Survival-related DLEU1 is associated with HPV infection status and serves as a biomarker in HPV-infected cervical cancer

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Abstract. Human papillomavirus (HPV) is the most common risk factor for the occurrence of cervical cancer (CC). In recent years, the important roles of long non-coding RNAs (lncRNAs) in CC have emerged, but studies on the relationship between lncRNAs and HPV-positive (HPV+) CC remain scarce. The present study aimed to investigate whether lncRNA deleted in lymphocytic leukemia 1 (DLEU1) is associated with HPV infection and explore the clinical significance of DLEU1 in HPV+ patients with CC. DLEU1 expression was detected by reverse transcription-quantitative PCR. The ability of DLEU1 to screen patients with CC from controls and differentiate individuals with different HPV infection status was evaluated by receiver operating characteristic analysis. The association of DLEU1 with the survival prognosis of patients with CC was assessed by Kaplan-Meier survival analysis and Cox regression analysis. The RNA Interactome Database was used to predict molecules interacting with DLEU1. The results indicated that DLEU1 expression was significantly upregulated in CC tissues and cell lines, particularly in those that were HPV+. In addition, DLEU1 had a high diagnostic value in discriminating patients with CC and differentiating between HPV+ and HPV- patients with CC, and had a certain ability to screen HPV+ controls. DLEU1 was correlated with HPV infection in CC patients. Furthermore, DLEU1 was indicated to be associated with survival prognosis in both total patients with CC and HPV+ patients with CC, and independently predict the prognosis of patients with CC. Most of the molecules interacting with DLEU1 were microRNAs. In conclusion, abnormal DLEU1 expression is associated with HPV infection and may serve as a diagnostic and prognostic biomarker for HPV+ patients with CC.

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Introduction

Cervical cancer (CC) has a high incidence in developing countries and is one of the most common causes of cancer-associated mortality in females (1-3), seriously threatening their health, life and safety (4). CC is divided into two subtypes, squamous cell carcinoma (SCC) and adenocarcinoma (5). High-risk human papillomavirus (HR-HPV) infection is considered to be the most crucial risk factor for CC and is closely related to the occurrence and development of CC (6). Although the morbidity and mortality of CC have decreased due to the implementation of prevention programs in recent years, the prognosis of CC is still not promising (7). Therefore, exploring the factors associated with HPV infection is expected to provide novel ideas for the diagnosis and treatment of CC.

Studies have pointed out that the differences in the pathological process between HPV infection-positive (HPV+) and -negative (HPV-) patients may be related to heterogeneous epigenetic changes in non-coding RNAs, including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs/miRs) (8). To date, various miRNAs associated with the development of HPV+ CC have been identified and reported (9,10). HPV infection is associated with the aberrant expression of certain lncRNAs (11), whilst reports on lncRNAs associated with HPV infection are limited. LncRNAs have roles in numerous diseases (12-16), including CC (17-19). The lncRNA deleted in lymphocytic leukemia 1 (DLEU1) has been indicated to have a role in promoting tumor progression in CC (20), and in the present study, data from the Cancer Genome Atlas (TCGA) database were analyzed to indicate that DLEU1 may be associated with the prognosis of patients with CC. However, whether the aberrant expression of DLEU1 in patients with CC is associated with HPV infection and what role it has in HPV-infected CC had remained elusive.

Therefore, the purpose of the present study was to determine the expression levels of DLEU1 in patients with CC with different HPV infection status, explore whether DLEU1 was related to HPV infection and investigate the clinical significance of DLEU1 in HPV-infected patients with CC. The present study provided a novel biomarker for the diagnosis and survival prognosis of HPV-infected patients with CC and indicated a novel target for the treatment of HPV-infected CC.

