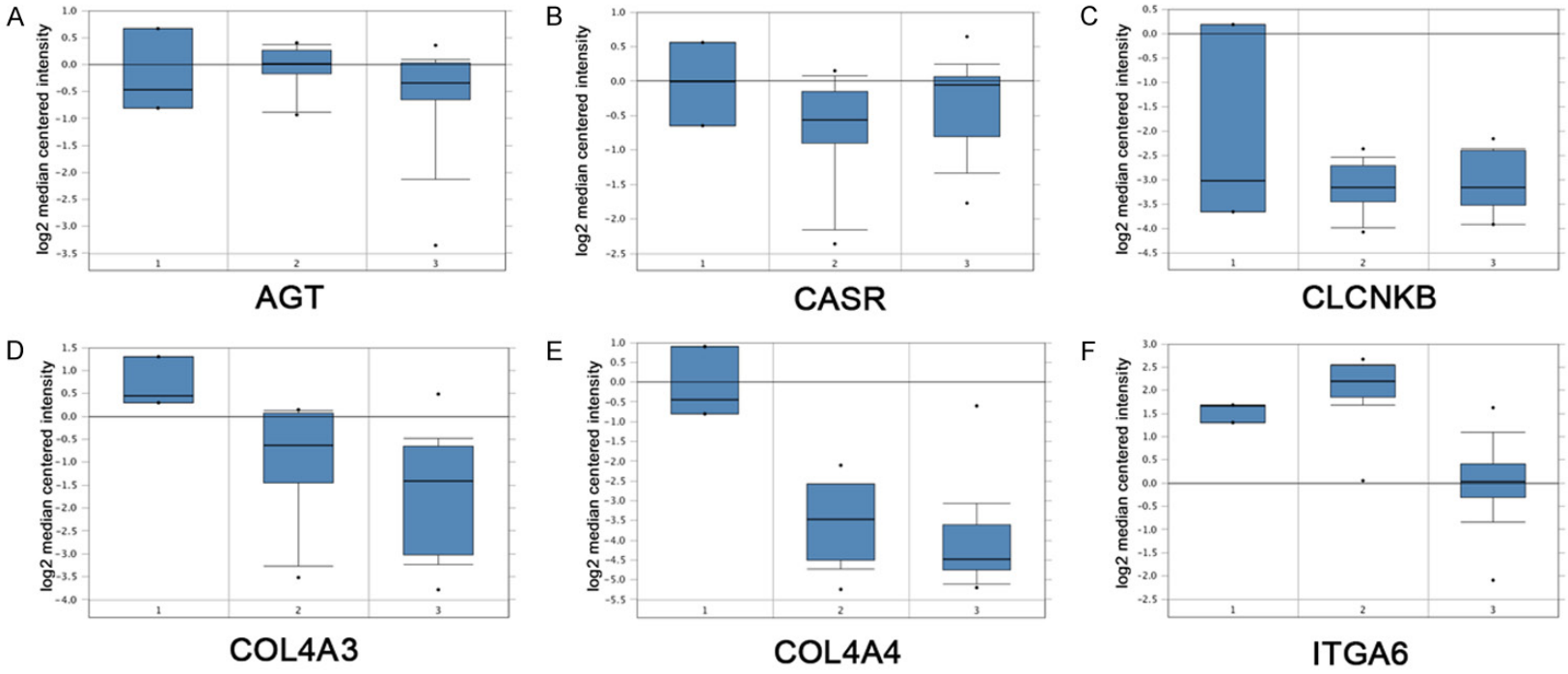


Figure 3. The network of the hub genes and their co-expression genes. The co-expression genes were green and purple nodes, whereas the hub genes were the others. The highest MCC gene was red node, and the lowest MCC gene was yellow node. MCC, maximal clique centrality.


A
log2 median centered intensity
AGT
B
log2 median centered intensity
CASR
C
log2 median centered intensity
CLCNKB
D
log2 median centered intensity
COL4A3
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COL4A4
F
log2 median centered intensity
ITGA6

