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Expression and clinical significance of UBE2V1 in cervical cancer

Zhishuai Ren [a,1], Zhendong Liu [b,1], Shenqian Ma [a,1], Junming Yue [c], Jinming Yang [d], Ruiya Wang [a], Yanzheng Gao [b,*], Yuqi Guo [e,f,**]

[a] People's Hospital of Zhengzhou University, Henan Provincial People's Hospital, Zhengzhou, 450003, Henan, China
[b] Department of Surgery of Spine and Spinal Cord, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, People's Hospital of Henan University, Zhengzhou, 450003, Henan, China
[c] Department of Pathology, The University of Tennessee Health Science Center, 19 S. Manassas St., Rm. 266, Memphis, TN, 38163, USA
[d] Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN, 37240, USA
[e] Department of Obstetrics and Gynecology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, People's Hospital of Henan University, Zhengzhou, 450003, Henan, China
[f] Henan International Joint Laboratory for Gynecological Oncology and Nanomedicine, Zhengzhou, 450003, Henan, China

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ABSTRACT

The majority of cervical cancer (CC) patients are caused by the high-risk human papillomavirus (HPV) infection Although they are preventable and controllable, the mortality rate is still high. It is essential to identify the biomarkers for early screening and diagnosis of CC to improve the prognosis of patients with CC. The conjugating enzyme 2 (E2) family members are the key components of ubiquitin protease system. However, the role of E2 family in CC remains unclear. We aimed to investigate the role of *UBE2V1*, a ubiquitin binding E2 enzyme variant protein (ube2v) without conserved cysteine residues required for E2s catalytic activity in CC. In this study, we first studied the expression of *UBE2V1* in CC by real time quantitative PCR (RT-qPCR), and then, the clinical information of 191 CC patients in TCGA database was retrieved to explore the relationship between the expression of *UBE2V1* and the occurrence and development of CC by examining the translational profile and methylation, the high expression of *UBE2V1* was well correlated to the poor prognosis of patients, indicating that *UBE2V1* is an independent risk factor for the prognosis of CC patients. The expression of *UBE2V1* was also correlated with clinical stages, tumor grades and TNM stages of CC. In addition, the expression of *UBE2V1* was slightly negatively correlated with the methylation at the multiple methylation sites. our study revealed the relationship between *UBE2V1* and the occurrence and development of CC from the level of transcriptional profile and DNA methylation. *UBE2V1* is a novel candidate biomarker for the diagnosis, screening and prognosis of CC.

1. Introduction

The incidence of CC ranks fourth in women worldwide, after breast cancer, colon cancer and lung cancer [1]. According to the latest cancer statistical report, there were 604000 newly diagnosed cases of CC and 342000 deaths worldwide in 2020 [1]. Almost all cases of CC are caused by HPV infection, of which, HPV types 16 and 18 are the most common [2,3]. With the development of HPV detection technology and the awareness and popularity of HPV vaccination, CC diagnosis and prevention have achieved gratifying results. However, HPV screening and HPV vaccination still pose major challenges. only approximately 49.5% of women aged 13–17 years receive HPV vaccination [4]. CC is the second leading cause of death in women aged 20–39 years [5]. One of the most important reasons for this outcome is the timely diagnosis of patients with CC and the inability to obtain treatment in a timely manner [6]. The new biomarkers may play a potential role in the preliminary screening of CC. As an auxiliary diagnostic and grading method for the preliminary cytological screening and the main HPV screening, it is helpful to further improve the screening of CC and precancerous lesions. An effective biomarker is significant for clinical diagnosis, treatment,

* Corresponding author. Department of Surgery of Spine and Spinal Cord, Henan Provincial People's Hospital; People's Hospital of Zhengzhou University, People's Hospital of Henan University, No.7, Weiwu Road, Zhengzhou, 450003, Henan, China,
** Corresponding author. Department of Obstetrics and Gynecology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, People's Hospital of Henan University, No.7, Weiwu Road, Zhengzhou, 450003, Henan, China.
E-mail addresses: yanzhenggaohn@gs.zzu.edu.cn (Y. Gao), yuqiguo@zzu.edu.cn (Y. Guo).
[1] These authors contributed equally.

