

# The clinical significance of *UBE2C* gene in progression of renal cell carcinoma

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## ABSTRACT

Renal cell carcinoma (RCC), with high morbidity and mortality, is one of the top ten serious cancers. Due to limited therapies and little knowledge about the mechanism underlying RCC, overall survival of RCC patients is poor. *UBE2C* is a member of ubiquitin modification system and promotes carcinogenesis in cancer, but its role in RCC is unknown. Based on the TCGA (The Cancer Genome Atlas) data, *UBE2C* was over-expressed in a total of 525 RCC tissues and displayed higher expression in advanced tissues (stage IV vs stage I,  $p < 0.05$ ). RT-qPCR and IHC analysis confirmed over-expression of *UBE2C* in 90 of clinical RCC tissues. Further, *UBE2C* was associated with clinical factors including TNM stage, gender, and pathological stage. And higher *UBE2C* expression predicted shorter overall survival and progression-free survival. Both univariate and multivariate COX analysis suggested *UBE2C* as a critical gene in RCC. Then GO and KEGG analysis showed that cell cycle and DNA replication pathways were two top signaling pathways affected by *UBE2C*. *In vitro* assay showed that knockdown of *UBE2C* in 786-O cells inhibited proliferation and migration significantly. Therefore, this study proves that *UBE2C* is an important gene in RCC and is essential to proliferation and migration of RCC.

**Key words:** *UBE2C*; GO analysis; KEGG analysis; renal cell carcinoma.

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**Contributions:** LW; study design, draft review; ZC, data acquisition and analysis, manuscript drafting. Both authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

**Funding:** This research was supported by Natural Science Foundation of Jiangxi Province (No.20192BAB205075 and No.20202BABL206119) and Health Commission of Jiangxi Province (No.20204518).

**Conflict of interest:** The authors declare that they have no competing interests, and all authors confirm accuracy.

**Availability of data and materials:** The data used to support the findings of this study are available from the corresponding author upon request.

**Ethical Approval:** Not applicable.

## Introduction

Renal cell carcinoma (RCC) is a malignant disease and originates from renal parenchyma urinary tubule epithelial system.<sup>1</sup> Among them, clear cell carcinomas the major type and accounts for about 70% of all RCC patients.<sup>1-3</sup> The main therapy for RCC is resection assisted with chemo-radiotherapy. But RCC is not so sensitive to radiotherapy or chemotherapy.<sup>3</sup> In recent years, target therapy is used to treat RCC and about 20-40% of RCC patients could benefit from target therapy.<sup>4,5</sup> However, long-term survival is not easily acquired.<sup>6</sup> According to the latest report released by AACR in 2019, the five-year survival rate for patients with distant metastasis is only 12%.<sup>7</sup> And about 16% of patients are diagnosed with distant metastasis. In addition, a total of 73,820 persons will be subjected to kidney cancer and about 14,000 will die.<sup>7</sup> This is partially due to the ignorance of mechanism underlying RCC.

Ubiquitin-conjugating enzyme E2C (UBE2C) is a member of the ubiquitin coupling enzyme E2 family and interacts with specific E3 enzymes, which leads to degradation of substrate proteins.<sup>8</sup> The substrate proteins of UBE2C are cell cycle-related proteins involved in regulation of cell mitosis. Recent studies indicated that *UBE2C* played an oncogenic role in several types of cancer. For example, the abnormal expression of *UBE2C* caused chromosomal instability and participated in development and progression in gastric cancer.<sup>9</sup> *UBE2C* is expressed abundantly in breast cancer,<sup>10</sup> colon cancer,<sup>11</sup> ovarian cancer,<sup>12</sup> and liver cancer.<sup>11</sup> And *UBE2C* was shown to be closely correlated with tumor stage, which suggests that UBE2C might be a new tumor marker and a promising therapeutic target in these tumors.<sup>10-13</sup> However, the expression of *UBE2C* in RCC and its clinical value remain unclear. The cancer genome atlas (TCGA) database is a public database consisted of cancer genomics from over 20,000 cases of primary cancer and matched normal samples spanning 33 cancer types.<sup>14</sup> The database contributes greatly to the research on elucidation of molecular mechanism underlying tumorigenesis.<sup>15</sup>

In order to evaluate the clinical significance of *UBE2C* in RCC, we extracted the clinical information of RCC patients and *UBE2C* expression profile in RCC tissues from TCGA database. Then the relationship of *UBE2C* with pathological factors and prognoses of RCC patients was analyzed. And the potential process as well as signaling pathways were explored.

## Materials and Methods

### Bioinformatics analysis and TCGA mining

In order to analyze the differential expression levels of *UBE2C* in RCC and normal renal tissues, TCGA database was extracted. The mRNA expression level of *UBE2C* from a total of 525 cases of RCC and 72 cases of normal renal tissues was downloaded from the TCGA database (<https://cancergenome.nih.gov/>). Then, RStudio (Version 1.1.442), a free and open-source data analysis software, was used to analyze the differential expression of *UBE2C* and to deduce the overall survival (OS) and disease-free survival (DFS) curves. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used in conjunction with DAVID 6.8 and Enrichment Map plug-in Cytoscape to visualize the significant pathways and differential expression genes (DEGs) in RCC.

### RCC tissues and pathological information of patients

A total of 90 RCC tissues and matched normal tissues were retrospectively collected to perform RT-qPCR analysis. All the patients had received radical operation in our hospital from July 2006 to December

2010. The last follow-up time was August 2017. The pathologic diagnosis of all cases was renal clear cell carcinoma after operation. This study was approved by the Ethics Committee of First affiliated Hospital of Gannan Medical University and written consent was collected from all patients.

