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ORIGINAL RESEARCH

Vitamin C Selectively Inhibits Kidney Renal Clear Cell Carcinoma Cell Growth by Suppressing the HIF-1 Pathway

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Aim: To observe the effect of vitamin C on Kidney renal clear cell carcinoma (KIRC) and investigate its mechanism.

Methods and Results: Firstly, 29 vitamin C direct target proteins (DPTs) were identified by Drug Bank 5.0, and the protein-protein interaction (PPI) network and signaling pathways of vitamin C DPTs were analyzed. The results showed that vitamin C was not only related to KIRC, but also to the HIF-1 pathway. Meanwhile, the top 300 highly expressed genes of KIRC were obtained by GEPIA. Next, We compared the genes of four vitamin C targets in the PPI network with highly expressed genes in KIRC. Interestingly, these common genes are also involved in HIF-1 pathway. Additionally, we utilized RNA-Seq technology to explore the differentially expressed genes in KIRC with vitamin C compared to those not intervened. We observed that these differentially expressed genes exhibited a close association with hypoxia. Finally, we observed the inhibitory effect of Vitamin C on KIRC by Cell Counting Kit-8 (CCK8) assay, real-time quantitative PCR, Western blotting, flow cytometry, and colony formation assay, and confirmed that Vitamin C inhibits the growth of KIRC cells through the HIF-1 pathway.

Conclusion: Through bioinformatics analyses, we identified the molecular mechanism of vitamin C's role in KIRC and verified it through a series of experiments. Combined bioinformatics analysis will play an important role in future drug-disease interaction studies.

Keywords: vitamin C, kidney renal clear cell carcinoma, HIF-1 signaling pathway, therapeutic target genes, integrated bioinformatical analysis

Introduction

Renal cell carcinoma (RCC) is the most common malignant renal tumor in adults. In men and women, RCC ranks as the sixth and ninth most common cancer, respectively, accounting for 5% and 3% of all tumor diagnoses.[1] Renal cell carcinomas have the highest incidence in people 70 years of age or older and originate in the renal tubules.[2] Statistically, clear cell renal cell carcinoma (ccRCC) comprises approximately 75% of all primary renal cell carcinoma.[3] Although cases of ccRCC that are confined to the primary lesion can be treated well by surgical, one-third of them still progress.[4] The 5-year survival rate for new and recurrent metastatic cases is only 15%. Although diagnosis and treatment of urologic tumors have improved rapidly since the 21st century, the lethality of chronic renal cell carcinoma remains high.

Vitamin C, an essential water-soluble vitamin first reported by Linus Pauling and Ewan Cameron in 1973 is also known as ascorbic acid or ascorbate plays a vital role in the growth and development of human beings.[5–7] As an electron donor, vitamin C actively participates in various redox and enzymatic reactions in the body, thus affecting iron metabolism and noradrenaline synthesis and other processes.[8,9] Humans lack the ability to synthesize vitamin C independently due to inactivating mutations in the gene responsible for encoding gulonolactone oxidase (GULO).[10] As a result, it is necessary for us to obtain vitamin C through dietary intake. Many studies have demonstrated the immense potential of vitamin C in cancer therapy by retarding tumor growth

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in vivo. High doses of intravenous vitamin C can effectively achieve anticancer effects, whether administered alone or in combination.[11–14] Additionally, to some extent, intravenous vitamin C can reduce chemotherapy-related side effects. Nevertheless, the processes connected to them in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway have not been elucidated.

Over the past 10 years, drug databases have developed rapidly as the level of network technology has improved and the needs of drug research and development have changed. They contain comprehensive information on drug action mechanisms and drug targets.[15] Cybermedicine has great potential to predict disease genes, identify gene modules, and characterize drug targets for complex diseases.[16] In-depth analysis of drug databases using high-throughput omics data will help us discover potential new drug targets.

In this work, first we looked up vitamin C in DrugBank 5.0, which gives access to 28 direct protein targets (DPTs). Next, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of vitamin C DPTs was further identified. We found that vitamin C is associated with several cancers, especially KIRC. The four most critical targeted genes for vitamin C in KIRC are EGLN1, EGLN2, EGLN3 and SLC2A1. In addition, the genes that were differentially expressed in KIRC compared to normal samples were obtained in GEPIA. These differentially expressed genes were then compared with the genes in the PPI network of the 4 vitamin C. Next, the biological processes of the co-expressed genes were analyzed by STRING. Interestingly, we found that these genes are involved in the biosynthetic process of HIF-1. We inferred that vitamin C may inhibit the growth of KIRC cells by inhibiting the HIF-1 pathway. Finally, we used CCK8 assay, real-time quantitative PCR, RNA-seq, Western blotting, colony formation assay and flow cytometry to further validate our thoughts.

Materials and Methods

Identifying the Direct Protein Targets (DPTs) of Vitamin C

DrugBank (https://www.drugbank.ca) is a drug-related database that was first proposed 18 years ago. The database contains comprehensive information on the mechanism of action of specific drugs as well as drug targets. It is used not only for drug design and drug metabolism prediction, but also for potential drug target discovery and general drug education.[15] Drug Bank drug proteomics data are manually validated by its team, while drug metabolomics data rely on manual literature searches by PolySearch2, a text mining tool that was developed by the Large Database Annotation Project co-developed. In this study, direct protein targets (DPTs) of vitamin C were obtained from DrugBank.

KEGG Pathways Analysis of Vitamin C DPTs

The STRING (Search Tool for the Retrieval of Interacting Genes) database (https://string-db.org/) collects protein-protein interaction data from various sources and integrates them systematically. This platform enables the prediction of gene-focused protein-protein interaction (PPI) networks, as well as signaling pathways, and more.[17] Cytoscape serves as a tool for analyzing network biology, integrating data, and visualizing networks. CluePedia, a Cytoscape plug-in, conducts linear and nonlinear statistical correlation analysis of experimental data, enriching it with relevant experimental data to unveil potential associations.[18] In this study, we constructed a PPI network of vitamin C DPTs using the STRING database and analyzed the signaling pathways of vitamin C DPTs, which were subsequently validated and visualized using Cytoscape 3.9.0.

