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Research Article
Identification of Target Genes Associated with Vitamin C Treatment in Renal Cell Carcinoma

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Abstract

Background and Objective: Renal Cell Carcinoma (RCC) is the most frequent urological tumor in women and the third most prevalent in men after prostate cancer and bladder cancer. Vitamin C, which is sometimes referred to as ascorbic acid (AsA), is one of the most essential water-soluble vitamins in everyday life. The AsA, when administered in large enough amounts, has been shown to have anti-cancer effects. The current study looked at how vitamin C works in RCC by combing through several open-source data sets. **Materials and Methods:** First, using DrugBank 5.1.10, 28 direct protein targets (DPTs) of vitamin C were identified. As a further step, the signaling pathways and protein-protein interaction (PPI) network of vitamin C DPTs was studied. From this, it was found that vitamin C is associated with RCC. After that, cBioPortal was used to categorize mutations in four vitamin C DPTs (SLC2A1, EGLN1, EGLN3 and EGLN2) associated with RCC. Then, 341 genes associated with RCC through the DISEASES database were identified. **Results:** It was determined that RCC in the vitamin C-related KEGG pathway is linked to the *SLC2A1*, *EGLN1*, *EGLN3* and *EGLN2* genes. Finally, five consistent coexpressed genes (*EGLN1*, *EGLN3*, *ALB*, *EGLN2* and *SLC2A1*) as potential therapeutic targets of vitamin C in RCC were found. Significant enrichment in the *EGLN3*, *EGLN1* and *EGLN2* genes was detected by GO analysis of the biological process. **Conclusion:** The molecular mechanism underpinning vitamin C's role in RCC might be better understood with the use of an integrated bioinformatics investigation. It can be said that integrated bioinformatic analysis, can shed light on future studies on the interaction between drugs and diseases.

Key words: Renal cell carcinoma, vitamin C, drugs, gene alterations, bioinformatics, genomic databases, pathways, cancer genomics, gene ontology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Metabolic reprogramming is a hallmark of a number of different cancers, including kidney cancer, which is one of the most researched types of the disease. Several different metabolic pathways, including oxygen and/or iron sensing, the tricarboxylic acid (TCA) cycle, glutamine metabolism and tumor energetics, are controlled by genes that are altered in kidney cancer. Accordingly, kidney cancer is now properly classified as a metabolic disease[1].

The most lethal kind of cancer that may affect the urinary system is called Renal Cell Carcinoma (RCC) and it accounts for around three percent of all cancers that affect adults. The risk of developing RCC is much greater in industrialized nations[2]. The RCC has three primary histological subtypes: Clear cell, papillary and chromophobe, as well as the less common collecting duct, medullary, hereditary leiomyomatosis and RCC-associated subtypes[3]. In addition to smoking, high blood pressure, obesity, being male and having a family history of kidney cancer, papillary renal cell carcinoma (PRCC) also has a distinct connection with the different degrees of renal impairment[4].

Clear-cell RCC accounts for almost all instances of the disease. Recent research has demonstrated that inactivation of the VHL tumor suppressor gene directly affects the pathological process of Clear Cell Renal Cell Carcinomas (ccRCC). Seventy percent of sporadic ccRCC cancers have VHL biallelic inactivation due to mutation, deletion, or hypermethylation of the promoter, resulting in the loss of its expression. An early start of bilateral kidney cancer is associated with a germline mutation in one allele of VHL in individuals with hereditary kidney cancer[5].

There is not yet a reliable biomarker available for the early detection, diagnosis, or prognosis of kidney malignancies at this time. There are a number of inflammatory indicators that are important in determining RCC prognosis, including the modified Glasgow Prognostic Score (mGPS), C-Reactive Protein (CRP) and the ratio of neutrophils, lymphocytes and monocytes. The combination of CRP and albumin, known as the C-reactive protein to albumin ratio (CAR), is a unique inflammation-based prognostic score that has recently demonstrated considerable predictive significance in RCC[6]. Patients with advanced RCC who are undergoing molecularly targeted treatment benefit from the use of the C-Reactive Protein (CRP)/albumin (Alb) and neutrophil lymphocyte ratio (NLR)/albumin (Alb) ratios as meaningful and independent prognostic indicators[7].

Researchers have shown that higher consumption of vitamin C has a protective effect against the development of RCC. Lack of 5-hydroxymethylcytosine (5 hmC) has been connected to the recurrence of RCC tumors, which in turn is linked to common cytosine methylation issues that promote tumor formation. A lack of 5 hmC may lower the likelihood of people with RCC surviving their disease. A group of enzymes called ten-eleven translocation (TET) may change 5-methylcytosine (5 mC) into 5hmC. Insufficient levels of the TET protein's cofactor, vitamin C, have been linked to a reduction in TET enzyme activity. Many people take vitamin C to lower their cancer risk since it is a powerful antioxidant. Additionally, vitamin C can significantly contribute to the antioxidant effect and increase 5 hmC levels[2].

Vitamin C boosts the activity of several enzymes and has a wide variety of other biological effects in almost all bodily tissues. For a long time, vitamin C deficiency was assumed to be a major contributor to cancer development. Vitamin C has a special effect on cancer because it turns on TET and Jumonji dioxygenases, which demethylate DNA and histones. High doses of intravenous vitamin C, prescribed more frequently and for years rather than weeks or months, have increased efficacy and anticancer activity, but even lower doses can reduce chemotherapy side effects and improve quality of life[8].

This study aimed to determine the functions of target genes in RCC by identifying direct protein targets of vitamin C through genomic databases. Investigating of pharmacogenetic effect of vitamin C using databases may help elucidate the molecular pathogenesis of RCC and provide targeted treatment options.

MATERIALS AND METHODS

Study area: The study was conducted between October and November, 2023 using data analysis techniques.