### RT-qPCR

Total RNA of renal cancer tissues and matched normal tissues was extracted with TRIzol (Invitrogen, USA) and treated with RNase-free DNase (Promega, USA). SYBR Green quantitative real-time PCR was performed by Stepone Real-time PCR system (Applied Biosystems 7000, USA). Beta-actin was used as the internal control. Relative expression level of each gene was calculated by the  $2^{-\Delta\Delta Ct}$  method, in which  $\Delta\Delta Ct = \text{Test group } \Delta Ct (CT_{\text{interested gene}} - CT_{\text{Beta-actin}}) - \text{Control group } \Delta Ct (CT_{\text{interested gene}} - CT_{\text{Beta-actin}})$ .

### Immunohistochemical analysis

Human tumor tissues were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned as 5  $\mu\text{m}$ . Each sectioned tissues were incubated with antibody against UBE2C (1:500, ab252940, Abcam, NY, USA) diluted with 0.01 M PBST buffer (0.01 M PBS, 0.001 $\times$ Triton X-100) for 12 h at 44°C followed by HRP-conjugated secondary antibody (1:100, Beyotime, Shanghai, China). Peroxidase conjugates were determined using 3,3'-diaminobenzidine solution.

### RNA interference assay (RNAi)

siRNA fragments targeting human *UBE2C* gene (siUBE2C: 5'-TCCTTTTGTGATTTCTGTATAG-3') was designed and synthesized. Random sequence was designed as negative control (NC). Then RCC typical cells 786-O cells were cultured in a 6-well plate and transfected with siUBE2C or NC (General Biosystems, Anhui, China) by Lipo reagent (Yeasen, Shanghai, China) for 4-6 h according to the manufacture's instruction. The knockdown efficiency of siUBE2C was tested by RT-qPCR method.

### Cell proliferation assay

Treated cells were seeded into a 96-well plate at  $5 \times 10^3$  cell/well and cultured consecutively for 96 h. At each designed time point, 10  $\mu\text{L}$  of CCK-8 agent was added and cultured for another 1-2 h. Then OD value at 450 nm was detected on microplate reader (Molecular Devices, CA, USA). The proliferation rate of cells was calculated according to the negative control.

### Cell invasion assay

Transwell rooms with 8- $\mu\text{m}$ -pore size membrane were pre-treated with 0.2 mL Matrigel 12 h before seeding tumor cells. Then a total of  $2 \times 10^4$  cells/well was seeded into upper room and DMEM medium without FBS was added. DMEM medium containing 15% FBS was added in the lower room. After culture for 24 h, cells on the upper room were removed and cells in the lower surface of upper room were fixed by 4% paraformaldehyde for 30 min and dyed with 0.5% crystal violet for 10 min. Then stained cells were observed under a microscope and counted.

### Statistical analysis

SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) was used to analyze the experimental data. All data was expressed as mean  $\pm$  SD. TCGA data were analyzed by the independent samples *t*-test to compare the differential levels of *UBE2C* mRNA between RCC and control. Chi-square test or Fisher's exact test was employed to analyze the relationship between *UBE2C* expression and clinicopathological factors of RCC patients. Kaplan-Meier analysis was used to produce the survival curve. Univariate and Cox multivariate survival analyses were performed to analyze the prognostic significance of *UBE2C* in RCC patients. The difference was considered statistically significant when the *p*-value was less than 0.05.