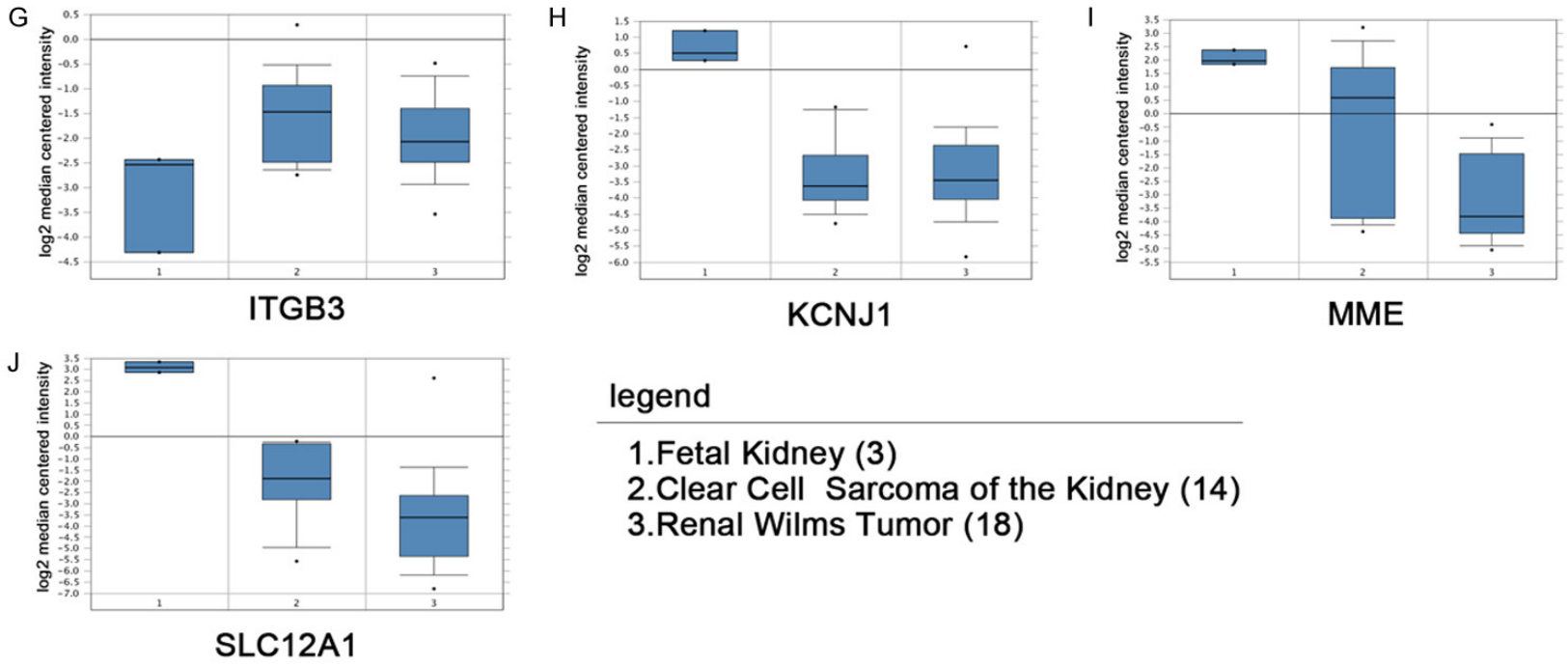

Figure 4. Gene expression levels between normal kidney and WT samples. In the Cutcliffe Renal dataset, the expression levels of *COL4A3* (D), *COL4A4* (E), *KCNJ1* (H), *MME* (I), and *SLC12A1* (J) in patients with WT were significantly lower than those in individuals with normal kidneys. There are no significantly differential expression levels of *AGT* (A), *CASR* (B), *CLCNKB* (C), *ITGA6* (F), and *ITGB3* (G) between normal kidney and WT samples. WT, Wilms tumor.