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grading and prognosis of cervical intraepithelial neoplasia, thus to decide whether it is needed for treatment or further to determine the nature of lesions using colposcopic biopsy. The false-positive results will lead to anxiety and over-treatment of patients. Therefore, it is important to identify the highly sensitive and specific biomarkers to improve the positive screening rate of CC and the accuracy of the diagnosis of precancerous lesions.

Ubiquitin-conjugated E2 enzyme variant protein is a unique subfamily of E2 protein family due to lacking of conserved cysteine for E2 catalytic activity [7,8]. The function of ubiquitin binding E2 enzyme variant protein is still unclear [9]. It is still controversal on the role of *UBE2V1* in cancer. *UBE2V1* was reported as an oncogene a tumor suppressor gene [7,10–12]. However, it is not clear on the role *UBE2V1* in CC.

Here, we report that the abnormally high expression of *UBE2V1* was well-correlated with poor prognosis and had a reliable predictive value for the survival time of CC patients. The *UBE2V1* expression is correlated with the clinical stages, tumor tissue grades, and TNM (tumor, node, metastasis) of CC patients. We therefore conclude that *UBE2V1* is a novel oncogene for CC diagnosis, screening as well as prognosis.

2. Materials and methods

2.1. Data sources

A total of 191 TCGA (https://portal.gdc.cancer.gov/) CC samples were retrieved from the TCGA database for analysis. The detailed clinical information of the corresponding patients is shown in Table S1.

2.2. Cell culture

A CC cell line (HELA cell) and a human normal cervical epithelial cell line (HUCEC cell) were obtained from the cell bank of Chinese Academy of Sciences. Both cells were cultured in DMEM medium (Procell, Wuhan, China) in a sterile wet cell incubator at 37 °C and 5% CO_2 (all medium contained 10% fetal bovine serum (Gibco), 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen)).

2.3. Real time quantitative PCR(RT-qPCR)

Total RNA was extracted from cultured cells using total RNA kit (Omega-BioTek). The concentration and purity of total RNA were measured by Thermo Scientific. After the total RNA was reversely transcribed into cDNA, the Novoprotein-SYBR-qPCR-Supermix-plus kit (Novoprotein, Shanghai, China) was used for fluorescent quantitative PCR amplification with *GAPDH* as the internal reference, and each sample was repeated for four times. PCR reaction conditions: 95 °C for 5 min, 95 °C for 20 s, annealing at 60 °C for 20 s, extension at 72 °C for 30 s, a total of 45 cycles. The primer sequences used were as follows: *GAPDH* forward 5 ' -CAAGGTCATCCATGACAACTTTG-3 ′ and *GAPDH* reverse 5 ' -GTCCACCAC CCTGTTGCTGTAG-3 '. *UBE2V1* forward 5 ' -TTCAAGCGTCTTACCTGAAGTC-3 ′ and *UBE2V1* reverse 5 ' -CCAACAGTCGAAATTGCGAG-3 '. The relative expression level of *UBE2V1* was determined by $2^{-\Delta\Delta CT}$ method, and the significance of the difference between the two groups was compared by paired *t*-test. $P < 0.05$ indicated that the difference was statistically significant.

2.4. Analysis of independent prognostic risk factors

Univariate and multivariate independent prognostic risk factors were analyzed using survival R software package. The clinical variables included were age, clinical stage, neoplastic histologic grade and TNM (tumor, node, metastasis). 191 CC patients with complete clinical information in TCGA dataset were included. HR is calculated and represented by forest plot.