Identification of Differentially Expressed Genes in KIRC and Normal Tissues

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia2.cancer-pku.cn/) is a web-based tool grounded on the TCGA and GTEx databases, offering features such as differential expression analysis, survival analysis, and gene similarity identification. GEPIA2, an advanced iteration, expands gene expression quantification from the gene to the transcript level.[19] Within GEPIA, we initiated Differential Expression Analysis, selecting renal clear cell carcinoma (KIRC) as the cancer type to reveal genes that exhibit differential expression patterns between tumor and normal samples.

Cell Culture and Reagents

Renal cancer cell lines 786-O and Umrc6 were obtained from Wuhan Punosai Life Science & Technology Co., Ltd. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere with 5% CO2. We procured the antibodies for our experiments from Cell Signaling Technologies. Vitamin C was dissolved in phosphate buffered saline (PBS), and the initial concentration was set to 100 mmol/L.

Cell Viability Assay

Renal clear cell carcinoma cell lines (Umrc6 and 786-O) were seeded into 96-well plates at a cell density of 1×10^3 per well. Various concentrations of vitamin C (0, 1, 2, 4, 8, 10, and 20 mmol/L) were added to the 96-well plates. After 24 hours of treatment, cell viability was assessed using a CCK8 assay and measured by absorbance at 490 nm with a multi-well microplate reader (BIO-TEC Inc, VA).

Colony Formation Assay

Renal clear cell carcinoma cell were seeded in 6-well cell culture dishes at a density of 500 cells per well and allowed to adhere overnight. The cells were then divided into control and drug administration groups. The second day, vitamin C was added to the administered group at concentrations of 1 mmol/L for Umrc6 and 1.5 mmol/L for 786-O. The medium was subsequently changed every 2–3 days, and after 7 days of incubation, colonies were formed. These colonies were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet solution, then rinsed and imaged. The number of colonies with a diameter >0.5 mm was counted using a 40× magnification (Nikon Eclipse Ti-S, Japan).

Apoptosis Assay

Initially, 1×10^5 cells were inoculated into each well of a six-well plate for overnight adhesion. The following day, fresh medium containing varying concentrations of vitamin C (0 mmol/L, 4 mmol/L, 8 mmol/L for Umrc6; 0 mmol/L, 10 mmol/L, 20 mmol/L for 786-O) was added. After 24 hours, the cells were treated according to the instructions provided in the PE Annexin V apoptosis detection kit (Wuhan Sevier Biosciences, Ltd.). Apoptosis in the cells was assessed using a FACSCalibur flow cytometer (BD Biosciences, USA). The total apoptosis rate was calculated as the combined percentage of early apoptotic cells (7-AAD -/PE Annexin V+) and late apoptotic cells (7-AAD +/PE Annexin V+).

RNA Sequencing Analysis

Total RNA was isolated from Umrc6 cells using Trizolreagent (Invitrogen, USA) according to the manufacturer's protocol. The samples were categorized into control and experimental groups. The experimental group was treated with fresh medium containing concentrations of 4 mmol/L vitamin C, whereas the control group was provided with medium devoid of vitamin C. Following a 24-hour treatment period, RNA sequencing analyses were conducted by Metware Biotechnology Co. (Wuhan, China).

Real-Time Quantitative PCR

Renal clear cell carcinoma cells were subjected to total RNA extraction using the Total RNA Fast Extraction Kit (Pioneer Bio, item no. RN03). Subsequently, cDNA was synthesized using the Prime Script RT reagent Kit (Perfect Real Time) (Takara). The synthesized cDNA was then amplified by RT-PCR. The cDNA amplification was performed by RT-PCR using primers custom-designed by Beijing Tsingke Bio-Technology Co. The desired system was prepared in 96-well plates using custom-designed primers and 2×Universal SYBR Green Fast qPCR mix (ABclonal) following the manufacturer's instructions. Subsequently, the assay was conducted on a real-time fluorescent quantitative PCR detection system (PikoReal). Relative expression levels were normalized to expression by GraphPad.

Western Blotting Analysis

Total protein was extracted using RIPA lysis buffer, and its concentration was determined by the BCA protein assay kit (Pierce, Rockford, USA) before proceeding to Western blotting. Electrophoresis was conducted using 10% PAGE precast gels (epizyme Bio, Shanghai, China), ensuring equal protein loading in each well. Following electrophoresis, proteins were transferred from the gel to a nitrocellulose membrane. The membrane was then incubated in a protein-free rapid closure solution (epizyme Bio, Shanghai, China) for 10 min, followed by overnight incubation with the primary antibody. The following day, the membranes were incubated with a horseradish peroxidase-coupled secondary antibody for 1 hour. Protein expression was detected using enhanced chemiluminescence (GE, Buckinghamshire, UK). Image processing was conducted using the ChemiDoc XRS imaging system (Bio-Rad, USA). GAPDH served as the internal loading control.

Statistics

The results are presented as mean ± standard deviation (SD). Statistical analysis was performed using Student's *t*-test with GraphPad Prism 6.0 software. A p-value of less than 0.05 was considered statistically significant. Each experiment was repeated at least three times.

Results

Identification and Analysis of Vitamin C's DPTs

Vitamin C is a vitamin used to correct vitamin deficiency and to increase the intestinal absorption of iron, in the output DB00126 from Drug Bank 5.0. Twenty-eight primary direct protein targets (DPTs) of Vitamin C were obtained, which were XylA, PLOD2, PHYH, PLOD3, BBOX1, DBH, PAM, P3H1, P3H2, P3H3, P4HA1, OGFOD1, OGFOD2, ALKBH2, ALKBH3, KDM5D, PLOD1, TMLHE, P4HTM, EGLN1, EGLN2, EGLN3, TXNRD1, ALB, SLC23A1, SLC2A1, SLC2A3, SLC2A4 (Table 1). The PPI (Protein-protein interaction) network and signaling pathways of 28 Vitamin C DPTs were generated by STRING (Figure 1A and Table 2). The top 4 KEGG pathways of DPTs were Lysine degradation, Renal cell carcinoma, HIF-1 signaling pathway and Pathways in cancer. The results showed that the most relevant solid tumor of Vitamin C was KIRC (p = 0.00035), and there were 4 DPTs of Vitamin C (EGLN1, EGLN2, EGLN3 and SLC2A1) associating with KIRC (Table 2). In addition, we used CluePedia to validate the association between vitamin C and KIRC again (Figure 1B). Not surprisingly, the 4 DPTs associated with Vitamin C and KIRC are consistent with the outcome of STRING.