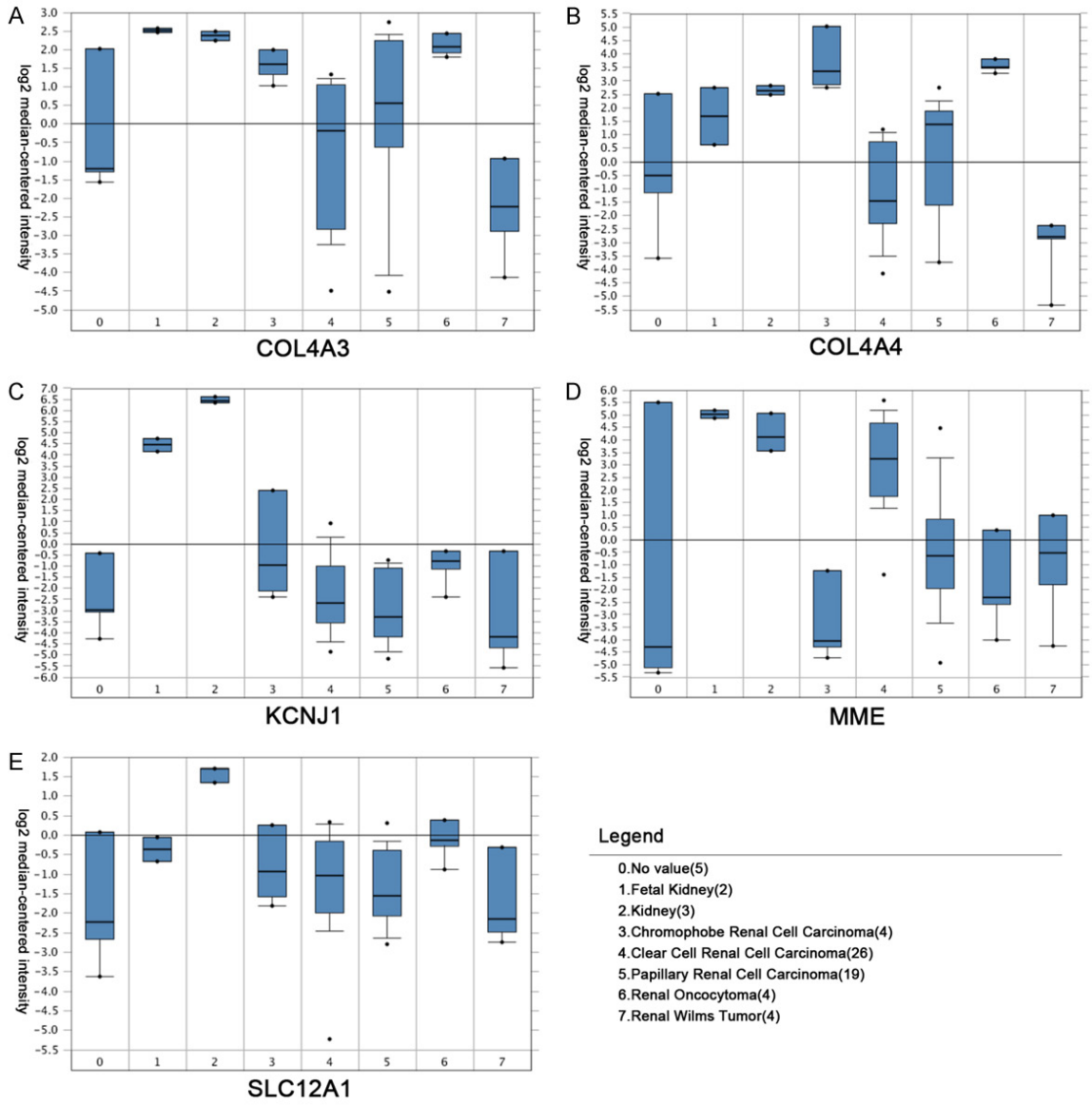

Figure 5. Gene expression levels between normal kidney and WT samples. In the Yusenko Renal dataset, the expression levels of *COL4A3* (A), *COL4A4* (B), *KCNJ1* (C), *MME* (D), and *SLC12A1* (E) in patients with WT were significantly lower than those in individuals with normal kidneys. WT, Wilms tumor.

patients and expression of *COL4A3, COL4A4, KCNJ1, MME,* and *SLC12A1* was analyzed. Kaplan-Meier curves were drawn using the UCSC online tool. Among a total of 132 WT patients, 66 patients were in the low-expression group and 66 patients were in the high-expression group. Low expression of *COL4A3, COL4A4,* and *KCNJ1* in WT patients was associated with a remarkably poor overall survival (**Figure 6**). Therefore, the expression of *COL4A3, COL4A4,* and *KCNJ1* is associated with clinical prognosis and may have a crucial role in the oncogenesis, development, and metastasis of WT.

Correlation analysis

The correlation among *COL4A3, COL4A4,* and *KCNJ1* was analyzed using the cBioPortal online platform. In WT samples, *COL4A3* expression was found to be positively correlated with that of *COL4A4* ($R = 0.8889$, $P<0.01$) and *KCNJ1* ($R = 0.6164$, $P<0.01$), and that of *COL4A4* was found to be positively correlated