2.5. CMap analysis

Based on 191 CC samples from TCGA database, we identified 10 genes related to the expression of *UBE2V1* by Pearson correlation analysis, 5 of which were positive and 5 were negative correlated genes. Then, we examined these positive and negative correlated genes in CMap database (https://portals.broadinstitute.org/cmap/) to identify potential drugs in inhibiting *UBE2V1* expression as screening criteria (enrichment < - 0.9 and $P < 0.05$). Finally, we collected the detailed information of these small molecule compounds through PubChem tool.

2.6. Statistical analysis

All data were analyzed by R software (v.4.0.3 version). Wilcoxon or Kruskal tests were used to reveal the correlation between *UBE2V1* and the clinical characteristics of patients with CC. Kaplan-Meier and Cox analyses were used to reveal the impact of *UBE2V1* on the prognosis of CC patients and whether *UBE2V1* has reliable diagnostic value for the prognosis of CC patients. Univariate Cox and multivariate Cox analyses were used to reveal whether *UBE2V1* is an independent risk factor for CC. The Pearson correlation coefficient between β value of dmCpG sites and FPKM value of located genes was calculated with cor. test in R and visualized. Pearson's correlation was used to identify genes co-expressing with *UBE2V1*. $P < 0.05$ indicates statistical significance.

3. Results

3.1. High expression of UBE2V1 in CC

Compared with normal cervical epithelial cells, *UBE2V1* was highly expressed in CC cell lines, and the results were statistically significant ($P < 0.05$) (Fig. 1A).

3.2. UBE2V1 expression is correlated with the survival time of CC patients

The survival time of 191 CC patients in TCGA database with high expression of *UBE2V1* was significantly shorter than that of CC patients with low expression of *UBE2V1* ($P < 0.05$) (Fig. 1B). to further examine whether *UBE2V1* has predictive value for the survival time of patients with CC, we calculated the relevant ROC and found that *UBE2V1* has a predictive value for the 3-year and 5-year survival time of patients with CC (Fig. 1C).

3.3. Relationship between expression of UBE2V1 and clinical features of CC

The expression of *UBE2V1* was positively correlated with clinical stage, tumor volume and lymph node metastasis (Fig. 1D, E, F).

3.4. UBE2V1 is an independent risk factor for the prognosis of CC patients

Clinical information from 191 CC patients in the TCGA database were included to perform univariate and multivariate survival analysis, and the results of this analysis indicated that *UBE2V1* was an independent risk factor indicator for the prognosis of CC patients ($P < 0.05$) (Fig. 2A and B).

3.5. The expression of UBE2V1 was negatively correlated with the methylation level of dmCpG sites

Thirteen dmCpG sites were selected from 191 samples of TCGA cohort, and the methylation level of each site was analyzed. Five of the 13 dmCpG sites were selected and analyzed for their correlation with the expression of *UBE2V1* (Fig. 2C). The results showed that the methylation levels of cg01710839 ($r = - 0.24$, $P = 0.00097$), cg09044633 ($r = - 0.23$,