Identification of the Potential Therapeutic Target Genes of Vitamin C in KIRC

Using the GEPIA on line database, we compared the KIRC samples with normal tissue samples and obtained the overexpressed genes. Overexpressed genes in KIRC are as shown in Figure 1C. After bioinformatics analysis, we derived the top 300 highly expressed genes. Next, we compared these 300 overexpressed genes with the four vitamin C DPTs interlinked in KIRC, and finally obtained three consistently highly expressed genes (Figure 1D). These three genes are EGLN3, SLC2A1, and SPARC, which might serve as mediators for potential therapeutic targets of vitamin C in KIRC. Therefore, we performed KEGG pathway enrichment analysis on these three genes, and the results showed that EGLN3 and SLC2A1 are related to the HIF-1 signaling pathway (Table 3).

Vitamin C Suppress Proliferation of KIRC Cells

First, the effect of vitamin C on the viability of KIRC cells was observed. When different concentrations of vitamin C were added to KIRC cells (Umrc6 and 786-O), we observed that the viability of KIRC cells was decreasing with increasing doses of vitamin C. The IC50 of the two cell lines, Umrc6 and 786-O, were 2.802 mmol/L and 12.37 mmol/L, respectively (Figure 2A). Thus, we can observe that vitamin C inhibits the proliferation of KIRC cells. We further confirmed the effect of vitamin C on the proliferative capacity of KIRC (Umrc6 and 786-O) by clone formation. The experimental results showed that when we treated KIRC cells with vitamin C, both the size and number of colonies were inhibited compared to untreated cells (Figure 2B).

Table 1 Identification of Direct Targets of Vitamin C Using DRUGBANK

Searched Drug (1/1) **DB_ID** **DB00126**	**Name** **Acetylsalicylic Acid**	**Target (28)** **Target Symbol**	**Uniprot ID**
		xylA	P24300
		PLOD2	O00469
		PHYH	O14832
		PLOD3	O60568
		BBOX1	O75936
		DBH	P09172
		PAM	P19021
		P3H1	Q32P28
		P3H2	Q8IVL5
		P3H3	Q8IVL6
		P4HA1	P13674
		OGFOD1	Q8N543
		OGFOD2	Q6N063
		ALKBH2	Q6NS38
		ALKBH3	Q96Q83
		KDM5D	Q9BY66
		PLOD1	Q02809
		TMLHE	Q9NVH6
		P4HTM	Q9NXG6
		EGLN1	Q9GZT9
		EGLN2	Q96KS0
		EGLN3	Q9H6Z9
		TXNRD1	Q16881
		ALB	P02768
		SLC23A1	Q9UHI7
		SLC2A1	P11166
		SLC2A3	P11169
		SLC2A4	P14672

Vitamin C Induces Apoptosis of KIRC

We also found that vitamin C induced apoptosis in KIRC cells. Apoptosis was increased in KIRC cells treated with vitamin C compared to the untreated group (Figure 3). Moreover, the apoptotic effect increased as the concentration of vitamin C increased. The above results suggest that vitamin C has the effect of inhibiting clone formation and inducing apoptosis in renal clear cell carcinoma cells.

Potential Pathways for Vitamin C to Inhibit the Growth of KIRC

We found that KIRC is strongly associated with the HIF-1 pathway, but the exact mechanism is still being explored. Umrc6 was incubated with medium with or without 4 mmol/L vitamin C for 24 hours, and total RNA was extracted from both groups. RNA sequencing analysis revealed differential gene expression between the vitamin C-treated and untreated groups of KIRC (Figure 4A). Subsequently, Disease annotation functional analysis was conducted on the down-regulated genes, revealing their close association with anoxia (Rich factor: 0.1818) (Figure 4B). Concurrently, GSEA (Gene Set Enrichment Analysis) identified a significant enrichment of hypoxia and apoptosis-process (Figure 4C). Furthermore, among the differentially expressed genes, SLC2A1 and HIF1A, pertinent to our study, were identified (Figure 4D). Consequently, our findings indicate that vitamin C may impede KIRC growth by modulating hypoxia.