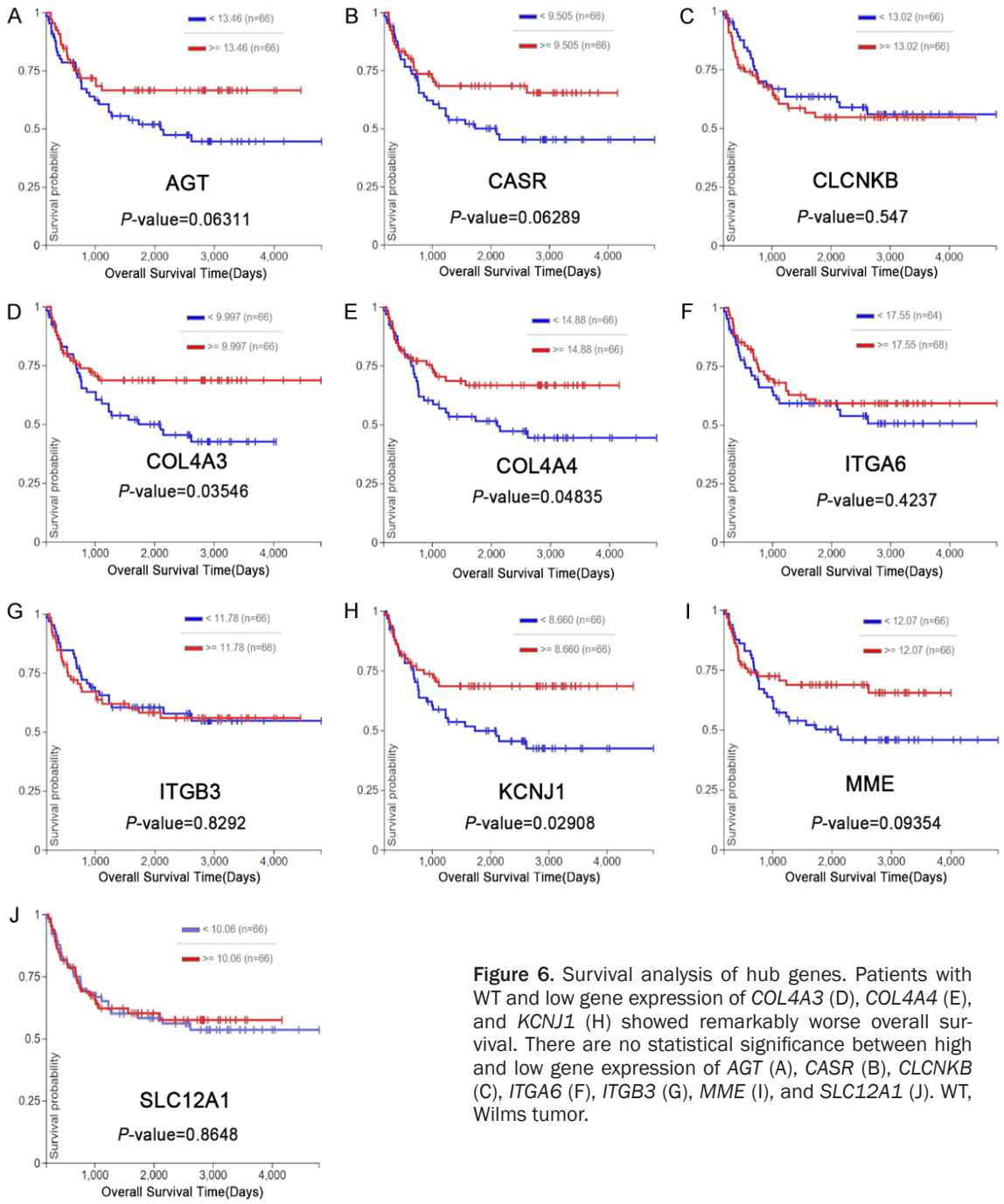

Figure 6. Survival analysis of hub genes. Patients with WT and low gene expression of *COL4A3* (D), *COL4A4* (E), and *KCNJ1* (H) showed remarkably worse overall survival. There are no statistical significance between high and low gene expression of *AGT* (A), *CASR* (B), *CLCNKB* (C), *ITGA6* (F), *ITGB3* (G), *MME* (I), and *SLC12A1* (J). WT, Wilms tumor.

with *KCNJ1* expression (R = 0.5657, P<0.01) (**Figure 7**).

Discussion

WT, also known as nephroblastoma, is the most common form of kidney cancer in children. It is caused by poorly differentiated mesenchymal renal stem cells [23]. This disease accounts for 90% of the renal tumors and 7% of all cancers in children [24]. WT therapy usually involves multiple modes of treatment including surgery, chemotherapy, and radiotherapy. The long-term survival rates of pediatric WT patients have gradually improved in recent years. However, chronic health conditions secondary to treatment, including renal failure, infertility, cardiotoxicity, restrictive lung disease, and subse-