Recognition of direct protein targets (DPTs) of vitamin C: DrugBank[9] is a comprehensive database of drug information, including extensive annotations on drug targets and mechanisms of action. *In silico* drug target discovery, drug design, metabolism and interaction prediction and other related fields rely on DrugBank. A variety of pharmacometric, pharmacometabolomic and pharmacoproteomic datasets are sourced from DrugBank.

DrugBank and other big database annotation efforts used PolySearch2 to facilitate manual pharmacometabolomic literature searches. The DrugBank curation team has carefully verified the pharmacoproteomic binding data in DrugBank.

DrugBank Online now offers 16,428 drug listings, including 2,751 authorized small-molecule medications, 1,605 biologics (proteins, peptides, vaccines and allergenics), 134 nutraceuticals and over 6,722 experimental (discovery-phase) pharmaceuticals. These drug entries are also connected to the sequences of 5,298 unique proteins (drug targets, enzymes, transporters and carriers). Each record has around two hundred data fields, half of which are dedicated to drug or chemical information and the other half to information on drug targets or proteins. DrugBank was queried for information on vitamin C's direct protein targets (DPTs).

Characterization of vitamin C DPT's protein-protein interaction (PPI) network and signaling pathways: An online application known as STRING (Search Application for the Retrieval of Interacting Genes)[10] was developed to analyze the protein-protein interaction (PPI) network. A string web-based tool was used to look at the possible interactions of 28 DPTs associated with vitamin C in RCC. The results showed that the interactions in the network were stronger (p<1.0e-16). The STRING was then used to study the DPT signaling pathways of vitamin C.

Exploration of genomic data for vitamin C DPTs in RCC: Multidimensional cancer genomic data is explored through the cBioPortal (cBio Cancer Genomics Portal)[11]. OncoPrint is a piece of software that allows for the visualization of alterations in gene sequences found in tumor samples. Total changes in four vitamin C DPTs (*SLC2A1*, *EGLN1*, *EGLN3* and *EGLN2*) associated with RCC were investigated using cBio Portal and OncoPrint. A filter was applied based on the frequency of genetic changes in the chosen cancer research. Changes were made in 967 (71.5%) of 1351 patients (1423 total samples).

Gene ontology study of vitamin C's possible therapeutic target genes in RCC: An application based on the R/Bioconductor package called ShinyGO[12] uses a large database of pathways. The GO enrichment results and gene features were visualized graphically with ShinyGO 0.77, which provides application program interface access to KEGG and STRING.

The GO analysis was used to find the functions of possible therapeutic target genes of vitamin C in RCC. *Homo sapiens* was first identified as the organism filter. The biological process GO class was then used as a filter and the most significant function of possible therapeutic target genes for vitamin C in RCC was determined to be the first of the GO terms.

RESULTS

Identification of DPTs of vitamin C: Vitamin C was identified in output DB06772 of DrugBank 5.1.10 as antioxidants, gastrointestinal acidifying agents, hydroxy acids, growth substances, protective agents and urinary acidifying agents. The 28 primary DPTs of vitamin C were identified, consisting of PLOD2, PHYH, PLOD3, BBOX1, DBH, PAM, P3H1, P3H2, P3H3, P4HA1, OGFOD1, OGFOD2, ALKBH2, ALKBH3, KDM5D, PLOD1, TMLHE, P4HTM, EGLN1, EGLN2, EGLN3, TXNRD1, ALB, SLC23A1, SLC23A2, SLC2A1, SLC2A3 and SLC2A4 (Table 1).

Vitamin C DPT protein-protein interaction and signaling pathways: The STRING was used to build the PPI network and signaling pathways of 28 vitamin C DPTs (Fig. 1, Table 2). Lysine degradation, renal cell carcinoma and the HIF-1 signaling pathway made up the top three DPTs, all of which were found in the KEGG pathways. The findings revealed that RCC was the most important form of cancer related to vitamin C (p = 4.63e-08) and 4 DPTs of vitamin C (SLC2A1, EGLN1, EGLN3 and EGLN2) were related to RCC (Fig. 2, Table 2).

Genetic changes in RCC vitamin C DPTs: The genetic alterations of these genes in RCC patients were explored via the cBio portal to determine the expression and function of four vitamin C DPTs (SLC2A1, EGLN1, EGLN3 and EGLN2) that have been related to RCC. Changes in cancer genomes and how SLC2A1, EGLN1, EGLN3 and EGLN2 are expressed in RCC were investigated. The 3 RCC studies include the TCGA Firehose Legacy study (Kidney Renal Papillary Cell Carcinoma), the TCGA Firehose Legacy study (Kidney Renal Clear Cell Carcinoma) and the Genentech study (Renal Non-Clear Cell Carcinoma). The changes in four genes (*SLC2A1*, *EGLN1*, *EGLN3* and *EGLN2*) ranged from 0.68% to 2.76% (Fig. 3).

Search for RCC-expressed genes: Using the DISEASES database, which provides information on connections between diseases and genes, 341 genes (z score: $\geq$4.2) associated with RCC were identified (Table 3). It was discovered that the majority of these 341 genes were enriched in KEGG pathways that were associated with the cell cycle, bladder cancer, melanoma, autoimmune thyroid disease, renal cell carcinoma and EGFR tyrosine kinase inhibitor resistance (Table 4).