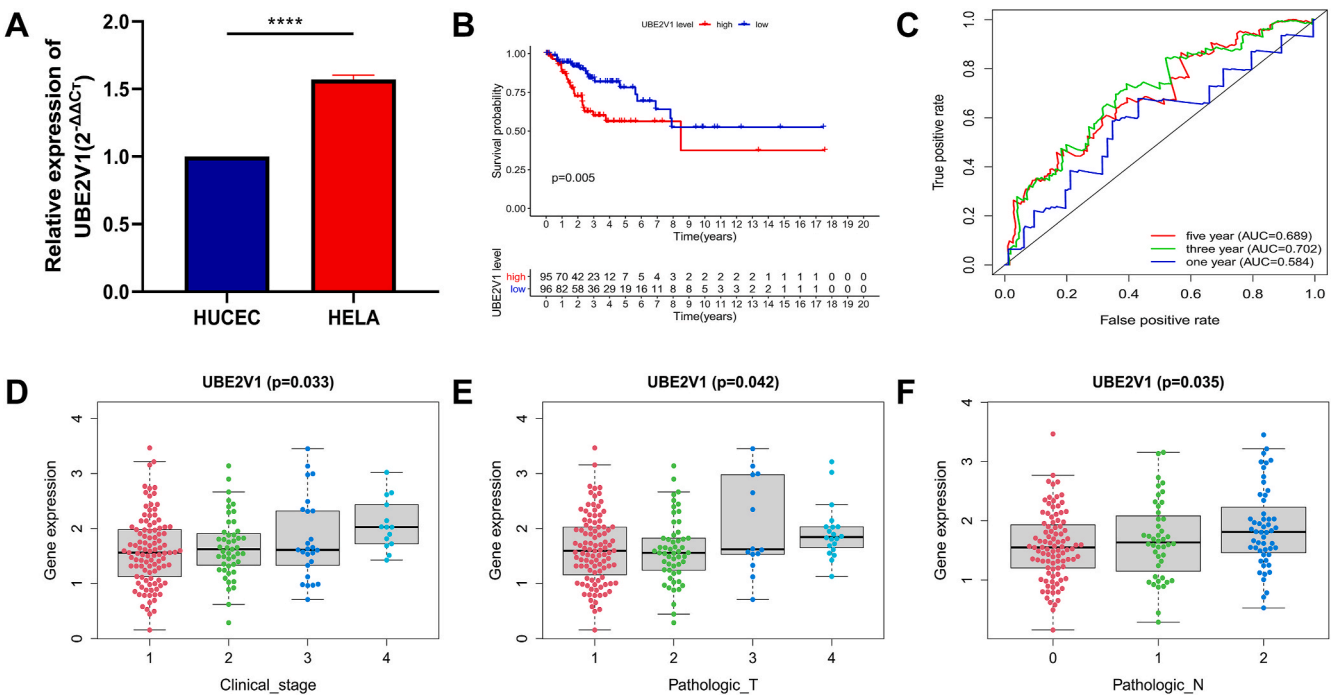

Fig. 1. *UBE2V1* is overexpressed in CC and predicts the poor prognosis of CC. A: *UBE2V1* is highly expressed in cervical cancer cell lines (****P < 0.0001). B: Overexpression of *UBE2V1* predicts poor prognosis of CC (P = 0.005). C: The expression of *UBE2V1* has a good predictive value for the 1-year, 3-year and 5-year survival time of patients with CC. D–F: The expression of *UBE2V1* is closely related to multiple clinical features of CC. D: Clinical stage. E: Lymph node metastasis. F: Tumor volume. AUC: Area Under Curve.

P = 0.0011), cg05827346 (r = - 0.20, P = 0.0067), cg22870172 (r = - 0.26, P = 0.00024) and cg15739997 (r = - 0.19, P = 0.01) were negatively correlated with the expression of *UBE2V1* (Fig. 2D–H).

3.6. Co-expression analysis results

We used Pearson tools to identify 5 genes that have a synergistic effect with the expression of *UBE2V1* (including *STAU1, STRINC3, ARFGEF2, TTPAL, and TOP1*.) and 5 genes that mutually inhibit the expression of *UBE2V1* (including *PMF1, EDF1, BLOC1S1, SARNP, and ATP5PO*). The names of these 10 genes and the correlation coefficients and P-value between these genes and *UBE2V1* are shown in Fig. 3A and B.

3.7. CMap analysis results

We identified three small molecule compounds that inhibit the expression of *UBE2V1*, including Azacitidine, Camptothecin, and Irinotecan through CMap, a public data platform. The relevant parameters are shown in Table 1. Immediately afterward, we obtained detailed information on these three small molecule compounds, including their molecular formulas and two-dimensional and three-dimensional structures, through PubChem, an online tool (Fig. 3C–E).