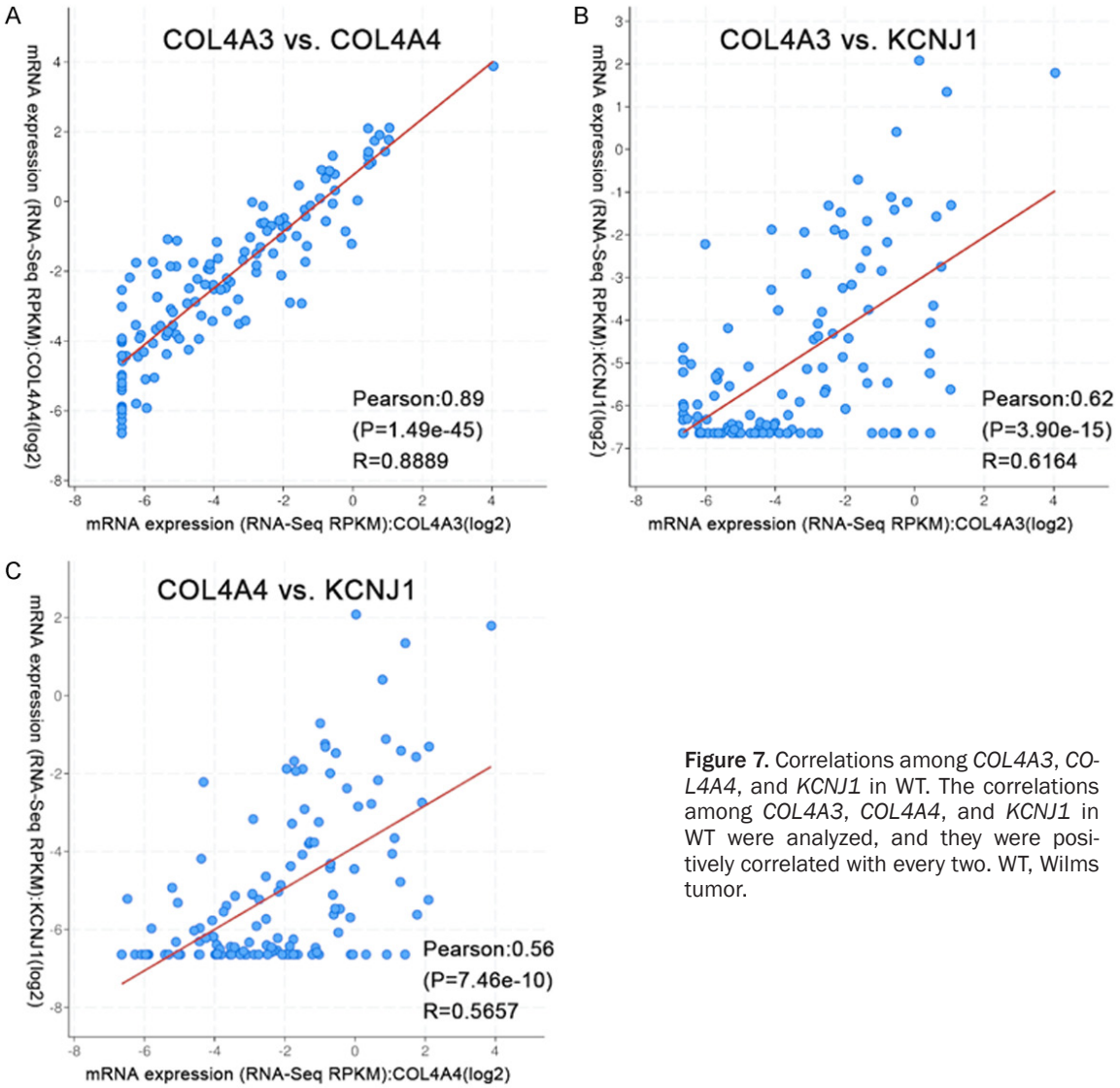

Figure 7. Correlations among *COL4A3*, *COL4A4*, and *KCNJ1* in WT. The correlations among *COL4A3*, *COL4A4*, and *KCNJ1* in WT were analyzed, and they were positively correlated with every two. WT, Wilms tumor.

quent malignant tumor development, affect nearly a quarter of the WT survivors [25, 26]. Therefore, candidate biomarkers are urgently needed for the diagnosis and effective treatment of this condition. Microarray technology provides an opportunity to explore gene variations in WT and is a useful approach for identifying new biomarkers in other cancers as well.

In this study, we identified the key biomarkers, *COL4A3, COL4A4,* and *KCNJ1*-all of which were downregulated in WT samples-through bioinformatic analysis. Our different approaches-through GEO, ONCOMINE, and TARGET database analyses-revealed that the expression of *COL4A3, COL4A4* and *KCNJ1* in these three databases was consistent. WT patients with low expression *of COL4A3, COL4A4,* and *KCNJ1* showed remarkably poor overall survival. Additionally, *COL4A3, COL4A4,* and *KCNJ1* positively correlated with each other in WT tissues. Therefore, *COL4A3, COL4A4,* and *KCNJ1* are possible clinically relevant genes with important roles in the carcinogenesis and development of WT.