## Results

### *UBE2C* was highly expressed in RCC

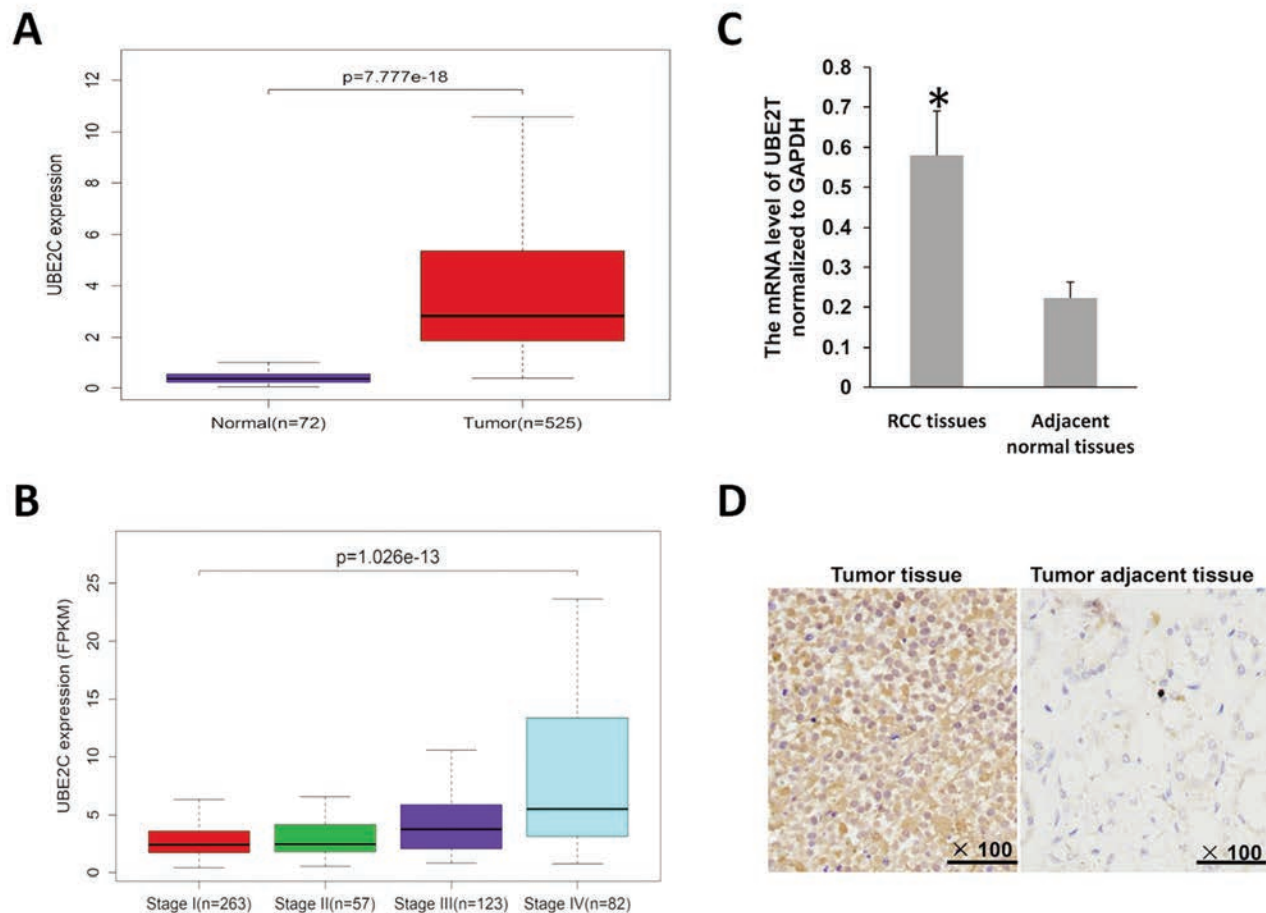
To explore the role of *UBE2C* in RCC, a total of 525 RCC cases and 72 normal cases from TCGA database (<https://cancergenome.nih.gov/>) were extracted for further analysis. The clinical information of the 525 RCC cases was displayed in Table 1. In brief, 187 cases were diagnosed as T3/T4 stage, accounted for 35.62% of total patients. About 205 cases were in stage III/IV, accounted for 39.05%. And 157 cases were dead, accounted for 29.9%. The mRNA level of *UBE2C* was significantly higher in RCC tissues than the control ( $p < 0.05$ ) (Figure 1A). The mean level of *UBE2C* in RCC was 5 folds of that in normal control. Furthermore, the expression level of *UBE2C* in stage IV patients was remarkably higher than that in stage I patients (Figure 1B).

To verify the bioinformatics data, the mRNA expression of *UBE2C* in 90 of RCC tissues and matched control was determined by RT-qPCR method. In consistency, *UBE2C* was expressed much higher in tumor tissues than the control and immunohistochemical (IHC) analysis confirmed this result (Figure 1C and 1D). Therefore, *UBE2C* was over-expressed in RCC tissues.

**Table 1.** Clinical parameters of RCC patients from TCGA database.

Covariates	Type	Stat
T	T1	269 (51.24%)
	T2	69 (13.14%)
	T3	176 (33.52%)
	T4	11 (2.1%)
N	N0	238 (45.33%)
	N1	15 (2.86%)
	NX	272 (51.81%)
M	M0	419 (79.81%)
	M1	77 (14.67%)
	MX	29 (5.52%)
Stage	Stage I	263 (50.1%)
	Stage II	57 (10.86%)
	Stage III	123 (23.43%)
	Stage IV	82 (15.62%)
Fustat	Alive (0)	368 (70.1%)
	Dead (1)	157 (29.9%)
Age	$\leq 60$	260 (49.52%)
	$> 60$	265 (50.48%)

T1-4, primary tumor; N0, no regional lymph node involved; N1, few regional lymph nodes involved; NX, regional lymph node unevaluated; M0, no distant metastasis; M1, distant metastasis; MX, distant metastasis unevaluated; the percentage in parentheses means the number in the total cases; fustat is a parameter in survival analysis: fustat 1 indicated alive, fustat 0 indicated dead.



**Figure 1.** *UBE2C* was over-expressed in RCC. A) Expression profiles of *UBE2C* extracted from TCGA database. B) The expression of *UBE2C* in RCC patients with different pathological stage. C) The mRNA level of *UBE2C* in a total of 90 RCC tissues and adjacent normal control by RT-qPCR assay. D) The IHC analysis of *UBE2C* expression in RCC tissues and matched control;  $*p < 0.05$  was considered as statistically significant.

## UBE2C was correlated to clinicopathological factors in RCC patients

Tumor is a malignant disease caused by multiple factors and genetic gene is a key factor. Driver gene is a critical genetic factor in tumor. In this study, RCC patients were separated as low and high group based on the expression of *UBE2C*. The cut value is 2-fold (*UBE2C* expression in RCC tissues to control tissues). Then chi-square test was performed to analyze the relationship of *UBE2C* level with clinical factors in RCC patients. As shown in Table 2, *UBE2C* expression was associated with TNM stage, pathological stage, gender and survival but not age in RCC patients.<sup>16</sup> Briefly, *UBE2C* was expressed at high level in 45.42% of T3 patients while at low level in 21.67% of T3 patients. But it was 38.93% vs 63.5% in T1 patients.