4. Discussion

CC is one of the important causes of cancer death in women, and it is the fourth most common cancer in women worldwide [13]. In all stages of CC, the 3-to 5-year survival rate of CC patients in many developed countries is less than 50%, which brings huge losses to individual families and Society [14]. The early diagnosis and screening of CC plays a decisive role in the prevention of CC. It is of far-reaching significance to find a new biomarker in the diagnosis and treatment of CC.

The function of *UBE2V1* has not been widely reported, and its role as a biomarker in tumor remains controversial. However, in this study, we found that the abnormal high expression of *UBE2V1* is well-correlated with the poor prognosis of patients with CC, has predictive value for the survival time of patients with CC. Previous studies showed that overexpression of *UBE2V1* in colon cancer leads to poor prognosis by promoting invasion and metastasis of colon cancer cells in vitro and in vivo [15]. Overexpression of ubiquitin binding enzyme complex *Ube2-v1-Ubc13* can promote the metastasis of breast cancer through the regulation of matrix metalloproteinase-1 gene mediated by nuclear factor NF- κ B, which leads to poor prognosis of breast cancer patients [12]. Therefore, we speculate that *UBE2V1* as an oncogene may also play a key role in the malignant progression of CC.

We found that *UBE2V1* can be an independent risk factor of CC through univariate and multivariate analysis based on the clinical information of CC patients in TCGA database. It is positively correlated with high clinical stages, higher tumor tissue grades and higher TNM stages of CC. As we all know, the clinical stages, histological grades and TNM stages of the tumor represent the malignant degree of the tumor. Therefore, we can infer that the expression of *UBE2V1* is positively correlated with the malignant degree of CC.

Gene co-expression network can associate unknown genes with specific biological processes, so as to determine the function and priority of candidate disease genes [16]. Through co-expression analysis, we found five genes with synergistic effect with *UBE2V1* and five genes with mutual inhibition with *UBE2V1*. Among them, studies have confirmed that Top1, as a member of topoisomerases family, is highly expressed in breast cancer, which is related to the poor prognosis of breast cancer patients [17]. *UBE2V1* is a co-expressed gene with *TOP1*. *TOP1* has been widely studied as an oncogene, which indirectly reveals the potential function of *UBE2V1* in the malignant progression of tumors.

In our study, we found that the expression of *UBE2V1* was weakly correlated with multiple DNA methylation sites. DNA methylation mainly occurs at cytosine phosphate guanosine dinucleotide (CpG), where cytosine is converted to 5-methylcytosine (5meC), which is considered to play an important role in the development of cancer [18]. The loss of DNA methylation is an early event in carcinogenesis.