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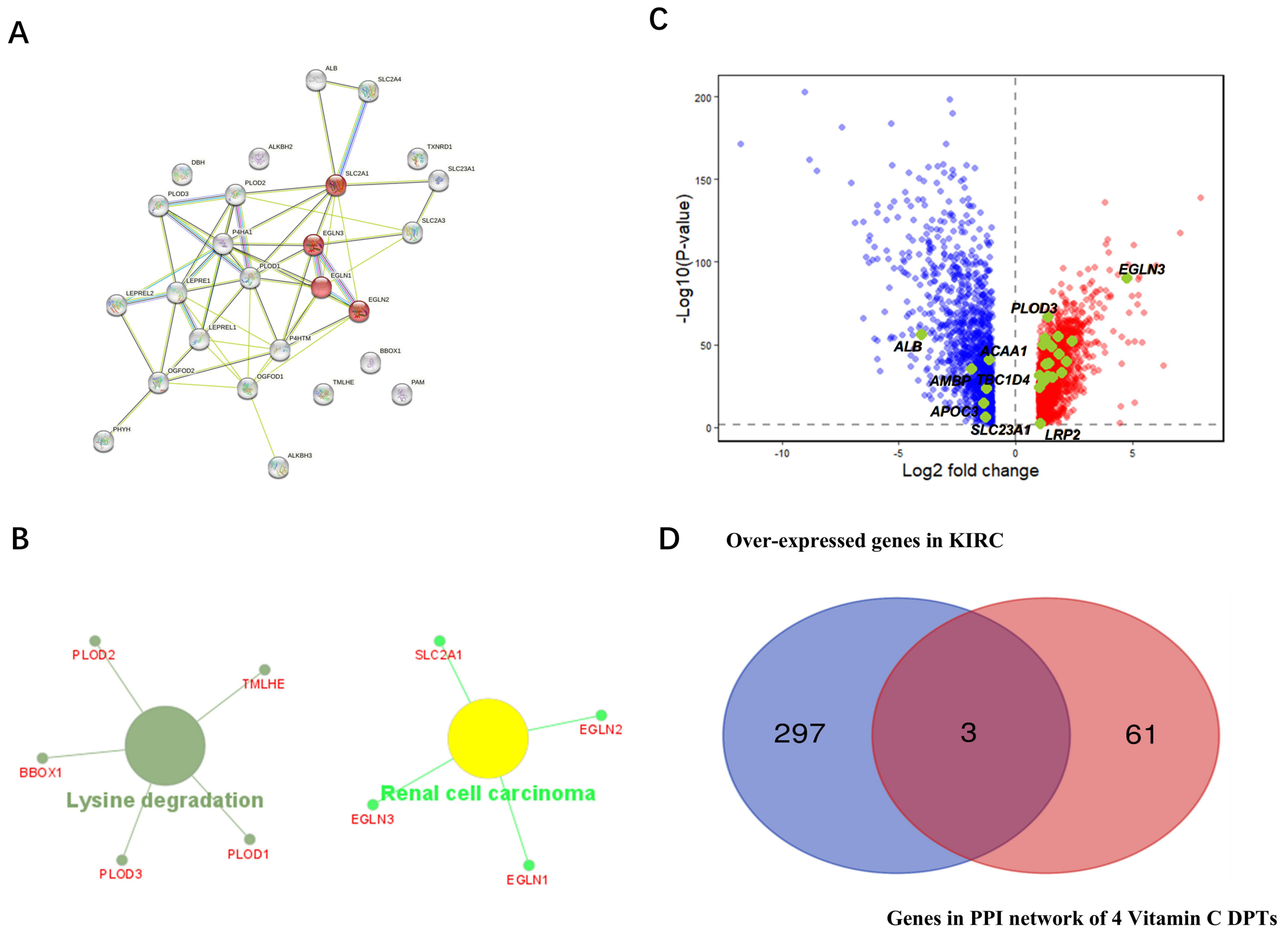

Figure 1 Identification of the potential therapeutic target genes of Vitamin C in KIRC. (**A**) PPI network of Vitamin C target-genes, 4 nodes in red color are related to KIRC. (EGLN1, EGLN2, EGLN3 and SLC2A1) (**B**) Visualization of KEGG pathways in 28 Vitamin C target-genes. (**C**) The overexpressed genes in KIRC. A not in blue color means least expressed, in red color means most expressed. (**D**) 3 genes that appeared both in the most significant target genes of Vitamin C in KIRC and in KIRC samples.

Vitamin C Suppresses HIF-1 Signaling Pathway in KIRC Cells

In the previous analysis, EGLN3 and SLC2A1 were found to be associated with the HIF-1 signaling pathway. SLC2A1 is localized at 1p34.2 and encodes the glucose transporter 1 protein (GLUT1). It was shown that hypoxia-inducible factor 1α (HIF-1α) is a transcription factor that upregulates glucose transporter protein (GLUT).[20] Under hypoxia, cancer cells rely even more on glucose transporters in order to accelerate glycolysis. Next, we examined the expression levels of HIF-1α and GLUT1mRNA in KIRC cells using qPCR. The expression levels of HIF-1α and GLUT1mRNA were significantly suppressed in KIRC cells treated with vitamin C compared to the untreated group (Figure 5A). Finally, Western blotting experiments further demonstrated that KIRC cells treated with vitamin C had reduced expression of HIF-1α and GULT1 protein (Figure 5B). Thus, we confirmed that vitamin C inhibits the growth of renal clear cell carcinoma through the HIF-1 pathway.

Table 2 List of KEGG Pathways in Vitamin C Associated KIRC

Pathway Description	Count	False Discovery Rate	Gene ID
Lysine degradation	5	7.20E-06	PLOD1, PLOD3, BBOX1, PLOD2, TMLHE
Renal cell carcinoma	4	0.00035	EGLN3, EGLN1, SLC2A1, EGLN2
HIF-1 signaling pathway	4	0.0014	EGLN3, EGLN1, SLC2A1, EGLN2
Pathways in cancer	5	0.0458	EGLN3, EGLN1, SLC2A1, TXNRD1, EGLN2

Table 3 List of KEGG Pathways in Co-Expressed Genes Identification from Two Gene Sets

Pathway Description	Count	False Discovery Rate	Gene ID
Renal cell carcinoma	8	3.74E-12	EGLN3, VHL, EPAS1, ARNT, EGLN1, SLC2A1, HIF1A, EGLN2
HIF-1 signaling pathway	7	4.96E-09	EGLN3, VHL, ARNT, EGLN1, SLC2A1, HIF1A, EGLN2
Pathways in cancer	10	1.54E-08	EGLN3, VHL, EPAS1, TP53, FN1, ARNT, EGLN1, SLC2A1, HIF1A, EGLN2
ECM-receptor interaction	6	7.92E-08	COL1A1, VTN, IBSP, COL1A2, FN1, SPP1
Proteoglycans in cancer	7	1.48E-07	COL1A1, VTN, LUM, TP53, COL1A2, FN1, HIF1A
Human papillomavirus infection	7	3.84E-06	COL1A1, VTN, IBSP, TP53, COL1A2, FN1, SPP1
Focal adhesion	6	4.36E-06	COL1A1, VTN, IBSP, COL1A2, FN1, SPP1
PI3K-Akt signaling pathway	7	4.73E-06	COL1A1, VTN, IBSP, TP53, COL1A2, FN1, SPP1