In stage I, 37.79% patients displayed high *UBE2C* expression when it was 62.36% in low *UBE2C* expression. But it was 29.01% vs 17.87% in stage III and 24.43% vs 6.84% in stage IV patients. Moreover, death rate of patients with high *UBE2C* expression is 41.98% but it was 17.87% in low *UBE2C* RCC patients. In 90 clin-

ical cases, Clinicopathological analysis demonstrated that *UBE2C* was correlated to TNM stage and pathological stage in RCC (Table 3). Conclusively, *UBE2C* displayed important clinic role in progression of RCC.

## UBE2C showed prognostic value in RCC

OS and progression-free survival (PFS) are two critical indicators in cancer. By mining TCGA database, we found that *UBE2C* expression was associated with OS and PFS in RCC patients. As shown in Figure 2A, RCC patients with higher *UBE2C* expression (n=262) showed much shorter OS than those with lower *UBE2C* expression (n=263) (p<0.05). And patients with lower *UBE2C* expression (n=262) displayed much better PFS than the control (Figure 2B, n=263) (p<0.05). By multivariate COX analysis, we found that patients with higher *UBE2C* expression exhibited worse OS than the control (p<0.001, HR=1.01, 95% CI 1.006-1.020) (Tables 3 and 4). In addition, pathological stage was associated with patient survival. Therefore, *UBE2C* might be a critical factor for predicting the prognosis of RCC patients.

**Table 2.** Correlation analysis of *UBE2C* expression with clinical parameters of RCC patients based on TCGA data.

Covariates	Type	Count	Low	High	p
Fustat	Alive (0)	368(70.1%)	216(82.13%)	152(58.02%)	2.87E-09
	Dead (1)	157(29.9%)	47(17.87%)	110(41.98%)	
Gender	Female	184(35.05%)	107(40.68%)	77(29.39%)	0.008775808
	Male	341(64.95%)	156(59.32%)	185(70.61%)	
T	T1	269(51.24%)	167(63.5%)	102(38.93%)	6.83E-10
	T2	69(13.14%)	38(14.45%)	31(11.83%)	
	T3	176(33.52%)	57(21.67%)	119(45.42%)	
	T4	11(2.1%)	1(0.38%)	10(3.82%)	
N	N0	238(45.33%)	116(44.11%)	122(46.56%)	0.009062443
	N1	15(2.86%)	2(0.76%)	13(4.96%)	
	N2	272(51.81%)	145(55.13%)	127(48.47%)	
M	M0	419(79.81%)	226(85.93%)	193(73.66%)	2.88E-08
	M1	77(14.67%)	16(6.08%)	61(23.28%)	
	M2	29(5.52%)	21(7.98%)	8(3.05%)	
Stage	Stage I	263(50.1%)	164(62.36%)	99(37.79%)	5.32E-11
	Stage II	57(10.86%)	34(12.93%)	23(8.78%)	
	Stage III	123(23.43%)	47(17.87%)	76(29.01%)	
	Stage IV	82(15.62%)	18(6.84%)	64(24.43%)	
Age	<=60	260(49.52%)	132(50.19%)	128(48.85%)	0.826927487
	<60	265(50.48%)	131(49.81%)	134(51.15%)	

The percentage in the parentheses means the number in the total cases or in each group; p-value less than 0.05 displays significant correlation of *UBE2C* expression with clinical factor.

**Table 3.** Univariate COX analysis of 525 RCC patients from TCGA database.

Term	HR	HR (lower 0.95)	HR (upper 0.95)	p-value
Gender	0.945597502	0.683103292	1.308959635	0.735990786
T	2.051550134	1.723523146	2.442008372	6.27E-16
N	0.867947038	0.739824998	1.018257105	0.082220867
M	2.483956418	1.930133225	3.196690987	1.56E-12
Stage	1.956212786	1.702382614	2.247889771	3.00E-21
Age	1.028451721	1.015156215	1.041921359	2.38E-05
<i>UBE2C</i>	1.018830727	1.012673308	1.025025585	1.62E-09

HR, hazard ratio.

### Identification of potential signaling pathways associated with *UBE2C* in RCC

Based on the differential expressed genes (DEGs) extracted from TCGA database, we explored the signaling pathways involved in development of RCC by GO and KEGG analysis. As seen in Figure 3A and Table 5, GO analysis revealed that DEGs were mostly distributed in cell division (n=86), mitotic nuclear division (n=67), and sister chromatid cohesion (n=45) process. KEGG analysis

revealed significance of *UBE2C* in about 17 signal pathways in which cell cycle was the most important pathway ( $p < 0.001$ ) (Figure 3B). About 40 DEGs were enriched in cell cycle (Table 6). Then a total of 13 DEGs was enriched in DNA replication and p53 signaling pathway (Figure 3B, Table 6). It is known to us that the signaling pathways including cell cycle, cell division, p53 signaling all play important roles in tumor. So it is conceived that *UBE2C* might regulate one or several of these pathways in RCC.

**Table 4.** Multivariate COX analysis of 525 RCC patients from TCGA database.

Term	HR	HR (lower 0.95)	HR (upper 0.95)	p-value
Gender	1.052653432	0.748571991	1.480257425	0.7679768
T	0.861615988	0.587843984	1.262889697	0.445157108
N	0.893264318	0.758286997	1.052268001	0.176881432
M	1.08647467	0.669831613	1.762274555	0.736802667
Stage	2.081291149	1.435882277	3.016802225	0.000108772
Age	1.033098721	1.017936353	1.048486935	1.58E-05
<i>UBE2C</i>	1.013312908	1.006346286	1.020327757	0.00017179

HR, hazard ratio.