Discovery of vitamin C-target genes in RCC: By comparing RCC-associated genes and interconnected genes with 28 vitamin C DPTs in RCC, 5 genes, namely *EGLN1*, *EGLN3*, *ALB*,

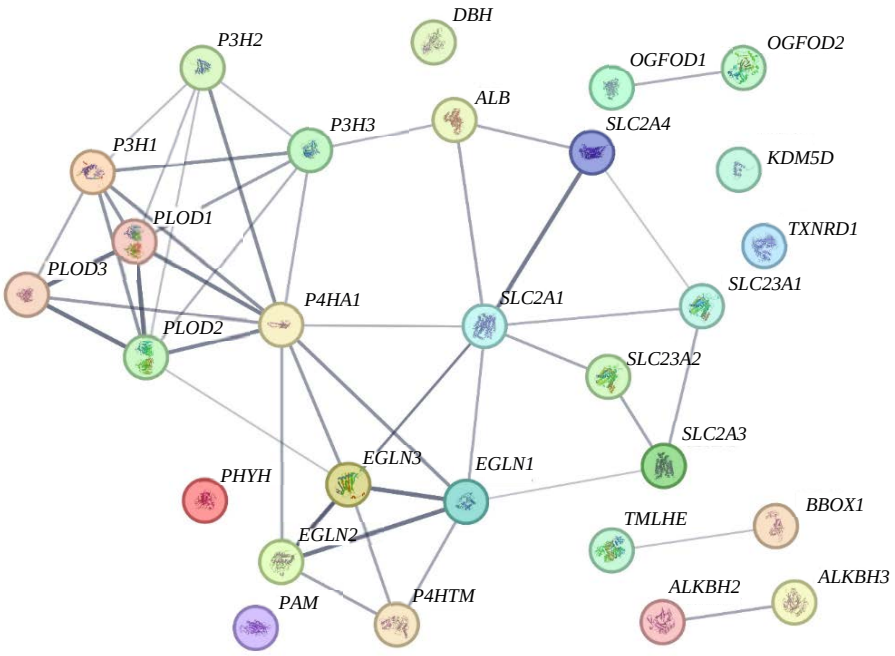

Fig. 1: Target genes for vitamin C and their protein-protein interaction (PPI) network (*EGLN2*, *EGLN3*, *TXNRD1*, *ALB*, *SLC23A1*, *SLC23A2*, *SLC2A1*, *SLC2A3*, *SLC2A4*, *PLOD2*, *PHYH*, *PLOD3*, *BBOX1*, *DBH*, *PAM*, *P3H1*, *P3H2*, *P3H3*, *P4HA1*, *OGFOD1*, *OGFOD2*, *ALKBH2*, *ALKBH3*, *KDM5D*, *PLOD1*, *TMLHE*, *P4HTM* and *EGLN1*)

Table 1: Use of DrugBank for the identification of vitamin C's direct targets

Searched_drug		Target	
DB_ID	Name	Target_symbol	Uniprot_ID
DB00126	Vitamin C	*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
		SLC23A2	Q9UGH3
		SLC2A1	P11166
		SLC2A3	P11169
		SLC2A4	P14672

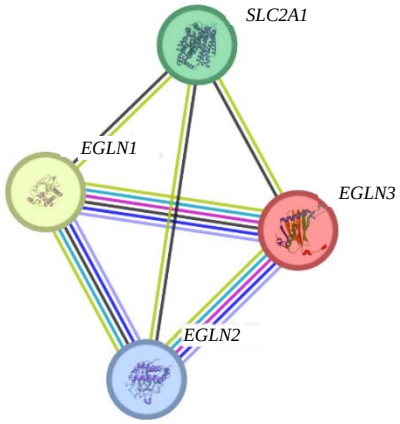

Fig. 2: Association of 4 DPTs of vitamin C (*SLC2A1*, *EGLN1*, *EGLN3* and *EGLN2*) with RCC

Table 2: DPT vitamin C-related KEGG pathways list

Pathway	Count	False discovery rate	Gene
Lysine degradation	5	1.03e-05	*PLOD1*, *PLOD2*, *PLOD3*, *TMLHE* and *BBOX1*
Renal cell carcinoma	4	0.00044	*SLC2A1*, *EGLN1*, *EGLN2* and *EGLN3*
HIF-1 signaling pathway	4	0.0016	*SLC2A1*, *EGLN1*, *EGLN2* and *EGLN3*

Table 3: Detection of RCC-associated genes and z-scores from the DISEASES database