Materials and methods

Patients and tissue collection. In the present study, 128 patients with CC who were admitted to Weifang People's Hospital (Weifang, China) for treatment between March 2012 and May 2016 were included and the patients' tumor tissues were collected. The inclusion criteria were as follows: i) Patients were pathologically diagnosed with CC; and ii) none of the patients with CC received chemotherapy, radiotherapy or other adjuvant treatments. Patients were excluded from the present study if they fulfilled the following criteria: i) Presence of other malignant tumors; and ii) current pregnancy or lactation. A total of 99 patients who underwent hysterectomy due to hysteromyoma at the same hospital during the same period were collected as controls and the participants in the control group were confirmed to have no CC or precancerous lesions. Cervical tissue samples were collected from controls at the time of hysterectomy. After the surgery, all tissue samples were immediately stored in liquid nitrogen until use. Samples of patients with CC and controls were subjected to HPV-DNA testing to determine the presence of HPV infection. There were 90 HPV+ patients and 38 HPV- patients among the 128 patients with CC; the 98 controls included 58 HPV+ samples and 41 HPV- samples. All patients attended the 5-year survival follow-up and their survival data were recorded. Each participant was signed an informed consent form.

Cell culture. The HPV16+ CC cell line SiHa [cat. no. HTB-35; American Type Culture Collection (ATCC)], the HPV18+ CC cell line HeLa (cat. no. CRM-CCL-2; ATCC), the HPV- CC cell line C33A (cat. no. HTB-31; ATCC) and the HPV- normal immortalized epithelial cell line HaCaT (cat. no. 300493/p800_HaCaT; Cell Line Services GmnH) were used in the present study. The cells were then cultured in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) with 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin. The conditions of the cell culture were 5% CO_2 and 37˚C.

Bioinformatics analysis. Gene Expression Profiling Interaction Analysis 2.0 (GEPIA 2.0; http://gepia2.cancer-pku.cn/#index) was used to evaluate the expression of DLEU1 in CC tissues and analyze the association of DLEU1 with overall survival of patients with CC based on the TCGA database. The molecules that interacted with DLEU1 were predicted using RNA Interactome Database (RNAInter; http://www.rna-society.org/raid/home.html).

RNA extraction. TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNA. Each sample was checked at least 3 times. The tissue was homogenized with a homogenizer after the addition of the lysate, followed by the addition of TRIzol reagent. To extract RNA from cells, the medium was discarded, cells were washed extensively with PBS to remove residual medium and then TRIzol was added directly into the cell culture dish to lyse the cells. After complete lysis, two-phase separation, RNA precipitation, RNA clean-up, RNA drying and dissolving RNA precipitation were performed. The obtained RNA solution was stored at -80˚C for further use. The purity and concentration of RNA were verified by a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Inc.) and RNA was used for further analysis when the optical density ratio of 260/280 nm was close to 2.0.

Reverse transcription-quantitative PCR (RT-qPCR). A total of 1 μg RNA was then reverse-transcribed into cDNA using a Reverse Transcription Kit (Takara Biotechnology, Co., Ltd.). SYBR-Green Real-time PCR Master Mix kit (Toyobo) in the 7900HT fast real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) were used for qPCR, which was performed to detect the expression of DLEU1. The thermal cycling conditions were as follows: 95˚C for 5 min, and then 40 cycles of 95˚C for 20 sec, 50˚C for 30 sec and 72˚C for 30 sec. The primers were all synthesized by GenePharma and the primer sequences were as follows: DLEU1 forward, 5'-CGTGCATTTAAAACCGCC-3' and reverse, 5'-TGTCTGCATTGTGACTCAATTC-3'; GAPDH forward, 5'-ATGATGACATCAAGAAGGTGGTG-3' and reverse, 5'-CCATGAGGTCCACCACCCTGTTG-3'. DLEU1 expression was normalized to GAPDH and was calculated using the $2^{-\Delta\Delta Cq}$ method (21).