Discussion

In this study, we employed a bioinformatics approach to investigate potential drug targets influencing KIRC at the protein level, which led us to focus on the HIF-1 signaling pathway. We further explored the underlying mechanisms through fundamental biological experiments. First, the bioinformatics analysis of vitamin C was divided into four steps: (i) Obtain the major DPTs of vitamin C in DrugBank 5.0. (ii) Analyze the PPI network and KEGG pathway of vitamin C DPTs using STRING. (iii) Identify the differentially expressed genes between KIRC and normal kidney tissues by GEPIA. (iv) Analyze the intersection of KIRC differentially expressed genes with vitamin C DPTs by STRING. In the first two steps of analysis, we obtained four major DPTs (EGLN1, EGLN2, EGLN3, and SLC2A1) associated with KIRC by vitamin C. And we found that the signaling pathway most closely associated with vitamin C DPTs was the HIF-1 signaling pathway. In the third step, we selected the first 300 overexpressed genes, and in the last step, we obtained three intersecting genes (EGLN3, SLC2A1, SPARC), we found that some of intersecting genes of KIRC participated in HIF-1 signaling pathway. Next, our bioinformatic analysis results were validated in the experimental data. The experimental data were mainly divided into two parts. On the one hand, clone formation, CCK8, and apoptosis assays confirmed that vitamin C could inhibit the growth and proliferation of KIRC cells, and that this was mainly achieved by inducing apoptosis; on the other hand, Western blot analysis and real-time quantitative PCR confirmed that vitamin C could selectively inhibit the HIF-1 signaling pathway in KIRC cells. Furthermore, we analyzed the potential pathways of action of vitamins to inhibit the growth of renal clear cell carcinoma using RNA-Seq. These results suggest that vitamin C can selectively inhibit the growth of renal clear cell carcinoma cells by inhibiting the HIF-1 pathway.

Based on these findings, this study proposes that the HIF-1 signaling pathway serves as a critical therapeutic target of vitamin C in KIRC. In RCC, the major source of morbidity is ccRCC, and likewise mortality.[1,21] The primary treatment modalities for clear cell renal cell carcinomas are surgery, targeted therapy, and immunotherapy. With the approval of combination regimens involving tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs), multiple Phase III clinical trials have demonstrated significant benefits for patients with metastatic or unresectable intermediate- and high-risk RCC. However, such combination therapies are also associated with a higher incidence of adverse events. Specifically, in the CLEAR trial, the incidence of grade ≥3 adverse events reached 82.4%, with approximately one-third of patients discontinuing treatment due to toxicity and nearly two-thirds requiring dose reductions.[22] Moreover, with the continued advancement of immunotherapy-based combinations, second-line treatment for metastatic RCC faces considerable challenges, and the limitations of existing therapeutic options have become increasingly evident. Therefore, it is necessary to explore therapeutic agents that can balance clinical efficacy with drug-related toxicity, thereby improving the overall treatment outcomes and quality of life in patients with RCC. Clear cell renal cell carcinomas are predominantly linked to mutations in the von Hippel-Lindau (VHL) oncogene. Although advancements have been achieved with VHL and HIF2α-targeted therapies in recent years, drug resistance often ensues, resulting in disease progression and impeding further development.[2] It has been shown that HIF1α plays a greater role than HIF2α in the process of kidney carcinogenesis.[23–25] It also provides us with new ideas to explore the treatment of renal clear cell carcinoma. Vitamin C, as an anticancer agent, has gained definite efficacy in many patients with solid tumors.[11,26–28] Vitamin C's anticancer mechanisms have been extensively studied, with its primary actions including the indirect generation of hydrogen peroxide, functioning as a cofactor to promote collagen synthesis, regulation of

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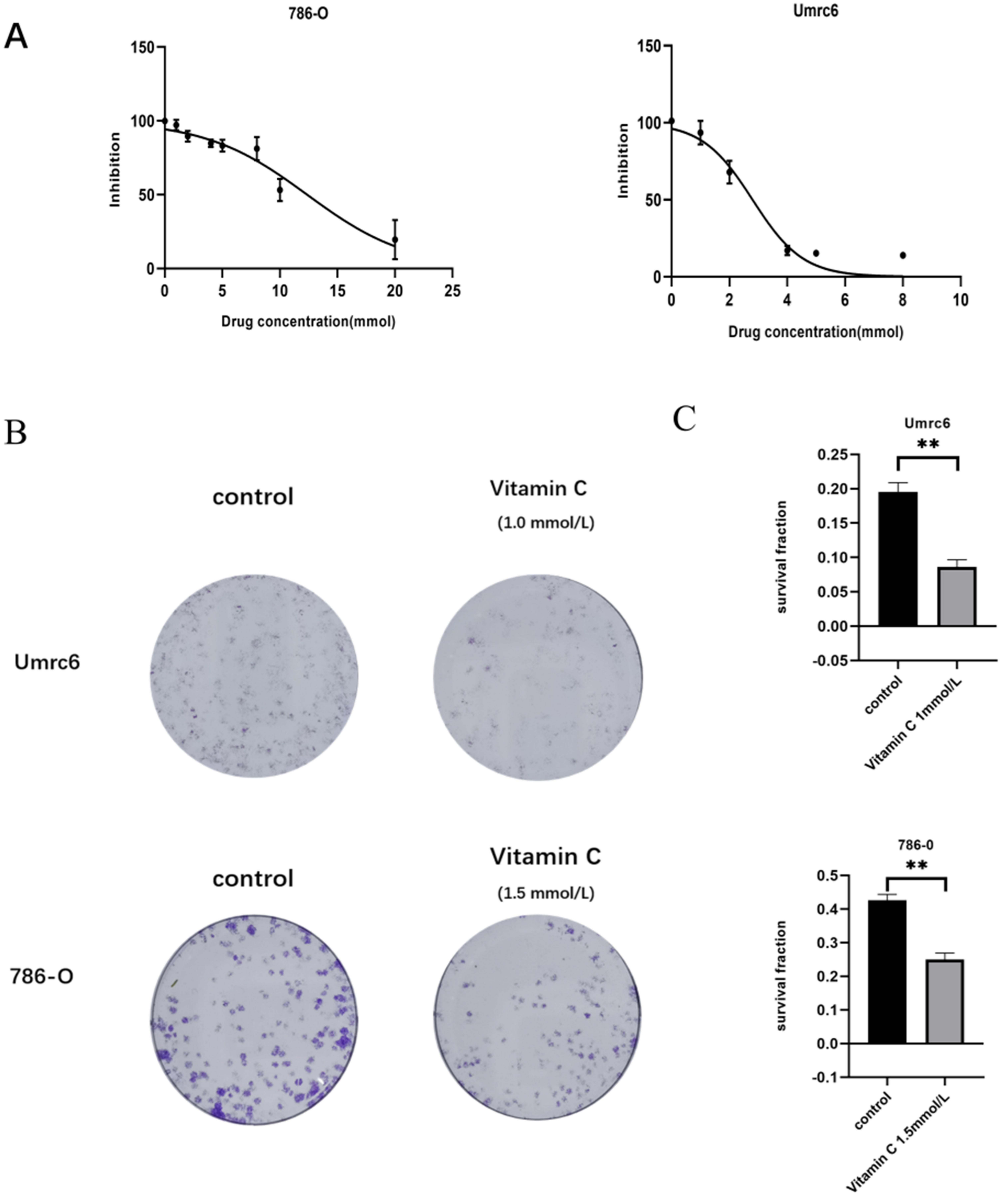