Gene	Z-score
CD274, CTLA4, IL2, PDCD1, CD8A, MTOR, CA9, HIF1A, AKT1, IFNA1, TFE3, EPAS1, PBRM1, TP53, VHL, MTUS1, MTUS2, EGFR, FH, KIT, KDR, CD4, PTEN, ERBB2, IFNG, SETD2, IFNA2, PDCD1LG2, MYC, FLT1, IFNA17, IFNA6, IFNA10, IFNA5, IFNA21, IFNA8, IFNA16, IFNA13, IFNA7, IFNA14, KRT7, IFNA4, CTNNB1, PRCC, BRAF, CDH1, STAT3, LAG3, PAX8, PDGFRB, GAPDH, HAVCR2, FLT3, MET, MME, CCND1, CD28, IL6, RET, BCL2, KRAS, AMACR, ACTB, CD80, FOXP3, FLT4, TNF, FLCN, PDPN, SLC49A4, CSF2, EGF, SDHB, MMP9, CCK, TSC2, IDO1, SRC, CD86, BAP1, AXL, CDKN2A, IL10, PXDN, ALK, PXDNL, SNAI1, TFEB, EIF5B, TNFRSF9, PDGFRA, ALB, EZH2, CD276, CASP3, ASPSCR1, ANXA5, CDH2, KDM5C, PIK3CA, SLC2A1, CXCR4, ESR1, ELOC, CD44, TIGIT, TGFB1, FN1, SLC25A16, MMP2, CD34, RPS6KB1, ZEB1, SDHC, CXCL8, SDHD, PECAM1, IL15, GZMB, CSF1R, ARNT, FGF2, SMARCB1, HGF, TNFRSF4, NCAM1, CDK4, CXCL12, PAX2, MAPK3, MITF, IL1B, BRCA1, H3-3B, IDH1, H3C12, PARP1, MDM2, H3C13, H3-4, H3-5, H3-2, IL4, FOLH1, CD40, ELOB, JUN, IGF1R, LDHA, MALAT1, TPX2, SNAI2, KLRK1, SDHA, WT1, EIF4EBP1, ITGAM, PRF1, CUL2, CEACAM5, AR, ARG1, MMUT, PMEL, CD19, RAF1, BCL2L1, ARID1A, KRT20, MUC1, EPCAM, NFKB1, ABL1, JAK2, PTPRC, RB1, FASLG, FCGR3A, FGFR1, TEK, CDH16, NTRK1, STAT1, CD70, ZEB2, CCL2, CTAG1B, VTCN1, GSK3B, TGFA, TERT, VEGFC, FKBP1A, METTL3, CXCL10, ATM, CD247, RPTOR, CDKN1A, DNMT1, FCGR3B, TCHP, PROM1, GATA3, BTLA, ICOS, CDK2, NRAS, EGLN1, NOTCH1, PKM, CRP, EGLN3, HSP90AA1, CXCL9, CDK1, E2F1, MCL1, HOTAIR, CD68, TNFRSF18, KLK3, IDO2, HSP90AB1, CD27, IDH2, STK11, PGF, CXCR2, PGR, CXCR3, CDK6, DDX53, FOXM1, POU5F1, CCL5, CSF1, SOX2, IGF1, CASP8, PTK2, TSC1, RICTOR, SMAD4, ICAM1, CD163, CASP9, FGF4, INS, FGF5, EPO, FGF3, SMARCA4, MAGEA3, BRCA2, FGF7, FGF9, FGF18, FGF17, EP300, CCR7, PTGS2, METTL14, CD33, FGF6, ARG2, TWIST1, FOXO3, FGF20, FGF22, IL1A, FGF8, EIF4E, FGF16, IL7, FGF10, NONO, NANOG, HRAS, ABCB1, NF2, AGO2, SMAD3, MSLN, ERBB3, RHOA, SDHAF2, KDM6A, KEAP1, RBX1, ABCG2, IL17A, BECN1, MAP2K1, CCNB1, HIF3A, PVT1, LGALS9, NF1, JAK1, AFP, EGLN2, SMAD2, DICER1, STAT5B, ANGPT2, B2M, IFNB1, CDH17, KRT19, SYP, RASSF1, BCL2L11, YAP1, SPDL1, ENTPD1, CYCS, VEGFD, STAT5A, FOXO1, MLH1, RHEB, CD40LG, IL18, SIRT1, FUT4, CD47, CAV1, ENG, IGF2, NT5E, SLC16A3, TGFBR2, TPBG, TNFSF10, PTPN11, THBS1, LOX, CCNA2, CDKN1B and *WTAP*	7.1- 4.2

Table 4: KEGG pathways involved in the DPT of vitamin C in the PPI

Pathway	Count	False discovery rate	Gene
Bladder cancer	26	2.49e-28	*RB1, E2F1, RAF1, RASSF1, MDM2, MYC, MMP2, CXCL8, THBS1, CDKN1A, ERBB2, KRAS, CCND1, CDH1, MMP9, THBS1, TP53, CDKN2A, HRAS, MAP2K1, NRAS, CDK4, EGFR, EGF, SRC* and *MAPK3*
Melanoma	44	2.71e-46	*MITF, MAP2K1, BRAF, CDKN1A, RB1, RAF1, CDK4, CDK6, E2F1, CDKN2A, PTEN, MDM2, CCND1, AKT1, TP53, EGFR, MAPK3, IGF1R, NRAS, HGF, IGF1, PIK3CA, HRAS, KRAS, EGF, CDH1, FGF2, FGF7, PDGFRB, FGF6, FGF18, PDGFRA, MET, FGFR1, FGF4, FGF5, FGF3, FGF20, FGF9, FGF22, FGF10, FGF17, FGF8* and *FGF16*
Autoimmune thyroid disease	25	1.74e-25	*FASLG, CD40, CD86, GZMB, CD28, CD80, CD40LG, PRF1, IL4, CTLA4, IL2, IFNA1, IL10, IFNA2, IFNA17, IFNA7, IFNA6, IFNA4, IFNA21, IFNA16, IFNA10, IFNA13, IFNA14, IFNA8* and *IFNA5*
Renal cell carcinoma	33	4.45e-33	*FLCN, TFE3, PRCC, MET, FH, SLC2A1, TGFB1, TGFA, HGF, EGLN2, ARNT, PTPN11, NRAS, HRAS, KRAS, AKT1, HIF1A, EPAS1, VHL, EGLN3, ELOC, EGLN1, EP300, JUN, MAPK3, PIK3CA, BRAF, RAF1, MAP2K1, CDKN1A, RBX1, ELOB* and *CUL2*
EGFR tyrosine kinase inhibitor resistance	38	1.32e-37	*AXL, PDGFRA, JAK1, PDGFRB, MET, KDR, TGFA, ERBB3, HGF, ERBB2, FGF2, NF1, HRAS, NRAS, MAP2K1, EGFR, EGF, JAK2, RAF1, KRAS, PTEN, SRC, MTOR, PIK3CA, STAT3, IL6, IGF1, BCL2, BCL2L1, FOXO3, EIF4EBP1, GSK3B, RPS6KB1, MAPK3, BCL2L11, BRAF, RAF1* and *AKT1*