Statistical analysis. All analyses were performed by SPSS 22.0 (IBM Corporation) and GraphPad Prism 7.0 software (GraphPad Software, Inc.). Values are expressed as the mean ± standard deviation. Differences in measurement data between two groups and among multiple groups were compared by Student's t-test and one-way analysis of variance followed by Tukey's test, respectively. Comparison between categorical variables was performed by the χ^2 test. Receiver operating characteristic (ROC) analysis was used to evaluate the ability of DLEU1 to screen patients with CC from controls, screen HPV+ controls from HPV- controls and screen HPV+ patients with CC from HPV- patients with CC. Kaplan-Meier survival analysis and the log-rank test were used to investigate the relationship between DLEU1 and the overall survival of patients with CC. Multivariate Cox regression analysis was used to evaluate the prognostic value of DLEU1 in patients with CC. All analyses were independently repeated at least 3 times. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Baseline characteristics of the participants. Table I presents the baseline characteristics of all participants included. There were no significant differences in age, body mass index (BMI) and HPV infection status between controls and patients with CC (all $P>0.05$). In addition, the tumor size of patients with CC was 3.6±0.9 cm. Furthermore, patients with CC were divided into 106 cases with SCC and 22 cases with adenocarcinoma, 96 cases with negative and 32 cases with positive lymph node metastasis or 90 cases with Federation of Gynecology and Obstetrics (FIGO) stage I-II and 38 cases with FIGO stage III-IV.

Expression of DLEU1 in patients with CC and cell lines. DLEU1 was expressed at a significantly higher level in the tumor tissues of patients with CC compared with that in normal controls ($P<0.001$; Fig. 1A). The results of the TCGA

Table I. Baseline characteristics of the participants.

Characteristic	Normal controls (n=99)	CC patients (n=128)	P-value
Age, years	46.4±3.0	47.1±2.8	0.104
BMI, kg/m^2	23.6±4.4	23.7±3.9	0.934
HPV infection			0.066
Negative	41	38	
Positive	58	90	
Tumor size (cm)	-	3.6±0.9	-
Histological type			-
SCC	-	106	
Adenocarcinoma	-	22	
Lymph node metastasis			-
Negative	-	96	
Positive	-	32	
FIGO stage			-
I-II	-	90	
III-IV	-	38	

Values are expressed as the mean ± standard deviation or n. CC, cervical cancer; BMI, body mass index; HPV, human papillomavirus; SCC, squamous cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics.