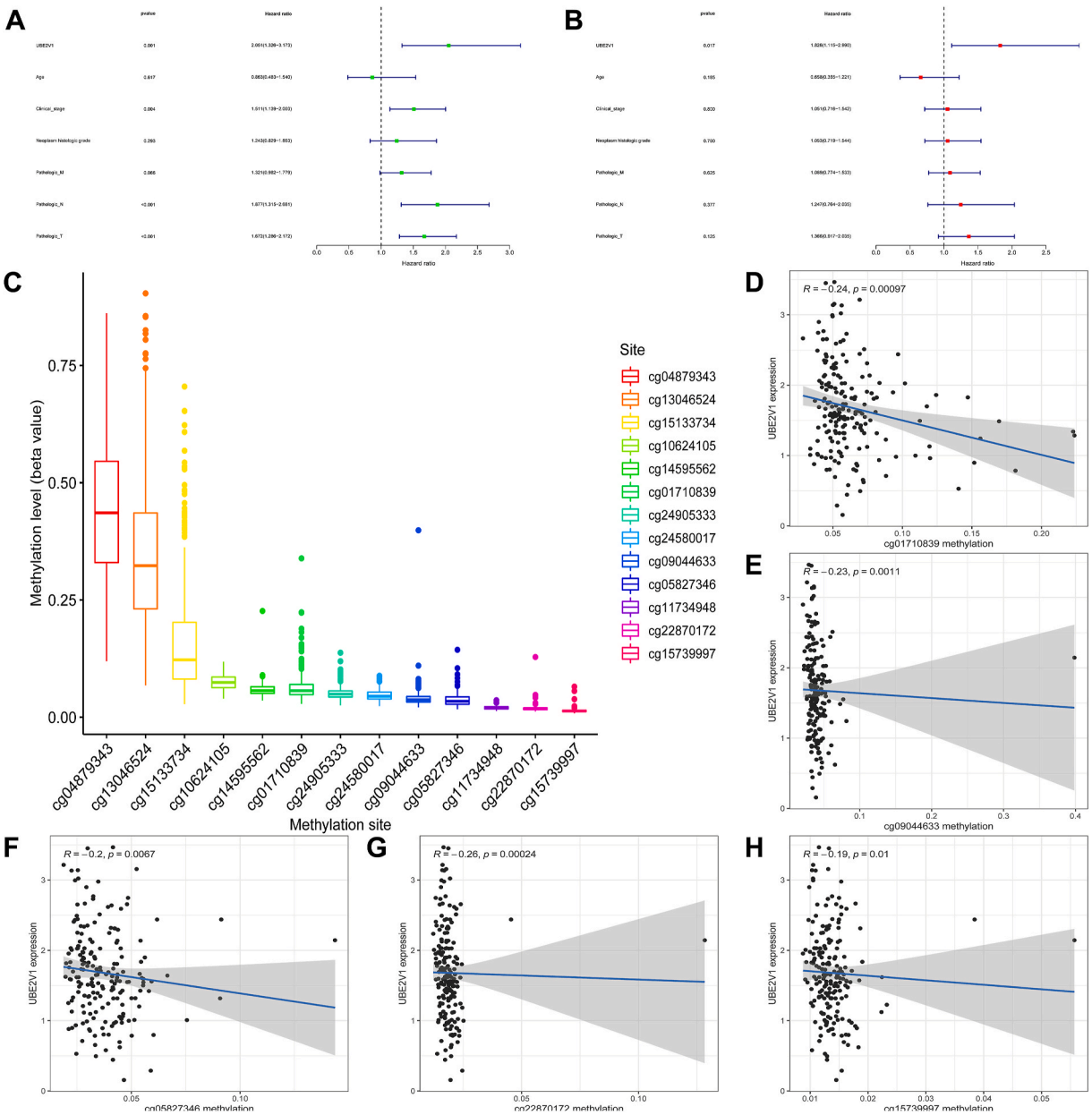

Fig. 2. The expression of *UBE2V1* can be used as an independent risk factor for the prognosis of CC patients and the relationship between the expression of *UBE2V1* and the methylation level of dmCpG sites. A: Univariate analysis. B: Multivariate analysis. C: Based on TCGA database, 13 dmCpG sites and their methylation levels were screened. D–H: The relationship between the expression of *UBE2V1* and the methylation levels of cg01710839 (D), cg09044633 (E), cg05827346 (F), cg22870172 (G) and cg1573997 (H).

Oncogenes are activated due to hypomethylation [19–21]. This supports the key role of *UBE2V1* as an oncogene in CC, and also reveals the possible specific mechanism and site of action of *UBE2V1* in the malignant progression of CC. As far as we know, this is the first study to reveal the expression and key role of *UBE2V1* in CC from the perspective of methylation level change.