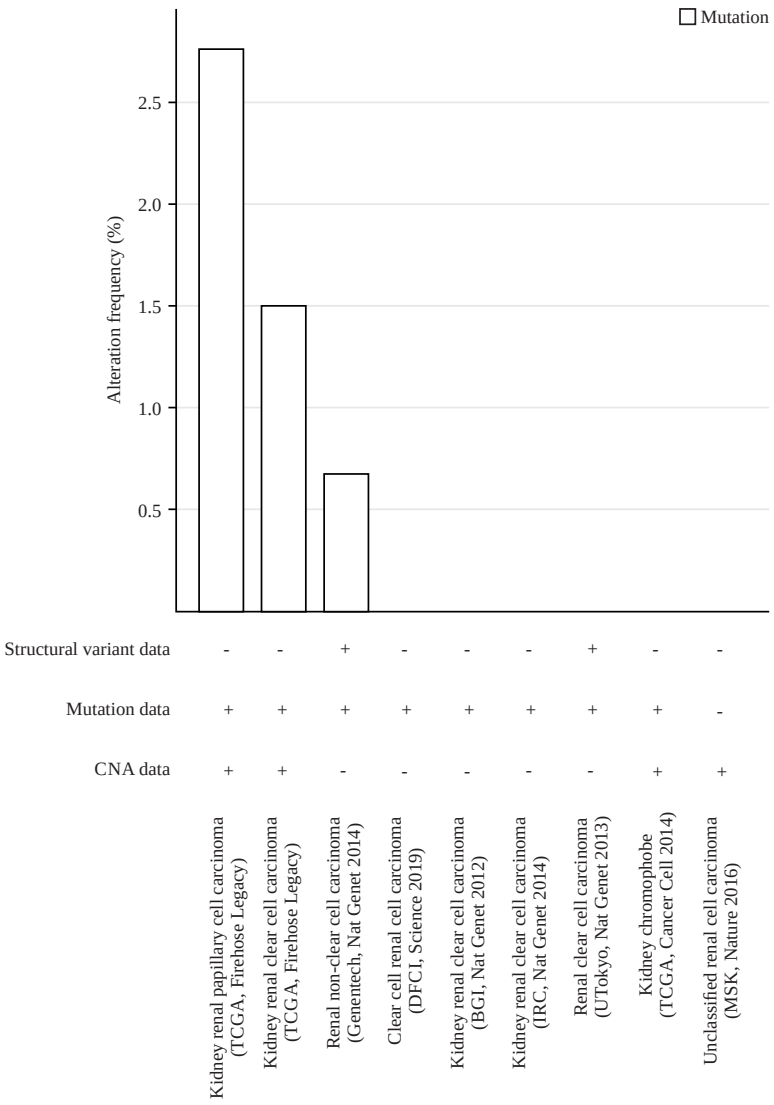

Fig. 3: Vitamin C-related gene *SLC2A1*, *EGLN1*, *EGLN3* and *EGLN2* genetic changes in RCC research are included in the cBio cancer genomics portal and summary of genomic datasets from nine RCC research projects on gene alterations (*SLC2A1*, *EGLN1*, *EGLN3* and *EGLN2*)

EGLN2 and SLC2A1, were found to be possible therapeutic targets of vitamin C in RCC (Fig. 5 and Table 5). These genes may function as cofactors, binders and substrates of vitamin C.

Vitamin C therapeutic target gene GO enrichment study in RCC: With enrichment analysis, the *EGLN1*, *EGLN3*, *ALB*, *EGLN2* and *SLC2A1* genes were associated with the Gene Ontology (GO) biological process from functional categories through the ShinyGO 0.77 database. Hypergeometric test nominal p-values are used to compute the false discovery rate (FDR). Significant enrichment of genes was found by GO analysis of the biological process (Table 6). The FDR cut off is taken as 0.05. In terms of biological processes, the most important of these is "peptidyl-proline hydroxylation to 4-hydroxy-L-proline" (FDR: 0.0000), which includes genes such as EGLN3, EGLN1 and EGLN2.

DISCUSSION

To further elucidate the molecular effect of vitamin C and its target proteins in RCC, comprehensive bioinformatics analysis was used in the current study. However, the following procedures were used in the vitamin C analysis: (I) Using DrugBank 5.1.10, identify vitamin C's major DPT, (ii) Apply STRING to analyze the vitamin C DPTs' protein-protein

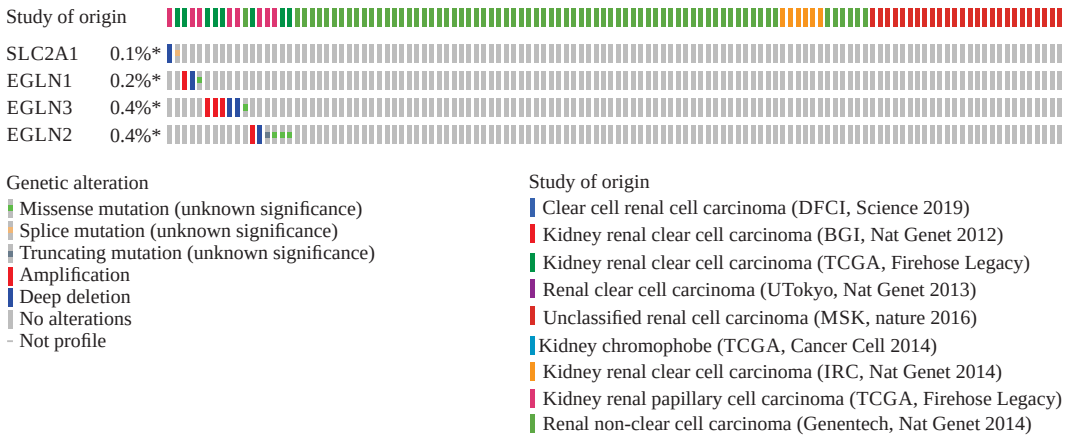

Fig. 4: OncoPrint: Change summarization diagram for a group of RCC samples using a query of 4 genes (*SLC2A1*, *EGLN1*, *EGLN3*, and *EGLN2*)