**Table 5.** GO enrichment analysis of differential expression genes in RCC.

Id	Term	Count	p-value	p adjust
GO:0051301	Cell division	86	6.50E-48	1.13E-44
GO:0007067	Mitotic nuclear division	67	6.75E-40	1.17E-36
GO:0007062	Sister chromatid cohesion	45	5.50E-37	9.54E-34
GO:0005654	Nucleoplasm	212	3.13E-33	4.47E-30
GO:0006260	DNA replication	47	2.50E-30	4.34E-27
GO:0000777	Condensed chromosome kinetochore	31	9.70E-23	1.38E-19
GO:0000082	G1/S transition of mitotic cell cycle	33	2.79E-22	4.84E-19
GO:0005515	Protein binding	415	5.73E-22	8.67E-19
GO:0005634	Nucleus	292	2.14E-21	3.05E-18
GO:0000776	Kinetochore	29	2.41E-21	3.45E-18

Count indicates the enriched genes in each bioprocess in Term.

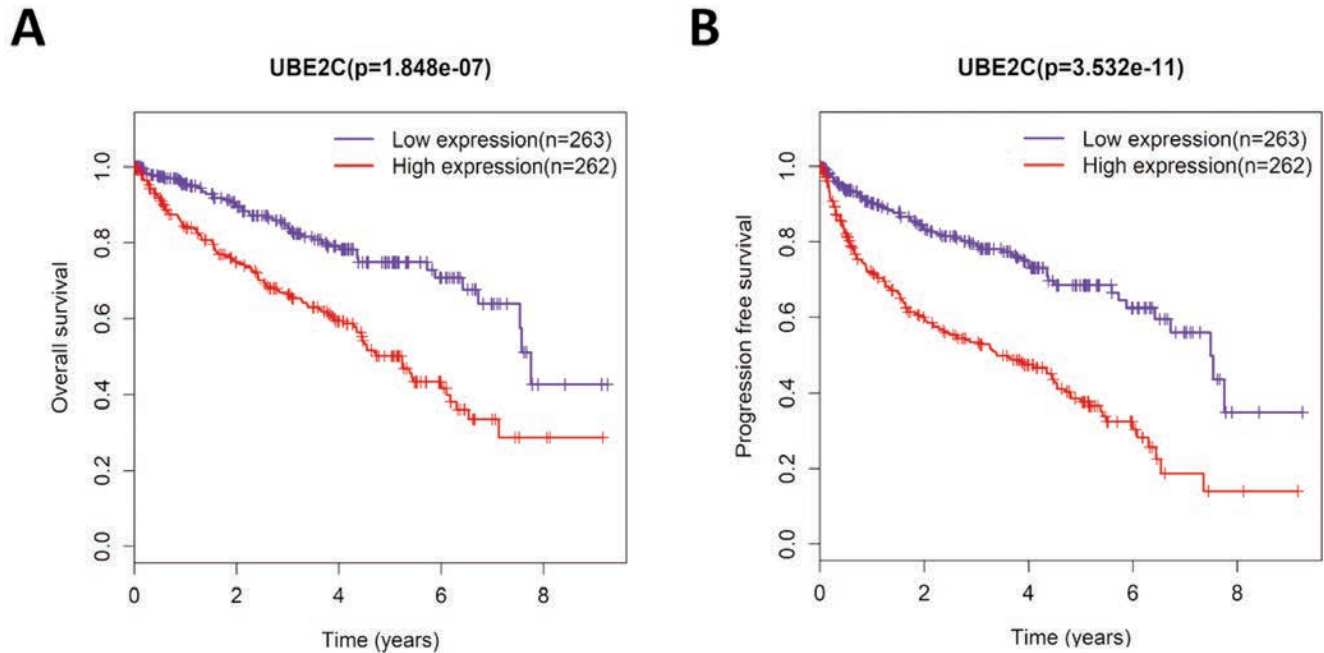
**Table 6.** GO enrichment analysis of differential expression genes in RCC.

ID	Description	Count	p-value	p adjust
hsa04110	Cell cycle	40	4.66E-28	9.97E-26
hsa03030	DNA replication	13	1.48E-10	1.59E-08
hsa03460	Fanconi anemia pathway	13	3.96E-08	2.83E-06
hsa04914	Progesterone-mediated oocyte maturation	17	7.11E-08	3.81E-06
hsa04114	Oocyte meiosis	18	4.11E-07	1.51E-05
hsa05166	Human T-cell leukemia virus 1 infection	27	4.24E-07	1.51E-05
hsa04115	p53 signaling pathway	13	1.41E-06	4.32E-05
hsa03410	Base excision repair	7	0.000142001	0.00379852
hsa04218	Cellular senescence	16	0.000204533	0.004863351
hsa03013	RNA transport	16	0.000436647	0.009344253
hsa03440	Homologous recombination	7	0.000583551	0.011352725
hsa05130	Pathogenic Escherichia coli infection	8	0.000730516	0.013027537
hsa03050	Proteasome	7	0.001041273	0.017140959
hsa03430	Mismatch repair	5	0.001170701	0.017895004
hsa05203	Viral carcinogenesis	16	0.002452518	0.034989252
hsa00240	Pyrimidine metabolism	10	0.003407413	0.045574151