Whether it is to study the pathological mechanism of cancer, or to find targeted biomarkers for cancer diagnosis and screening, the ultimate goal is the treatment of cancer. Through CMap online tool, we found three small molecule compounds to potentially inhibit the expression of *UBE2V1*: azacitidine, camptothecin and irinotecan for the treatment of CC. Studies have shown that high doses of azacytidine can inhibit cell proliferation and DNA synthesis [22]. Low dose azacytidine can lead to DNA demethylation and improve the clinical treatment of cancer [23]. At present, azacytidine has been approved by FDA, and has been widely used in the treatment of myelodysplastic syndrome (MDS) and achieved good therapeutic effect [24,25]. Camptothecin is a broad-spectrum anticancer drug, which can induce apoptosis of cancer cells by preventing the progress of replication forks, so it is widely used in the treatment of a variety of cancers [26]. Although camptothecin has a strong anti-cancer effect, because of offtargets lead to serious adverse effects, camptothecin derivatives with low toxicity were synthesized [27]. Irinotecan, as a semi synthetic water-soluble camptothecin derivative, was approved for cancer treatment as early as 1994. It has been widely used in the treatment of non-small cell lung cancer, advanced colon cancer, pancreatic cancer, biliary tract cancer, gastric cancer, CC, etc. It can be used alone or in combination [28,29]. The three drugs we found through CMAP have been widely used in the treatment of various cancers, including CC. The inhibitory effects of these three drugs on *UBE2V1* need to be further verified, *UBE2V1* may be an important gene

A

Gene	Correlation coefficient	P-value
STAU1	0.704	1.86E-39
SERINC3	0.696	2.91E-38
ARFGEF2	0.692	1.31E-37
TTPAL	0.687	5.90E-37
TOP1	0.681	4.55E-36
PMF1	-0.484	2.09E-16
EDF1	-0.484	2.36E-16
BLOC1S1	-0.486	1.53E-16
SARNP	-0.49	8.00E-17
ATP5PO	-0.499	1.70E-17