Different changes in the genome, such as mutations and amplifications, deep deletions, and structural variants, are summed up and shown with color-coding based on the percentage change in genes that were affected in each tumor sample and a gene is shown in each row, and a tumor sample is shown in each column

Table 5: Comparison of two gene expression datasets for the detection of co-expressed genes

Features	Total	Target genes
Co-express genes of X and Y	5	*EGLN1, EGLN3, ALB, EGLN2* and *SLC2A1*
X: PPI network of genes for vitamin C's 28 DPTs	23	*P4HA1, BBOX1, PAM, SLC23A1, P4HTM SLC23A2, ALKBH2, P3H1, KDM5D, P3H2 SLC2A3, TMLHE, ALKBH3, OGFOD2, PHYH TXNRD1, PLOD3, PLOD2, DBH, P3H3 SLC2A4, OGFOD1* and *PLOD1*
Y: Expressed genes in RCC	336	*IL17A, ACTB, MMP2, CD44, RPTOR, IFNA4, ITGAM, KRT19, FH, MTOR, TNFRSF18, CD34, CXCL10, SDHC, PDPN, MTUS2, ENG, FASLG, H3C12, WT1, CXCR4, SNAI1, ARG2, IGF1, CD163, TNFRSF4, IFNA14, KDR, IDO1, CCL5, NTRK1, TP53, ABCG2, CDK6, PTEN, PDGFRB, CYCS, TPX2, EP300, MITF, ENTPD1, SETD2, INS, STK11, TNF, FLT1, TGFBR2, BTLA, RAF1, CCNB1, TIGIT, ZEB1, LOX, MSLN, KLRK1, EIF4E, HOTAIR, PECAM1, SMAD2, IFNA1, EGF, CTAG1B, IL1A, CASP9, TCHP, PDCD1, FGF3, CD4, FGFR1, EIF5B, MET, AGO2, AXL, CDKN1A, SDHB, IFNA7, CD276, METTL14, VEGFC, GATA3, PMEL, BRCA2, CUL2, PKM, TFE3, NANOG, JAK1, NONO, FLCN, CD33, KIT, BCL2L11, BRCA1, LDHA, CD27, AKT1, JAK2, IFNA17, IL10, PDGFRA, H3C13, STAT3, CD8A, H3-5, IGF2, PGF, TEK, CD80, CD47, TSC1, VEGFD, DNMT1, CA9, SPDL1, ASPSCR1, IDH1, PTPN11, SMAD3, KDM5C, PTGS2, POU5F1, SDHD, B2M, ARG1, NF1, FOXP3, TFEB, YAP1, CDH16, FGF4, MALAT1, MDM2, DDX53, FGF7, CTNNB1, MAP2K1, EGFR, ICOS, NFKB1, SIRT1, DICER1, IFNA6, FCGR3A, H3-3B, SDHAF2, IL1B, TNFRSF9, HIF3A, NT5E, FGF17, IL18, IFNA2, FOLH1, CXCR3, CCND1, FOXM1, PIK3CA, SLC49A4, FLT3, ALK, PDCD1LG2, EZH2, RBX1, CTLA4, FGF9, IL15, IL2, ERBB3, ATM, ESR1, ERBB2, ARNT, PRF1, CEACAM5, CDKN1B, CDK4, CDK1, CDH1, PTK2, PXDNL, IFNG, IFNA5, SLC25A16, BECN1, CSF1, TNFSF10, SMARCB1, IL4, CD28, SMARCA4, MAGEA3, MUC1, H3-4, EPAS1, BCL2, MLH1, KRT20, HRAS, FGF2, ELOB, STAT5B, FOXO3, PXDN, CXCL12, RHEB, CCK, IFNB1, GAPDH, IFNA10, IDH2, SMAD4, PVT1, CDKN2A, TGFB1, MME, AMACR, IGF1R, PAX2, CXCR2, METTL3, RICTOR, IFNA13, ICAM1, CXCL9, MYC, IDO2, THBS1, CAV1, H3-2, CSF1R, JUN, BCL2L1, FGF18, CD40, CD19, LGALS9, ABL1, E2F1, KEAP1, MAPK3, NF2, CCNA2, FGF8, GZMB, FCGR3B, SLC16A3, CASP8, TSC2, FGF6, AR, SNAI2, MTUS1, FGF22, CDH2, HAVCR2, KDM6A, NCAM1, FLT4, FGF20, HSP90AB1, LAG3, PRCC, KRAS, MMUT, CD86, BRAF, TWIST1, EPO, EIF4EBP1, NOTCH1, CD274, GSK3B, FUT4, VTCN1, KLK3, FOXO1, FKBP1A, RHOA, CRP, MCL1, CCL2, TERT, PAX8, CD247, HIF1A, CD40LG, HGF, IFNA21, STAT1, RASSF1, TPBG, RET, TGFA, ARID1A, IL6, PROM1, IFNA16, IL7, ANXA5, FGF5, RB1, SOX2, STAT5A, SDHA, NRAS, CXCL8, VHL, BAP1, CCR7, CD68, CD70, EPCAM, ZEB2, AFP, SRC, WTAP, CDK2, IFNA8, FN1, ABCB1, PBRM1, PGR, CASP3, HSP90AA1, FGF10, KRT7, ANGPT2, ELOC, CSF2, FGF16, RPS6KB1, SYP, MMP9, PARP1, PTPRC* and *CDH17*

Table 6: ShinyGO 0.77 Gene Ontology enrichment analysis (biological process)