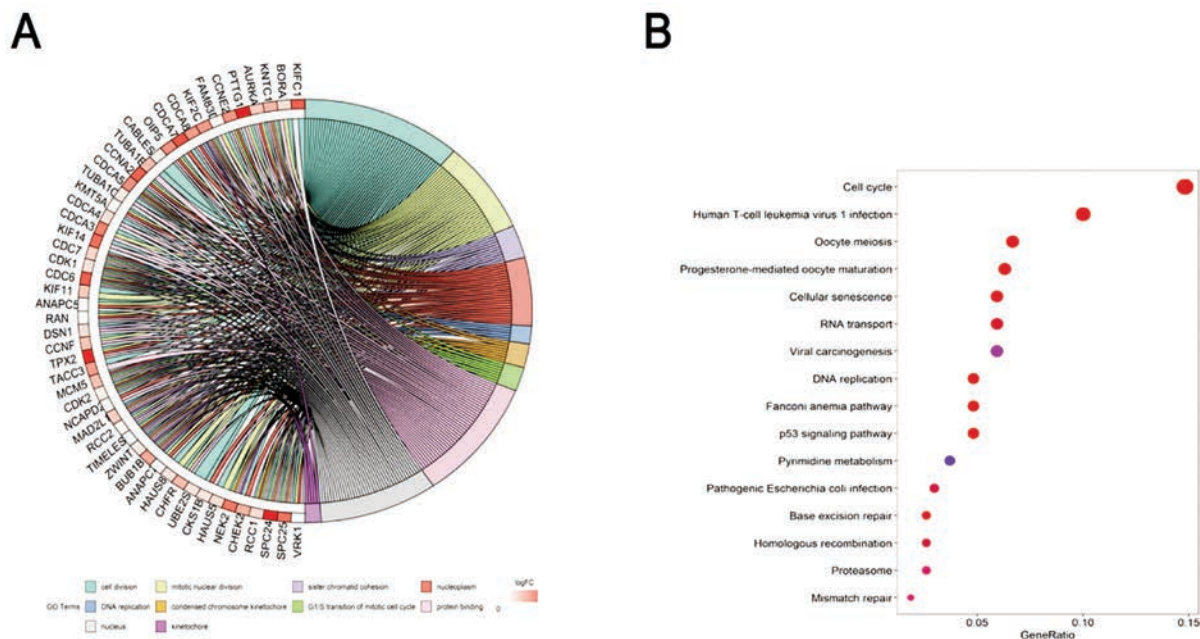
### UBE2C is important for cell proliferation in RCC

To verify the real function of *UBE2C* in RCC, *UBE2C* was knocked down in 786-O cells. The knockdown efficiency of *UBE2C* in 786-O cells was over 66% (Figure 4A). Then by CCK-

8 assay, we found that the growth of 786-O cells was dramatically inhibited at third day of culture. The proliferation fold in *UBE2C* knockdown group was only 1.53 but it was 3.49 in the control (Figure 4B). Therefore, *UBE2C* was essential to the growth of RCC cells.



**Figure 2.** Overall survival (OS) and progression-free survival (PFS) curves of RCC patients based on the expression of *UBE2C*. A) OS curve based on TCGA data. B) PFS curve based on TCGA data.



**Figure 3.** GO and KEGG analysis of *UBE2C*-associated pathways. A) GO analysis showed enrichment of differential expression genes. B) KEGG analysis showed the enriched signaling pathway.

## UBE2C contributes to cell migration in RCC

Tumor cells often have potent migration ability according to the previous study. In transwell assay, we found that 786-O cells couldn't transmit through the chamber effectively. The number of transmitted cells in UBE2C knockdown group was decreased by 44% compared to the control (Figure 4 C,D). The results suggested that UBE2C conferred migration ability to 786-O cells.