Svetlana et al. [27] identified that genes, including those coding for adhesion molecules-*COL4A3* and *CDH5*-were downregulated in early non-small-cell lung cancer. Further, Nie et al. [28] suggested that *COL4A3* overexpression is associated with the development of gastric cancer. The upregulation of *COL4A3* was associated with other tumors, including WT, breast cancer, and ovarian cancer [29-31]. Wang et al.

[32] reported that *COL4A4* expression is reduced in patients with clear cell renal cell carcinoma (ccRCC) compared to that in paracancerous tissues, whereas patients with ccRCC and high expression of *COL4A4* exhibit remarkably longer overall survival. Chattopadhyay et al. [33] suggested that genes *(KRT4* and *COL4A4*) coding for proteins involved in the extracellular matrix tissue and cell communication pathways are significantly downregulated in esophageal cancer. Li et al. [34] reported that the expression of *COL4A4* is markedly downregulated in esophageal squamous cell carcinoma. Guo et al. [35] reported that *KCNJ1* has low expression and is associated with poor prognosis in ccRCC; moreover, it plays an important role in the growth and metastasis of this carcinoma. Valletti et al. [36] reported that low *KCNJ1* expression in patients with ccRCC can be used in the preliminary diagnosis of the condition and prediction of the prognosis and therapeutic response.

In our study-in addition to *COL4A3, COL4A4,* and *KCNJ1*-we identified seven other hub genes associated with WT, including *SLC12A1, CASR, CLCNKB, AGT, ITGB3, ITGA6,* and *MME*. Dysregulation of these genes has also been reported in glioma, hyperparathyroidism, Bartter syndrome, and gastric, lung, bladder, and breast cancers; thus, these genes may represent valuable biomarkers for tumor diagnosis, treatment, and prognosis [37-43]. *COL4A3, COL4A4,* and *KCNJ1* were downregulated in WT tissues and were associated with worse overall survival, suggesting that these genes might serve as biomarkers and therapeutic targets for WT. Nonetheless, further studies are required to investigate the function of these genes in WT.

Conclusion

The purpose of this study was to identify DEGs that might be involved in the carcinogenesis or progression of WT. In our study, newly identified DEGs and hub genes provided an opportunity to understand the mechanism underlying the carcinogenesis and development of nephroblastoma. *COL4A3, COL4A4,* and *KCNJ1* may be the key genes in WT and could represent targets for the diagnosis and treatment of the disease. As we have adopted a bioinformatic approach, further biological experiments are required to better understand the role of these genes in WT.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Guochang Bao, Department of Urology, Affiliated Hospital of Chifeng University, Chifeng 024000, China; Urology Research Center, Chifeng University, No. 42, Wangfu Street, Songshan District, Chifeng 024000, China. Tel: +86-13947690228; Fax: +86-476-5973310; E-mail: guochangbao58@163.com

References

[1] Ayatollahi H, Sadeghian MH, Naderi M, Jafarian AH, Shams SF, Motamedirad N, Sheikhi M, Bahrami A and Shakeri S. Quantitative assessment of Wilms tumor 1 expression by real-time quantitative polymerase chain reaction in patients with acute myeloblastic leukemia. J Res Med Sci 2017; 22: 54.

[2] Stokes CL, Stokes WA, Kalapurakal JA, Paulino AC, Cost NG, Cost CR, Garrington TP, Greffe BS, Roach JP, Bruny JL and Liu AK. Timing of radiation therapy in pediatric Wilms tumor: a report from the national cancer database. Int J Radiat Oncol Biol Phys 2018; 101: 453-461.

[3] Charlton J, Pavasovic V and Pritchard-Jones K. Biomarkers to detect Wilms tumors in pediatric patients: where are we now? Future Oncol 2015; 11: 2221-2234.

[4] Chu A, Heck JE, Ribeiro KB, Brennan P, Boffetta P, Buffler P and Hung RJ. Wilms' tumour: a systematic review of risk factors and meta-analysis. Paediatr Perinat Epidemiol 2010; 24: 449-469.

[5] Cunningham ME, Klug TD, Nuchtern JG, Chintagumpala MM, Venkatramani R, Lubega J and Naik-Mathuria BJ. Global disparities in Wilms tumor. J Surg Res 2020; 247: 34-51.

[6] Millar AJW, Cox S and Davidson A. Management of bilateral Wilms tumours. Pediatr Surg Int 2017; 33: 737-745.