Enrichment FDR	Genes	GO terms	Pathways
0.0000	*EGLN3, EGLN1* and *EGLN2*	GO:0018401	Peptidyl-proline hydroxylation to 4-hydroxy-L-proline
0.0012	*EGLN3, EGLN1, SLC2A1* and *EGLN2*	GO:0001666	Response to hypoxia
0.0012	*EGLN3, EGLN1* and *EGLN2*	GO:0061418	Regulation of transcription from RNA polymerase II promoter in response to hypoxia
0.0063	*EGLN3, ALB, EGLN1, SLC2A1* and *EGLN2*	GO:0033554	Cellular response to stress
0.0362	*EGLN3, EGLN1* and *EGLN2*	GO:1901214	Regulation of neuron death

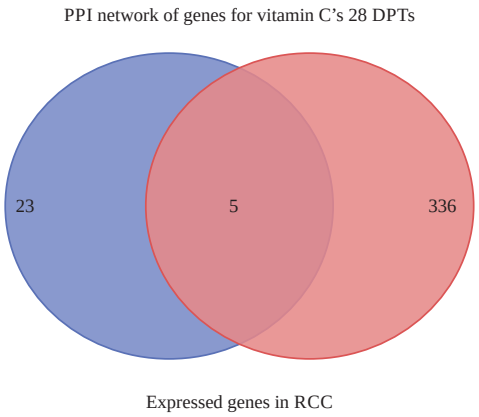

Fig. 5: Five genes found in RCC and vitamin C target genes

interaction (PPI) network and signaling pathways, (iii) Use the cBio portal to identify genetic variants and put them to the test, (iv) Search the DISEASES database for genes linked to RCC, (v) Compare two gene sets to find co-expressed genes and (vi) Conduct a GO enrichment study of biological processes involving vitamin C therapeutic target genes for RCC. In the first two steps, 28 primary vitamin C DPTs (PLOD2, PHYH, PLOD3, BBOX1, DBH, PAM, P3H1, P3H2, P3H3, P4HA1, OGFOD1, OGFOD2, ALKBH2, ALKBH3, KDM5D, PLOD1, TMLHE, P4HTM, EGLN1, EGLN2, EGLN3, TXNRD1, ALB, SLC23A1, SLC23A2, SLC2A1, SLC2A3 and SLC2A4) were found (Table 1). Of these, 4 (SLC2A1, EGLN1, EGLN3 and EGLN2) were linked to RCC. In the third stage, the genetic alteration in SLC2A1 in RCC samples was found to be deep deletion and splice mutations; the genetic change in EGLN1 was missense mutations, amplifications and deep deletion; the genetic change in *EGLN3* was amplification, deep deletion and missense mutations and the genetic change in EGLN2 was amplification and deep deletion (Fig. 4).

Finally, the genes (*EGLN1*, *EGLN3*, *ALB*, *EGLN2* and *SLC2A1*) co-expressed in interconnected genes with four vitamin C target genes in RCC samples as potential therapeutic targets of vitamin C in RCC. These five genes (*EGLN1*, *EGLN3*, *ALB*, *EGLN2* and *SLC2A1*) have been found to play a significant role in renal cell carcinomas, which was consistent with current results.

The term "renal cell carcinoma" really refers to a collection of cancers rather than a singular illness. Each subtype has discrete histologic hallmarks, which are accompanied by a unique genetic profile, varied clinical manifestations and individualized responses to treatment[13]. The short arm of chromosome 3p is related to structural abnormalities in both hereditary and nonhereditary (sporadic) forms of renal cancer. Four genetic disorders related to RCC include Von Hippel-Lindau (VHL), hereditary papillary renal carcinoma, familial renal oncocytoma associated with Birt-Hogg-Dube syndrome and hereditary renal carcinoma[14].

The prevalence of PRCC among RCC cases is second only to that of clear-cell RCC. A better prognosis is expected for those diagnosed with PRCC rather than CCRCC. The PRCC is classified into two subgroups, types 1 and 2, distinguished by distinct histological and genetic characteristics. Type 2 PRCC is a more advanced tumor stage with a worse survival rate than type 1. This means that effective biomarkers for early diagnosis and prognosis prediction are needed. The CRP is an acute-phase protein that has been generally established to be a valuable biomarker for clinical prediction of RCC. Tissue development associated with tumors may cause inflammation, which then results in the production of CRP. Several studies have identified a unique biomarker-CRP/Albumin Ratio (CRP/Alb)-in RCC that deserves additional study[15]. Recent years have seen a surge in interest in the role antioxidants play in the primary prevention of cancer. Vitamin C is one of the most well-known antioxidants because of its vital function in cellular metabolism. Results from a meta-analysis of studies reveal that vitamin C use may decrease the risk of RCC[16]. The catalytic activity of TET enzymes is regulated by vitamin C, as shown in recent research and this is how somatic cells may be reprogrammed. Similarly, vitamin C controls TET1 activity and keeps ES cells in a blastocyst-like condition. As a result, vitamin C may have an anti-tumor impact in its capacity as an epigenetic reagent[17].

Most non-metastatic RCC patients may be treated with surgery, but 40% relapse quickly, raising the topic of extra perioperative therapy. Multiple studies have evaluated adding systemic treatment after surgery in locally advanced RCC to meet this clinical requirement. Due to the high adverse event profiles of adjuvant antiangiogenic tyrosine kinase inhibitors,

inhibitors, have been developed. Adjuvant treatment regimens that enhance outcomes and raise cure rates for patients with non-metastatic RCC will need more exploration into combination therapies with immunotherapy, neoadjuvant methods and patient selection[18]. Understanding the pathophysiology of clear-cell RCC was greatly aided by the discovery of mutations in the Von Hippel-Lindau (VHL) gene and the involvement of increased angiogenesis. In addition, the PI3K-Akt-mTOR pathway's deregulation in RCC has provided new insights into the disease's molecular biology. As expected, addressing angiogenesis and mTOR pathways with particular medicines has improved clinical results. Vascular Endothelial Growth Factor Receptor (VEGFR) Tyrosine Kinase Inhibitors (TKIs) such as sunitinib, pazopanib, axitinib and sorafenib; anti-VEGF monoclonal antibody (mAb) bevacizumab and mTOR inhibitors temsirolimus and everolimus are all examples of such drugs[19].