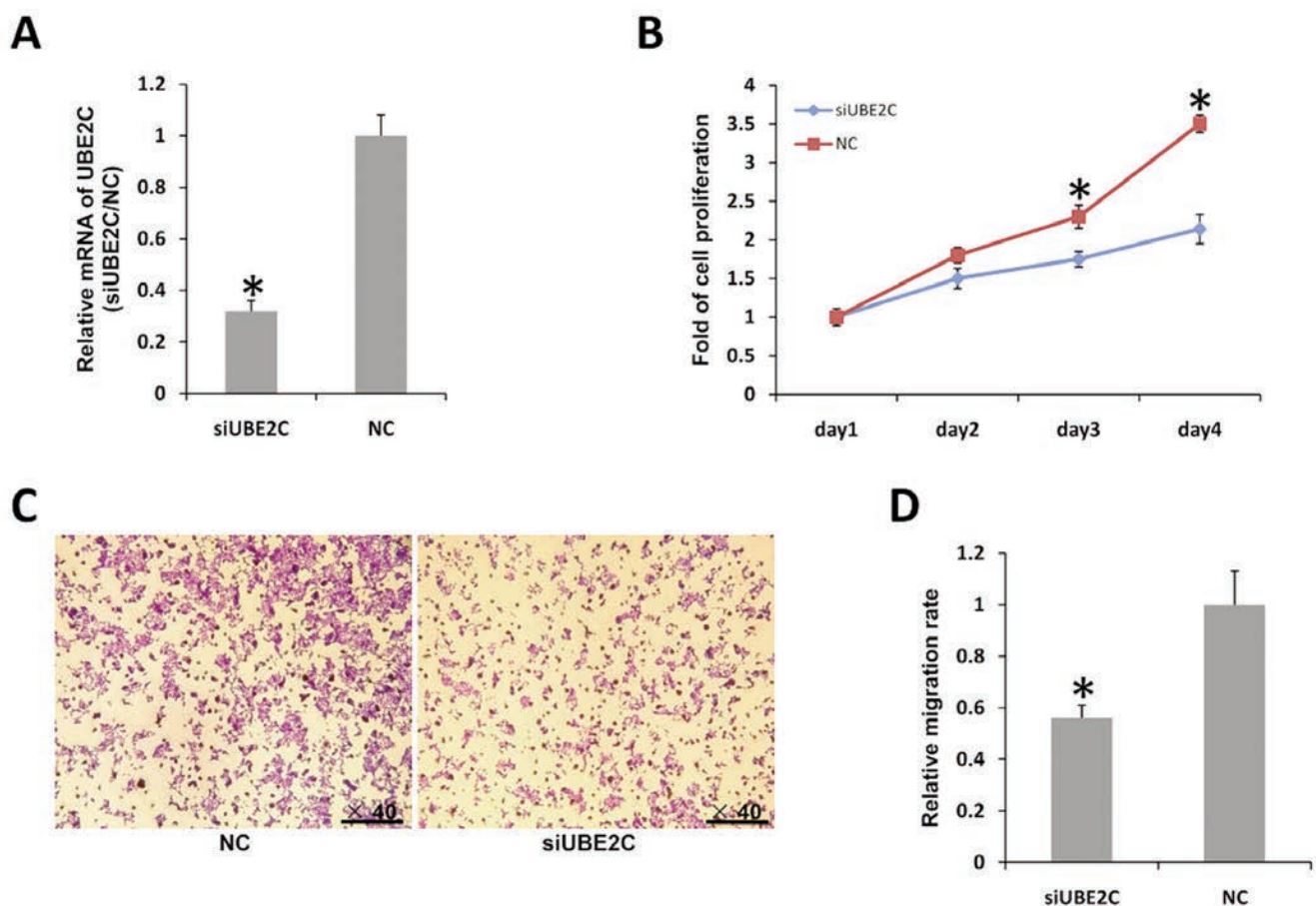
## Discussion

UBE2C is an important gene in cancer. For example, in prostate cancer, silence of UBE2C prevents cell growth.<sup>17-19</sup> Methylation of UBE2C lead to gene instability.<sup>18</sup> Over-expression of UBE2C is closely correlated to invasion and pathological stage in ovarian cancer.<sup>12</sup> In this study, UBE2C was firstly demonstrated to be clinically associated with progression and prognosis in RCC patients. This was consistent with previous studies in other cancers such as ovarian, gastric cancer.<sup>12,19</sup>

UBE2C is also shown to be associated with poor prognosis in cancers.<sup>8,13,20,21</sup> In this study, we demonstrated that patients with high UBE2C expression displayed short survival time and decreased PFS, suggesting that UBE2C might be a prognostic factor in RCC. This was further supported by multivariate COX

analysis, which suggested UBE2C as a critical gene in RCC. Taken together, UBE2C played critical roles in development and progression of RCC and might be a new biomarker for diagnosis or prognosis in advanced RCC.

RCC is a malignant disease affected by multiple factors and a series of signaling pathways contributed to the initiation or progression of RCC.<sup>22,23</sup> In this study, the DEGs between tumor tissues and normal tissues were extracted from the TCGA databases followed by GO enrichment analysis. As stated above, the most correlated process was cell division in which 86 DEGs were enriched. Then 67 DEGs were enriched in mitotic nuclear division and 47 DEGs in DNA replication process. The three processes are known to play important roles in embryonic development or homeostasis.<sup>24,25</sup> Aberrant in these processes often causes severe diseases or abnormalities such as cancer. So, it is possible that UBE2C might also regulate cell division, DNA replication, and mitotic nuclear division in RCC. In KEGG analysis, 40 DEGs was enriched in cell cycle pathway while 13 DEGs in DNA replication pathway. Accelerated cell division was common in cancer and was associated with unlimited cell proliferation and accelerated cell cycle. DNA replication was also an active process in cancer. Here, KEGG analysis showed that cell cycle and DNA replication were two top pathways in RCC. Indeed, active DNA replication process provides basis for cell cycle transition. This will further contribute to the division and proliferation of cancer cells. As a result, we



**Figure 4.** Knockdown of UBE2C inhibited cell proliferation and migration in 786-O cells. A) Knockdown efficiency of UBE2C in 786-O cells determined by RT-qPCR technology. B) The proliferation curve of 786-O cells treated with siUBE2C or NC in CCK-8 assay for 96 h. C,D) Transmitted cells stained by crystal violet in transwell assay; \*p<0.05 was considered as statistically significant.

deduce that *UBE2C* might regulate cell cycle and DNA replication pathway in RCC.

The bioinformatics analysis showed that *UBE2C* played important roles in clinic in RCC. This was further supported by *in vitro* assay in 786-O cells. As stated in the above, knockdown of *UBE2C* by RNNi significantly inhibited cell proliferation and migration in 786-O cells. It is known that potent proliferation and migration ability are two common traits in cancer.<sup>26,27</sup> So, the preliminary data in 786-O cells further confirmed the role of *UBE2C* in RCC. However, more experiments are needed to support the oncogenic role of *UBE2C* in RCC and the mechanism that how *UBE2C* promotes progression in RCC. In future, we plan to further detect the role of *UBE2C* in more than two strain cell lines in RCC, especially the effects on cell apoptosis, cell cycle, and cell invasiveness. In addition, the *in vivo* role of *UBE2C* in RCC is a very important proof to support this study.

In summary, this study proves the clinical role of *UBE2C* and suggests it to be an important prognostic factor in RCC. *UBE2C* contributes to the proliferation and migration in RCC.