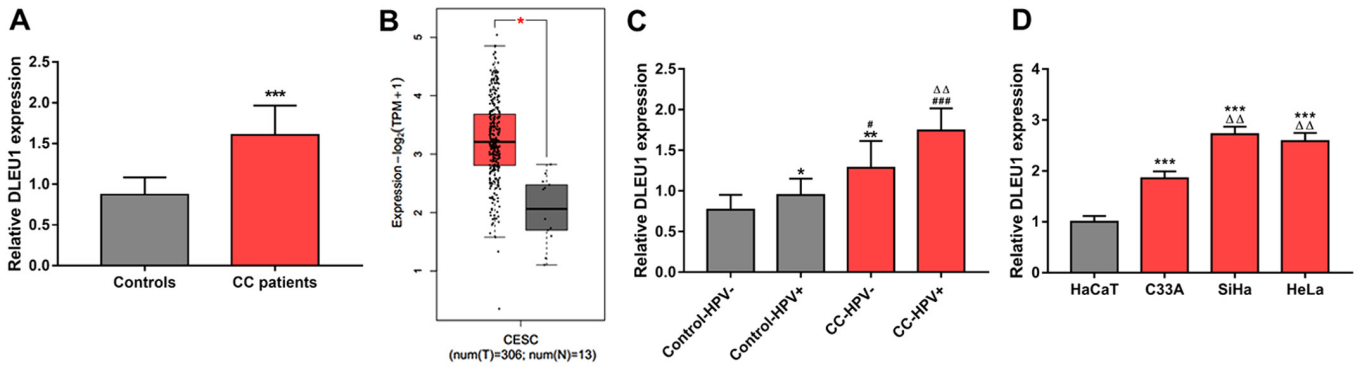


Figure 1. Expression of DLEU1 in patients with CC and cell lines. (A) Expression of DLEU1 in patients with CC and controls. (B) Expression of DLEU1 was increased in CC tissues according to the analysis results of TCGA data. (C) Expression of DLEU1 in HPV+ and HPV- controls and in patients with CC with HPV+ and HPV- status. (D) Expression of DLEU1 in a normal cell line (HaCaT), two HPV+ CC cell lines (SiHa and HeLa) and an HPV- CC cell line (C33A). *P<0.05, **P<0.01, ***P<0.001 vs. Controls or CC tissues from TCGA data or Control-HPV- or HaCaT; $^{\#}$P<0.05, $^{\#\#\#}$P<0.001 vs. Control-HPV+; $^{\Delta\Delta}$P<0.01 vs. CC-HPV- or C33A. CC, cervical cancer; CESC, cervical squamous cell carcinoma; TCGA, the Cancer Genome Atlas; HPV, human papillomavirus; DLEU1, long noncoding RNA deleted in lymphocytic leukemia 1; T, tumor; N, normal; num, number.

data analysis were consistent with the results of the present study, indicating that DLEU1 was significantly upregulated in CC tissue samples (P<0.05; Fig. 1B). As indicated in Fig. 1C, HPV+ controls had a significantly higher level of DLEU1 than HPV- controls (P<0.05); furthermore, patients with CC with HPV- status had higher levels of DLEU1 than both HPV+ and HPV- controls, but the difference from HPV+ controls was somewhat smaller (all P<0.05); in addition, DLEU1 levels were the highest in patients with CC with HPV+ and the difference of DLEU1 expression between HPV+ patients with CC and HPV- patients with CC also reached statistical significance (all P<0.01). Analysis of DLEU1 levels in cells similarly indicated significantly increased DLEU1 in both HPV- CC cells and HPV+ CC cells compared with that in normal cells; furthermore, DLEU1 expression was higher in HPV+ CC cells than that in HPV- CC cells (all P<0.01; Fig. 1D). The raw data of DLEU1 expression are provided in Table SI.

Differentially expressed DLEU1 used to distinguish patients with CC with different HPV infection conditions. Through ROC analysis, the diagnostic value of DLEU1 was explored. The results of Fig. 2A suggested that DLEU1 was able to screen patients with CC from controls with an area under the ROC curve (AUC) of 0.951. In the control group, DLEU1 had a certain utility in differentiating between HPV+ and HPV- populations (Fig. 2B). The results of Fig. 2C suggested that DLEU1 had an ability to discriminate between HPV+ and HPV- patients with CC with an AUC of 0.867.

Table II. Association between DLEU1 expression and clinicopathological characteristics of patients with cervical cancer.

Characteristic	Total (n=128)	Low DLEU1 (n=62)	High DLEU1 (n=66)	P-value
Age, years				0.973
<47	58	28	30	
≥47	70	34	36	
BMI, kg/m^2				0.715
<24	66	33	33	
≥24	62	29	33	
HPV infection				0.003
Negative	38	26	12	
Positive	90	36	54	
Tumor size, cm				0.034
<4	81	44	37	
≥4	47	18	29	
Histological type				0.437
SCC	106	53	53	
Adenocarcinoma	22	9	13	
Lymph node metastasis				0.008
Negative	96	53	43	
Positive	32	9	23	
FIGO stage				0.001
I-II	90	52	38	
III-IV	38	10	28	

BMI, body mass index; HPV, human papillomavirus; SCC, squamous cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; DLEU1, long noncoding RNA deleted in lymphocytic leukemia 1.