[7] Aldrink JH, Heaton TE, Dasgupta R, Lautz TB, Malek MM, Abdessalam SF, Weil BR, Rhee DS,

Baertschiger R and Ehrlich PF; American Pediatric Surgical Association Cancer Committee. Update on Wilms tumor. J Pediatr Surg 2019; 54: 390-397.

[8] Gratias EJ, Dome JS, Jennings LJ, Chi YY, Tian J, Anderson J, Grundy P, Mullen EA, Geller JI, Fernandez CV and Perlman EJ. Association of chromosome 1q gain with inferior survival in favorable-histology Wilms tumor: a report from the children's oncology group. J Clin Oncol 2016; 34: 3189-3194.

[9] Edgar R, Domrachev M and Lash AE. Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002; 30: 207-210.

[10] Abdueva D, Wing M, Schaub B, Triche T and Davicioni E. Quantitative expression profiling in formalin-fixed paraffin-embedded samples by affymetrix microarrays. J Mol Diagn 2010; 12: 409-417.

[11] Yusenko MV, Zubakov D and Kovacs G. Gene expression profiling of chromophobe renal cell carcinomas and renal oncocytomas by Affymetrix GeneChip using pooled and individual tumours. Int J Biol Sci 2009; 5: 517-527.

[12] Ludwig N, Werner TV, Backes C, Trampert P, Gessler M, Keller A, Lenhof HP, Graf N and Meese E. Combining miRNA and mRNA expression profiles in Wilms tumor subtypes. Int J Mol Sci 2016; 17: 475.

[13] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM and Sherlock G. Gene ontology: tool for the unification of biology. The gene ontology consortium. Nat Genet 2000; 25: 25-29.

[14] Kanehisa M. The KEGG database. Novartis Found Symp 2002; 247: 91-252.

[15] Smoot ME, Ono K, Ruscheinski J, Wang PL and Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 2011; 27: 431-432.

[16] Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pagès F, Trajanoski Z and Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 2009; 25: 1091-1093.

[17] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ and von Mering C. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017; 45: D362-D368.

[18] Cutcliffe C, Kersey D, Huang CC, Zeng Y, Walterhouse D and Perlman EJ; Renal Tumor Committee of the Children's Oncology Group. Clear cell sarcoma of the kidney: up-regulation of neural markers with activation of the sonic hedgehog and Akt pathways. Clin Cancer Res 2005; 11: 7986-7994.

[19] Yusenko MV, Kuiper RP, Boethe T, Ljungberg B, van Kessel AG and Kovacs G. High-resolution DNA copy number and gene expression analyses distinguish chromophobe renal cell carcinomas and renal oncocytomas. BMC Cancer 2009; 9: 152.

[20] Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM and Hau ssler D. The human genome browser at UCSC. Genome Res 2002; 12: 996-1006.

[21] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1.

[22] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012; 2: 401-404.

[23] Spreafico F and Bellani FF. Wilms' tumor: past, present and (possibly) future. Expert Rev Anticancer Ther 2006; 6: 249-258.

[24] Treger TD, Chowdhury T, Pritchard-Jones K and Behjati S. The genetic changes of Wilms tumour. Nat Rev Nephrol 2019; 15: 240-251.

[25] Termuhlen AM, Tersak JM, Liu Q, Yasui Y, Stovall M, Weathers R, Deutsch M, Sklar CA, Oeffinger KC, Armstrong G, Robison LL and Green DM. Twenty-five year follow-up of childhood Wilms tumor: a report from the childhood cancer survivor study. Pediatr Blood Cancer 2011; 57: 1210-1216.

[26] Wong KF, Reulen RC, Winter DL, Guha J, Fidler MM, Kelly J, Lancashire ER, Pritchard-Jones K, Jenkinson HC, Sugden E, Levitt G, Frobisher C and Hawkins MM. Risk of adverse health and social outcomes up to 50 years after Wilms tumor: the British childhood cancer survivor study. J Clin Oncol 2016; 34: 1772-1779.

[27] Metodieva SN, Nikolova DN, Cherneva RV, Dimova II, Petrov DB and Toncheva DI. Expression analysis of angiogenesis-related genes in Bulgarian patients with early-stage non-small cell lung cancer. Tumori 2011; 97: 86-94.

[28] Nie XC, Wang JP, Zhu W, Xu XY, Xing YN, Yu M, Liu YP, Takano Y and Zheng HC. COL4A3 expression correlates with pathogenesis, pathologic behaviors, and prognosis of gastric carcinomas. Hum Pathol 2013; 44: 77-86.