## References

- Nabi S, Kessler ER, Bernard B, Flaig TW, Lam ET. Renal cell carcinoma: a review of biology and pathophysiology. *F1000Res* 2018; 7:307.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *Ca Cancer J Clin* 2018;68:7-30.
- Vecchio SJD, Ellis RJ. Cabozantinib for the management of metastatic clear cell renal cell carcinoma. *J Kidney Cancer VHL* 2018;5:1-5.
- Conti A, Santoni M, Amantini C, Burattini L, Berardi R, Santoni G, et al. Progress of molecular targeted therapies for advanced renal cell carcinoma. *BioMed Res Int* 2013;2013: 419176.
- Flippot R, Escudier B, Albiges L. Immune checkpoint inhibitors: toward new paradigms in renal cell carcinoma. *Drugs* 2018;78:1443-57.
- Sanchez-Gastaldo A, Kempf E, Aranzazu GDA, Duran I. Systemic treatment of renal cell cancer: A comprehensive review. *Cancer Treat Rev* 2017;60:77-89.
- Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2019. *CA Cancer J Clin* 2019;69:7-34.
- Hao Z, Zhang H, Cowell J. Ubiquitin-conjugating enzyme UBE2C: molecular biology, role in tumorigenesis, and potential as a biomarker. *Tumour Biol* 2012;33:723-30.
- Zhang J, Liu XY, Yu GZ, Liu L, Wang JJ, Chen XY, et al. UBE2C is a potential biomarker of intestinal-type gastric cancer with chromosomal instability. *Front Pharmacol* 2018;9:847.
- Han QL, Zhou C, Liu F, Xu GH, Zheng R, Zhang X. MicroRNA-196a post-transcriptionally upregulates the UBE2C proto-oncogene and promotes cell proliferation in breast cancer. *Oncol Rep* 2015;34:877-83.
- Takahashi Y, Ishii Y, Nishida Y, Ikarashi M, Nagata T, Nakamura T, et al. Detection of aberrations of ubiquitin-conjugating enzyme E2C gene (UBE2C) in advanced colon cancer with liver metastases by DNA microarray and two-color FISH. *Cancer Genet Cytogen* 2006;168:30-5.
- Martinez-Canales S, Miguel LDR, Nuncia-Cantarero M, Paez R, Amir E, Gyorffy B, et al. Functional transcriptomic annotation and protein-protein interaction analysis identify EZH2 and UBE2C as key upregulated proteins in ovarian cancer. *Cancer Med* 2018;7:1896-907.
- Loussouarn D, Campion L, Leclair F, Campone M, Charbonnel C, Ricolleau G, et al. Validation of UBE2C protein as a prognostic marker in node-positive breast cancer. *Br J Cancer* 2009;101:166-73.
- Wang Z, Jensen MA, Zenklusen JC. A Practical Guide to The Cancer Genome Atlas (TCGA). *Methods Mol Biol* 2016;1418:111-41.
- Cheng PF, Dummer R, Levesque MP. Data mining the cancer genome atlas in the era of precision cancer medicine. *Swiss Med Wkly* 2015;145:w14183.
- Guinan P, Sobin LH, Algaba F, Badellino F, Kameyama S, MacLennan G, et al. TNM staging of renal cell carcinoma. *Cancer* 1997;80:992-3.
- Wei SQ, Li CY, Li BG, Li JM, Wang H. UBE2C siRNA inhibits proliferation and invasion of human castration resistant prostate cancer PC3 cells. *Tumor* 2015;11:1200-7.
- Wang HY, Zhang CP, Rorick A, Wu DY, Chiu M, Thomas-Ahner J, et al. CCI-779 inhibits cell-cycle G2/M progression and invasion of castration resistant prostate cancer via attenuation of UBE2C transcription and mRNA stability. *Cancer Res* 2011;71:4866-76.
- Zhang HQ, Zhao G, Ke B, Ma G, Liu GL, Liang H, et al. Overexpression of UBE2C correlates with poor prognosis in gastric cancer patients. *Eur Rev Med Pharmacol Sci* 2018;22:1665-71.
- Mo CH, Gao L, Zhu XF, Wei KL, Zeng JJ, Chen G, et al. The clinicopathological significance of UBE2C in breast cancer: a study based on immunohistochemistry, microarray and RNA-sequencing data. *Cancer Cell Int* 2017;17:83.
- Hutson TE, Fiqlin RA. Renal cell cancer. *Cancer J* 2007;13:282-6.
- Yuan LS, Chen L, Qian KY, Qian GF, Wu CL, Wang XH, et al. Co-expression network analysis identified six hub genes in association with progression and prognosis in human clear cell renal cell carcinoma (ccRCC). *Genom Data* 2017;14:132-40.
- Aze A, Maiorano D. Recent advances in understanding DNA replication: cell type-specific adaption of the DNA replication program. *F1000Res* 2018;7:F1000 Faculty Rev-1351.
- Deneke VE, Puliafito A, Krueger D, Narla AV, De Simone A, Primo L, et al. Self-organized nuclear positioning synchronizes the cell cycle in drosophila embryos. *Cell* 2019;177:925-41.
- Bjorklund M. Cell size homeostasis: metabolic control of growth and cell division. *Biochim Biophys Acta Mol Cell Res* 2019; 866:409-17.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57-70.
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646-74.

Received for publication: 9 November 2020. Accepted for publication: 10 February 2021.

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*European Journal of Histochemistry* 2021; 65:3196

doi:10.4081/ejh.2021.3196