Figure 2 Vitamin C suppress proliferation of KIRC cells. (**A**) The growth of KIRC (Umrc6 and 786-O) was inhibited by Vitamin C in a dose-dependent manner. (**B**) The impact of Vitamin C on clone formation capability of KIRC. (**C**) Quantitative analysis of colone formation capacity shown in (**B**) **P <0.01.

HIF responses, and modulation of TET enzyme activity. In addition, vitamin C exerts antioxidant and anti-inflammatory effects that counteract oxidative stress and inflammatory responses within the tumor microenvironment, thereby further promoting cancer cell apoptosis and inhibiting tumor growth. The preliminary study by Kurbache et al reported that vitamin C

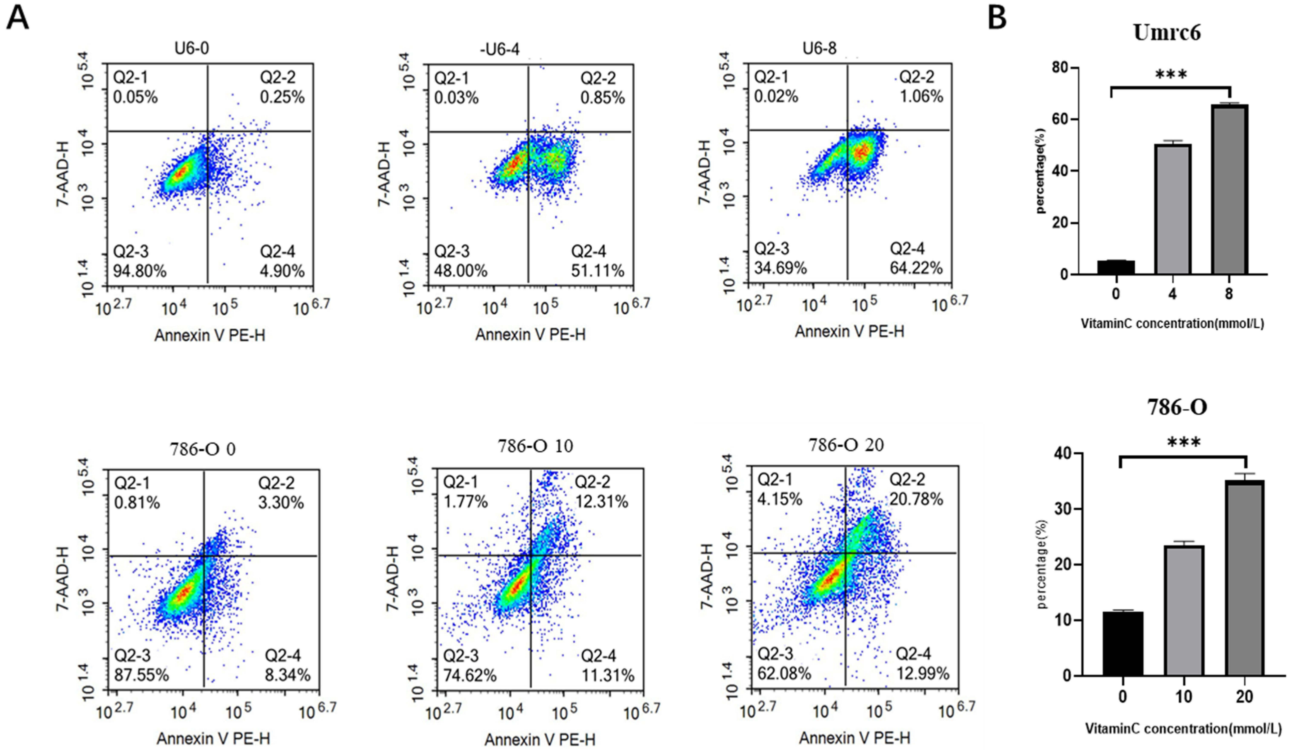

Figure 3 Vitamin C-induced apoptosis of KIRC. (**A**) Apoptosis assay results showing cells cultured with varying concentrations of vitamin C. (**B**) Corresponding statistical analysis of apoptosis rates. ***P <0.001.