[29] Tao YF, Lu J, Du XJ, Sun LC, Zhao X, Peng L, Cao L, Xiao PF, Pang L, Wu D, Wang N, Feng X, Li YH, Ni J, Wang J and Pan J. Survivin selective inhibitor YM155 induce apoptosis in SK-NEP-1 Wilms tumor cells. BMC Cancer 2012; 12: 619.

[30] Georgiou GK, Igglezou M, Sainis I, Vareli K, Batsis H, Briasoulis E and Fatouros M. Impact of breast cancer surgery on angiogenesis circulating biomarkers: a prospective longitudinal study. World J Surg Oncol 2013; 11: 213.

[31] Siamakpour-Reihani S, Owzar K, Jiang C, Turner T, Deng Y, Bean SM, Horton JK, Berchuck A, Marks JR, Dewhirst MW and Alvarez Secord A. Prognostic significance of differential expression of angiogenic genes in women with high-grade serous ovarian carcinoma. Gynecol Oncol 2015; 139: 23-29.

[32] Wang J, Zhang C, He W and Gou X. Construction and comprehensive analysis of dysregulated long non-coding RNA-associated competing endogenous RNA network in clear cell renal cell carcinoma. J Cell Biochem 2019; 120: 2576-2593.

[33] Chattopadhyay I, Phukan R, Singh A, Vasudevan M, Purkayastha J, Hewitt S, Kataki A, Mahanta J, Kapur S and Saxena S. Molecular profiling to identify molecular mechanism in esophageal cancer with familial clustering. Oncol Rep 2009; 21: 1135-1146.

[34] Li J, Wang X, Zheng K, Liu Y, Li J, Wang S, Liu K, Song X, Li N, Xie S and Wang S. The clinical significance of collagen family gene expression in esophageal squamous cell carcinoma. PeerJ 2019; 7: e7705.

[35] Guo Z, Liu J, Zhang L, Su B, Xing Y, He Q, Ci W, Li X and Zhou L. KCNJ1 inhibits tumor proliferation and metastasis and is a prognostic factor in clear cell renal cell carcinoma. Tumour Biol 2015; 36: 1251-1259.

[36] Valletti A, Gigante M, Palumbo O, Carella M, Divella C, Sbisà E, Tullo A, Picardi E, D'Erchia AM, Battaglia M, Gesualdo L, Pesole G and Ranieri E. Genome-wide analysis of differentially expressed genes and splicing isoforms in clear cell renal cell carcinoma. PLoS One 2013; 8: e78452.

[37] Bralten LB, Kloosterhof NK, Gravendeel LA, Sacchetti A, Duijm EJ, Kros JM, van den Bent MJ, Hoogenraad CC, Sillevis Smitt PA and French PJ. Integrated genomic profiling identifies candidate genes implicated in glioma-genesis and a novel LEO1-SLC12A1 fusion gene. Genes Chromosomes Cancer 2010; 49: 509-517.

[38] Marx SJ and Sinaii N. Neonatal severe hyperparathyroidism: novel insights from calcium, PTH, and the CASR gene. J Clin Endocrinol Metab 2020; 105: dgz233.

[39] Wang C, Chen Y, Zheng B, Zhu M, Fan J, Wang J, Jia Z, Huang S and Zhang A. Novel compound heterozygous CLCNKB gene mutations (c.1755A>G/c.848_850delTCT) cause classic Bartter syndrome. Am J Physiol Renal Physiol 2018; 315: F844-F851.

[40] Zhang C, Liang Y, Ma MH, Wu KZ and Dai DQ. KRT15, INHBA, MATN3, and AGT are aberrantly methylated and differentially expressed in gastric cancer and associated with prognosis. Pathol Res Pract 2019; 215: 893-899.

[41] Hong SK, Lee H, Kwon OS, Song NY, Lee HJ, Kang S, Kim JH, Kim M, Kim W and Cha HJ. Large-scale pharmacogenomics based drug discovery for ITGB3 dependent chemoresistance in mesenchymal lung cancer. Mol Cancer 2018; 17: 175.

[42] Jin H, Ying X, Que B, Wang X, Chao Y, Zhang H, Yuan Z, Qi D, Lin S, Min W, Yang M and Ji W. N^6-methyladenosine modification of ITGA6 mRNA promotes the development and progression of bladder cancer. EBioMedicine 2019; 47: 195-207.

[43] Boberg DR, Batistela MS, Pecharki M, Ribeiro EM, Cavalli IJ, Lima RS, Urban CA, Furtado-Alle L and Souza RL. Copy number variation in ACHE/EPHB4 (7q22) and in BCHE/MME (3q26) genes in sporadic breast cancer. Chem Biol Interact 2013; 203: 344-347.