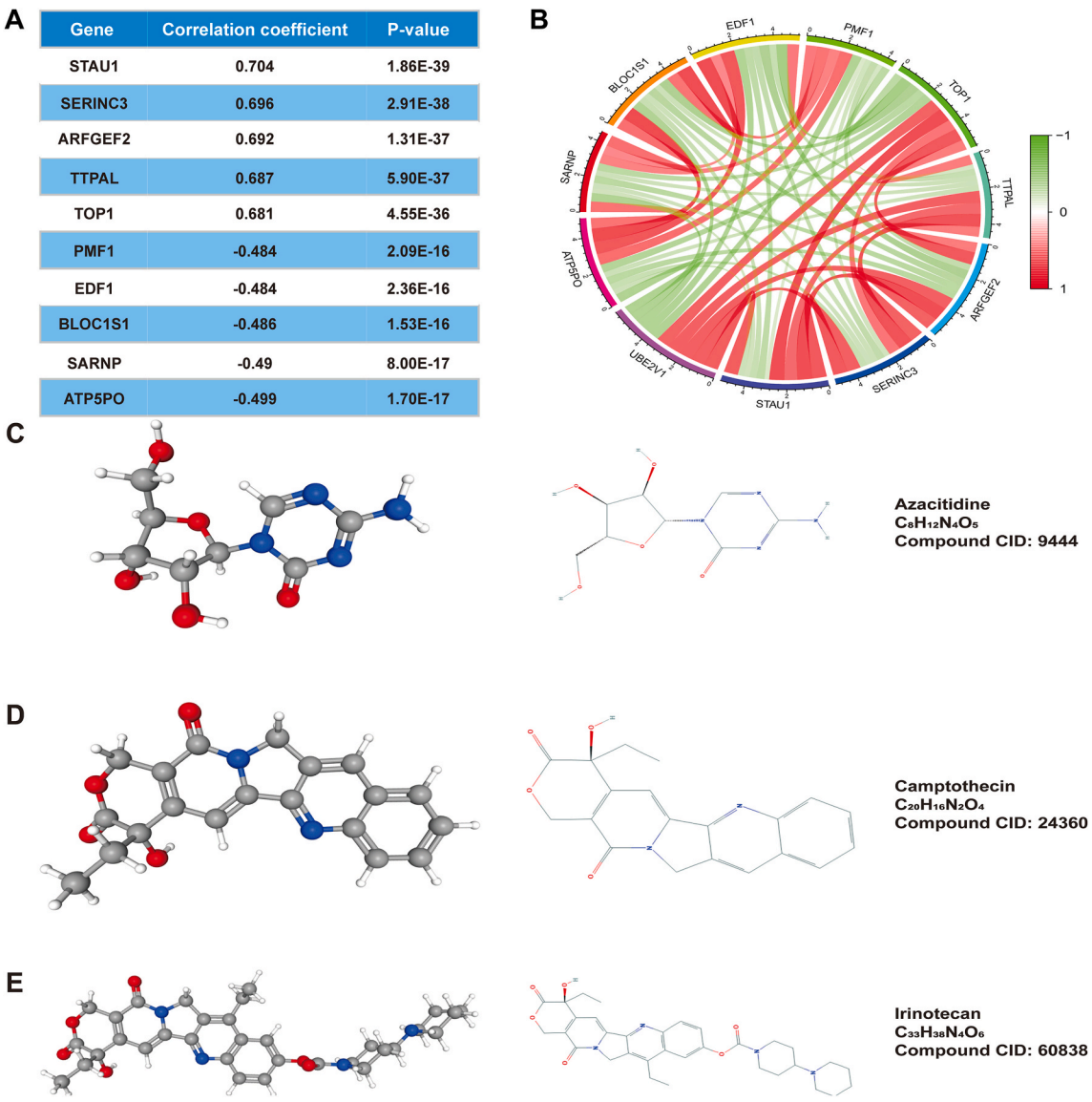

Fig. 3. Genes associated with *UBE2V1* expression based on co-expression analysis (A, B) and small molecular compounds with targeted inhibitory effect on *UBE2V1* expression were identified by CMap (C–E). C : Azacitidine. D: Camptothecin. E: Irinotecan.

Table 1
Small molecule compounds predicted by CMap.

NO.	CMap name	enrichment	p
1	Azacitidine	-0.973	0.00006
2	Camptothecin	-0.977	0.00004
3	Irinotecan	-0.915	0.00106

Enrichment < -0.9, p < 0.05. CMap: connectivity map.

target of these drugs in the treatment of CC.

Based on the clinical information in TCGA database, we revealed the correlation between the abnormal high expression of *UBE2V1* and the malignant progression of CC from the aspects of transcriptional profile and DNA methylation and found that *UBE2V1* is a new potential biomarker for CC screening and prognosis. But our research still has some limitations. First, the incidence rate of CC is closely related to the level of health care in China. The number of deaths in CC is less than 85% in the undeveloped or developing countries. The mortality rate of CC in low-income and middle-income countries is about 18 times that of the rich countries. The incidence of central and South America is the highest in the world [13]. In this study, we lack the data of ethnic and regional classification, which is likely to cause bias in the results. In addition, in the study of the correlation between *UBE2V1* and TNM staging of CC, there is a lack of M staging data. The lack of some specific clinical data is an unavoidable drawback of public databases. Therefore, we have carried out relevant research from the aspects of transcriptional profile and DNA methylation to ensure the reliability of our data.

5. Summary

Our study revealed for the first time that overexpression of *UBE2V1* predicted poor prognosis of CC from transcriptional profile and DNA methylation levels, and was closely related to clinical stages, tissue grades and TNM stages of CC. We also found methylation sites of *UBE2V1* that may play a role in the malignant progression of CC, which provided insight for the development of *UBE2V1* as a new target for diagnosis and treatment of CC.

Author contributions

ZSR, ZDL and SQM conceived the idea of the manuscript, provided the general concept and inputs for each specific section, and drafted part

of the manuscript. JMY, JMY and RYW collected and collated the relevant data. YQG and YZG provided project funding and critical comments on the manuscript. Finally, all the authors read and approved it for publication.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101108.

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