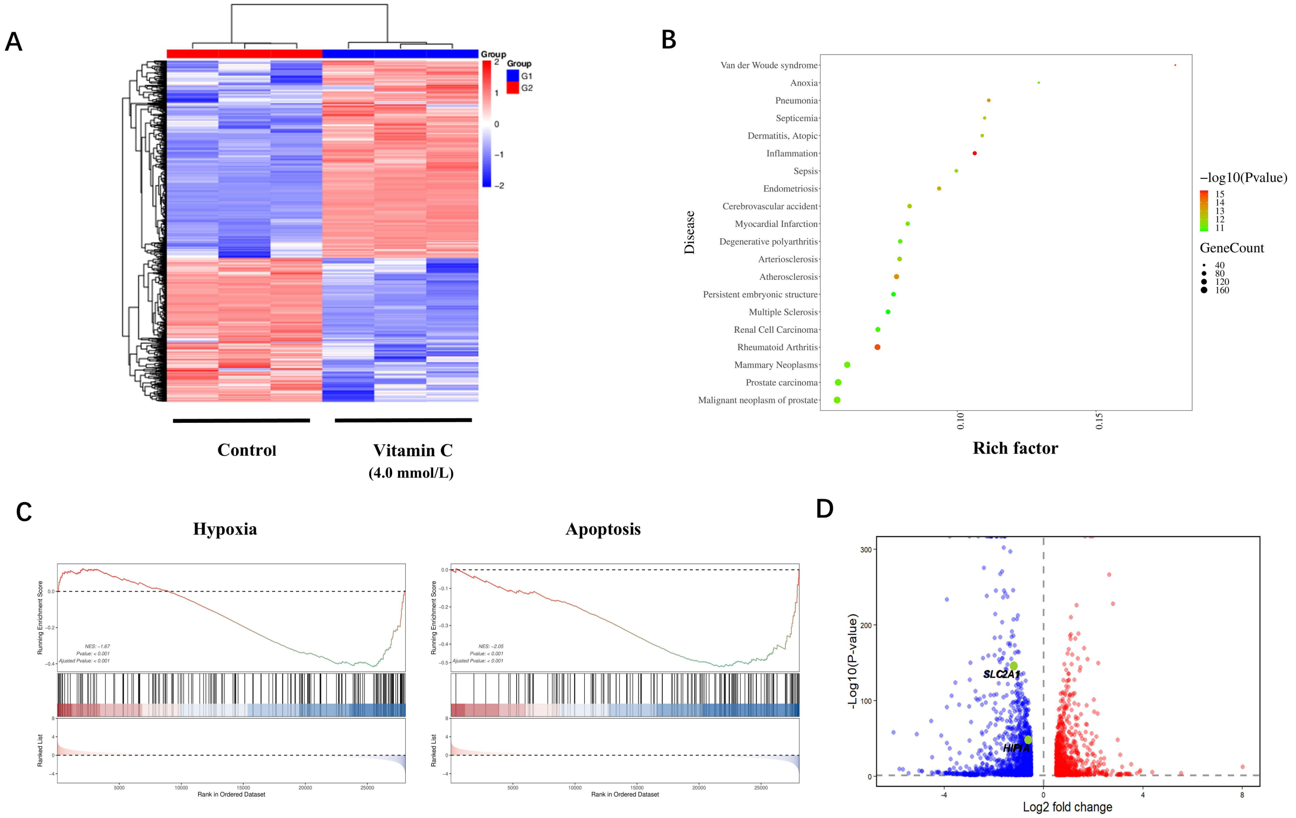

Figure 4 Differentially expressed genes in KIRC in vitamin C treated and untreated groups. (**A**) Differentially expressed genes (DEGs) between vitamin C-treated and control groups in KIRC. (**B**) Disease functional annotation analysis of KIRC DEGs. (**C**) Gene set enrichment analysis (GSEA) showing significant enrichment in hypoxia and apoptosis pathways. (**D**) Heatmap of DEGs in KIRC: blue (downregulated), red (upregulated), with key genes (SLC2A1 and HIF1A) highlighted in green.

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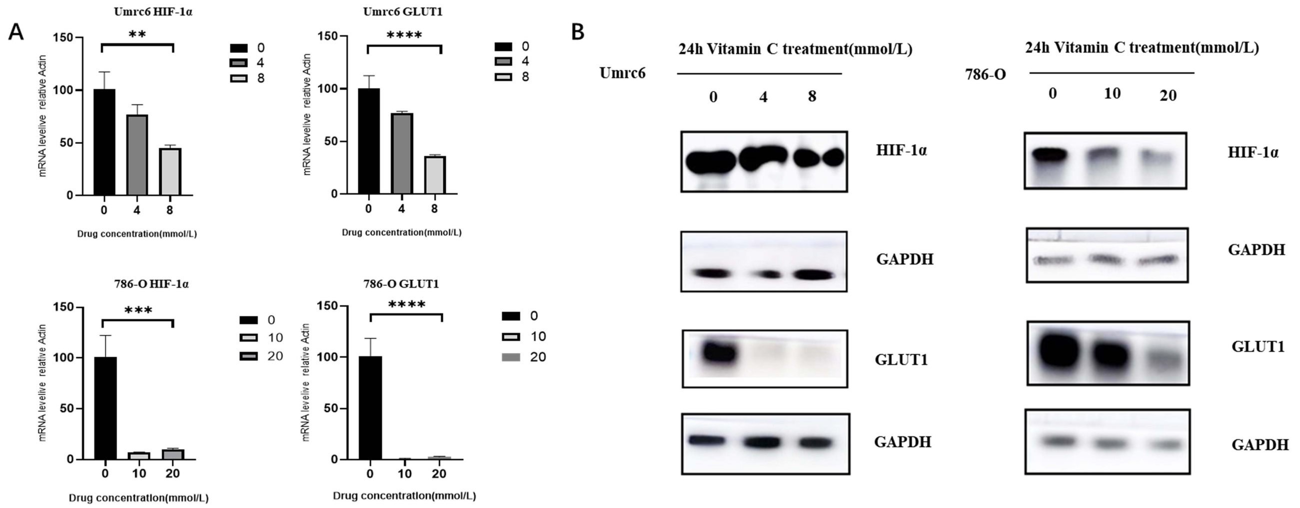

Figure 5 Relative (**A**) mRNA and (**B**) protein levels of HIF-1α and GLUT1 were down-regulated in the vitamin C-treated group compared with the NC group. **P <0.01, ***P <0.001, ****P <0.0001.

Abbreviation: NC, normal control.

significantly enhanced the antitumor activity of several chemotherapeutic agents, such as cisplatin and paclitaxel, in human breast cancer cells under in vitro conditions.[29] In addition, vitamin C can improve the efficacy of immune checkpoint inhibitors by modulating the tumor microenvironment (TME) and enhancing immune cell function.[30] Furthermore, due to its antioxidant properties, vitamin C can effectively alleviate oxidative damage induced by radio- and chemotherapy, thereby reducing both the incidence and severity of treatment-related adverse events.[31] In addition, some studies have shown that the therapeutic effect of vitamin C on KIRC, with some specificity.[32] However, the molecular mechanisms and signaling pathways underlying the therapeutic effects of vitamin C on renal clear cell carcinoma are not clear. Many solid tumors grow very rapidly, but the surrounding neoplastic capillaries are unable to provide sufficient oxygen, leading to varying degrees of hypoxia in the tumors, in which case they activate the transcription factor HIF-1α to promote better survival,[33,34] and the expression of HIF-1α is usually higher in patients with a low survival rate.[35] Therefore, the HIF-1 pathway is closely associated with tumor growth. Studies have shown that vitamin C inhibits tumor growth by degrading the HIF-1 target proteasome.[8,9] It has also been shown that HIF-1α is overexpressed in renal clear cell carcinoma.[36] However, the ability of vitamin C to inhibit the growth of renal clear cell carcinoma through the HIF-1 pathway has not been reported. This study elucidated this by a combination of bioinformatics and experimental methods.