The *EglN* family members (*EglN1*, *EglN2* and *EglN3*) may hydroxylate HIF-α *in vitro*; however, EglN1 is the predominant hydroxylase in most cell types under normal circumstances[20]. Two paralogs of *EglN*, *EglN2* (PHD1) and *EglN3* (PHD3), are gaining attention as possible cancer targets due to their HIF-independent involvement in cell proliferation and death[21]. It has been shown that somatic pairing is connected to significant alterations in gene expression within paired areas. Oxygen triggers the release of prolyl-hydroxylase *ELGN2*, an enzyme with central control over HIF degradation. The *ELGN2* overexpression in renal oncocytoma boosted ubiquitin-mediated HIF degradation and decreased the expression of multiple HIF target genes, including the death-promoting BNIP3L gene[22]. The EGLN3 and BNIP3 have been identified to significantly interact with the treatment arm and may be predictive of response to treatment[23].

The EGLN1 is the main hypoxia/oxygen sensor for HIF, but all three isoforms have enzyme properties that match their sensing roles, with Km values for O_2 that are higher than what cells and tissues should have. The *EGLN2* and *EGLN3* participate in HIF regulation in certain settings[24]. The ccRCC is one of the most common types of kidney cancer. Identifying genes that direct cellular changes and cell transformation in the pathogenesis of ccRCC is a complex process. Microarray datasets of ccRCC to integrated bioinformatics analysis and identified key genes and molecular pathways involved in ccRCC progression. By detecting the gene expression levels of hub genes in all 12 datasets in the GEO database, SLC2A1 was found to be upregulated[25]. Compared to normal tissues, malignant tissues in RCC samples showed substantially increased expression of SLC2A1 mRNA[26].

Tamukong *et al.*[23] discovered that the CRP/Alb ratio has a predictive effect on overall survival (OS) in patients with clear cell renal cancer, though further research is still necessary to determine its prognostic value in RCC. Researchers looked back at past data on patients with RCC to see how useful the CRP/Alb ratio was for predicting outcomes and how it related to clinical outcomes. Before surgery, the CRP/Alb ratio was found to be a reliable indicator of how long patients with RCC would live after a radical or partial nephrectomy. Clinicians may also utilize the preoperative CRP/Alb ratio to predict the disease-free survival of localized RCC patients following curative therapy and select high-risk patients for closer follow-up[27]. Recent research suggests that pretreatment serum albumin may be strongly correlated with the prognosis of cancer patients, particularly RCC patients. Low pretreatment blood albumin levels are associated with a poor prognosis in renal cell carcinoma patients and may be evaluated for risk classification and tailored therapy[28]. Evidence from the ALB gene suggests that individuals with metastatic RCC who have low blood albumin levels have a shorter progression-free survival. The ALB has been found to have a potential inhibitory impact on the progression of ccRCC[29].

Data obtained from the Human Protein Atlas show that EGLN1 plays a role as a prognostic marker in cervical cancer, EGLN3 in liver and renal cancers, ALB in stomach cancer, EGLN2 in cervical and pancreatic cancers and *SLC2A1* in renal, liver, pancreatic, lung and urothelial cancers. These five genes (EGLN1, EGLN3, ALB, EGLN2 and SLC2A1) have been linked to the GO Enrichment Analysis biological process. They are all involved in how cells react to stress.

Therefore, these 5 genes (*EGLN1*, *EGLN3*, *ALB*, *EGLN2* and *SLC2A1*) are involved in the development of RCC. A combination of bioinformatics tools was used to look at these genes as possible vitamin C therapy targets in RCC. It can be said that there is an inverse relationship between tissue ascorbate and markers of HIF pathway activation in cells with RCC. The results obtained may be helpful in elucidating the genetic mechanism of vitamin C in RCC. Determining drug-target interactions is very important in elucidating the molecular genetics of RCC. It is possible to obtain insight into the role that vitamin C plays in the prevention and treatment of illnesses by using a variety of computational models to find distinct genes that are related to vitamin C. It may provide opportunities for future experimental studies to determine the possible effects of drugs on diseases using integrated bioinformatics analysis methods. Thus, these methods can enable the identification of mechanisms for diseases associated with drug-related genes.

CONCLUSION

According to this, integrated bioinformatics analyses are used to identify genetic alterations in RCC due to vitamin C use. In line with the data obtained, it can be said that the *EGLN1*, *EGLN3*, *ALB*, *EGLN2* and *SLC2A1* genes may be effective as therapeutic targets in elucidating the pathogenesis of RCC and demonstrating the clinical functional relationship on drug metabolism. Moreover, pharmacogenetic studies contribute to the advancement of personalized medicine, with vitamin C available for the prevention of RCC and as an additional treatment option.

SIGNIFICANCE STATEMENT

The current study aims to investigate the anti-cancer effects of vitamin C by identifying potential therapeutic target genes for vitamin C associated with RCC using open-source data sets. The study showed that RCC is the most important type of cancer associated with vitamin C and that the *SLC2A1*, *EGLN1*, *EGLN3* and *EGLN2* genes, which are direct protein targets of vitamin C, are associated with RCC. As a result of the comparison of two gene expression datasets, it was concluded that the *EGLN1*, *EGLN3*, *ALB*, *EGLN2* and *SLC2A1* genes, which are possible therapeutic targets of vitamin C in RCC, may be cofactors, binders and substrates of vitamin C. These data may provide opportunities to elucidate the molecular mechanisms of various cancer types associated with drug-related genes, especially RCC.

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