Notably, vitamin C, as a low-cost and low-toxicity therapeutic option, demonstrates considerable potential as an anticancer agent. In recent years, multiple preclinical studies and early-phase clinical trials have shown that high-dose intravenous vitamin C exhibits significant antitumor activity across various solid tumor models, with a favorable safety profile. In a meta-analysis involving over 17,000 breast cancer cases, Harris et al reported that vitamin C was associated with a significant reduction in both overall mortality and cancer-specific mortality among breast cancer patients (RR 0.85, 95% CI 0.72–0.91 and RR 0.85, 95% CI 0.74–0.99).[37] Moreover, the transcription factor HIF-1α, stably expressed in tumor cells, plays a crucial role in the Warburg effect, enhancing glycolysis by up-regulating various proteins including glucose transporter protein (GLUT), hexokinase (HK), and others.[38] Glucose transporters play a crucial role in facilitating glucose uptake by cancer cells. GLUT1 expression has been correlated with tumor progression and prognosis in various cancers, such as rectal cancer.[39,40] Furthermore, Anders Lidgren et al discovered that GLUT1 expression was detected in the majority of patients through immunohistochemical staining of RCC tumor tissues and significantly correlated with HIF-1α expression.[41] HIF-1α expression level in ccRCC patients serves as a significant indicator of cancer-specific survival (CSS).[42] Drugs that inhibit glucose metabolism, whether in vivo or in vitro, demonstrate some inhibitory effects on tumor growth.[43] In this study, we observed a significant reduction in the expression of both HIF-1α and GLUT1 in KIRC with vitamin C. Vitamin C is anticipated to serve as a potential therapeutic agent for targeting KIRC tumor cells that heavily rely on elevated glucose uptake and glycolysis. Certainly, further studies are urgently needed to validate this prediction and elucidate its mechanism in more detail. One approach could involve the use of siRNAs or shRNAs targeting HIF1A to investigate its role in the process. By specifically knocking down the expression of HIF1A, researchers can assess its impact on glucose metabolism and tumor growth, providing valuable insights into potential therapeutic strategies.

Furthermore, this study provides experimental evidence supporting the potential use of vitamin C as an adjuvant therapy for KIRC. Tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs) represent the main treatment strategies for advanced KIRC. However, drug resistance remains a major limitation to their efficacy, partly due to HIF-1–mediated metabolic reprogramming and immune evasion.[44] Our findings suggest that vitamin C may enhance the metabolic state of KIRC cells by inhibiting the HIF-1 signaling pathway, potentially increasing tumor cell sensitivity to TKIs and ICIs. Previous studies have also shown that vitamin C can modulate the tumor immune microenvironment, enhance T cell activity, and reduce immunosuppressive states. Moreover, high-dose vitamin C has demonstrated potential synergy with PD-1/PD-L1 inhibitors in various tumor models.[14] Therefore, future studies should further explore the combination of vitamin C with existing targeted and immunotherapeutic strategies and evaluate its clinical applicability in KIRC patients.

This study has several limitations that should be acknowledged. We observed that the IC50 value of 786-O cells (12.37 mmol/L) was relatively higher compared with that of Umrc6 cells (~2.8 mmol/L). This discrepancy may be explained by the distinct biological backgrounds of the two cell lines. Specifically, 786-O cells are VHL-deficient and are known to undergo profound metabolic reprogramming, which provides greater metabolic flexibility and stress tolerance, thereby requiring a higher concentration to achieve growth inhibition. In contrast, Umrc6 cells displayed a more sensitive phenotype, likely due to differences in drug uptake, efflux capacity, and intracellular redox regulation. Importantly, although the IC50 value in 786-O appears relatively high, it still falls within the range reported for metabolic modulators in vitro. Furthermore, the consistent inhibitory trend observed across both cell lines strengthens the reliability of our findings. More significantly, the cell-line-specific differences themselves are informative, as they reflect the intrinsic heterogeneity of RCC and suggest that genetic and phenotypic backgrounds may critically influence treatment responsiveness.

Moreover, the conclusions of this study are primarily based on in vitro cell experiments. The pharmacokinetic properties, optimal dosage, administration routes, and safety profile of vitamin C in vivo require further investigation to determine its stability and efficacy under physiological conditions. Future studies should incorporate animal models and clinical samples from patients to systematically evaluate the therapeutic potential of vitamin C in KIRC. In addition, the precise molecular mechanisms by which vitamin C inhibits the growth of KIRC remain incompletely understood. The regulation of the HIF-1 signaling pathway is complex and may be influenced by multiple metabolic and signaling networks. It also remains to be determined whether vitamin C indirectly affects KIRC growth via other metabolic pathways (such as mTOR and AMPK) or signaling cascades (such as NF-κB and PI3K/AKT). Future work employing gene-editing or RNA interference techniques to specifically investigate the role of HIF-1α in mediating the anticancer effects of vitamin C will help systematically elucidate its molecular mechanisms. Complementary functional studies and in vivo validation will further substantiate these hypotheses, providing a stronger theoretical foundation for metabolic-targeted therapies in KIRC.

Conclusions

In conclusion, the integration of bioinformatics analysis and experimental validation utilizing existing drug databases not only facilitates the identification of potential beneficial drug groups but also enables the validation of therapeutic targets and associated signaling pathways in cancer. In comparison to prior experimental studies, this integrated approach offers significant time and resource savings while also presenting novel perspectives for investigating drug-disease related action pathways.

Data Sharing Statement

The research data used in this paper can be obtained from the DrugBank (https://www.drugbank.ca) and GEPIA (http://gepia2.cancer-pku.cn/) websites.

Ethics Approval

This study used only publicly available, de-identified data from TCGA and GTEx via GEPIA, which are exempt from additional IRB approval under national legislation (Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects, 2023, China).

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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