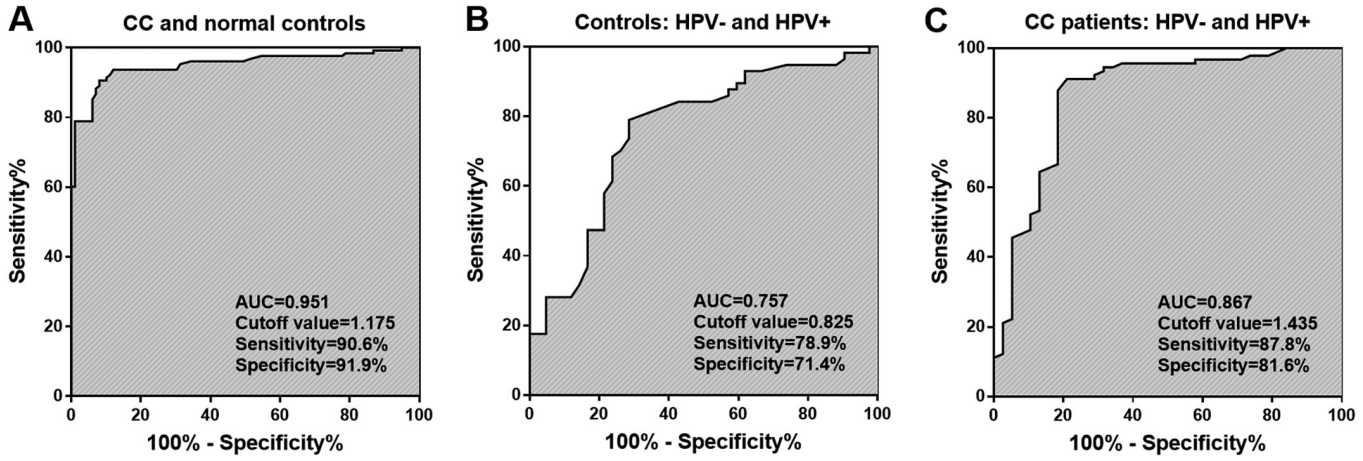


Figure 2. Ability of DLEU1 expression to distinguish patients with CC and controls with different HPV infection status. (A) DLEU1 had a high diagnostic value in screening patients with CC from controls. (B) DLEU1 had a certain ability to screen controls with HPV+ from controls with HPV- status. (C) DLEU1 had diagnostic value in the screening of patients with CC with HPV+ from HPV- patients with CC. CC, cervical cancer; HPV, human papillomavirus; AUC, area under the ROC curve; ROC, receiver operating characteristic; DLEU1, long noncoding RNA deleted in lymphocytic leukemia 1.

Association between DLEU1 expression and clinicopathological characteristics of patients with CC. As presented in Table II, DLEU1 was indicated to be associated with tumor size, HPV infection, lymph node metastasis and FIGO stage in patients with CC (all P<0.05). However, no association was obtained between DLEU1 expression and age, BMI or histological type (all P>0.05).

As indicated in Fig. 3A, there was no significant difference in DLEU1 expression between patients with CC with SCC and adenocarcinoma (P>0.05). Furthermore, the expression of DLEU1 was significantly increased in patients with CC with positive vs. negative lymph node metastasis (P<0.001; Fig. 3B) and patients with CC with FIGO stage III-IV vs. I-II (P<0.001; Fig. 3C).

Table III. Multivariate Cox regression analysis for the survival of patients with cervical cancer.

Variable	HR	95% CI	P-value
Age (≥47 vs. <47)	1.231	0.812-1.698	0.329
BMI (≥24 vs. <24)	1.189	0.703-1.777	0.632
HPV infection (positive vs. negative)	1.121	0.728-1.803	0.417
Tumor size (≥4 vs. <4)	1.437	0.841-2.046	0.223
Histological type (adenocarcinoma vs. SCC)	1.237	0.938-1.603	0.129
Lymph node metastasis (positive vs. negative)	1.692	1.115-2.388	0.042
FIGO stage (III-IV vs. I-II)	2.124	1.560-2.974	0.006
DLEU1 (high vs. low)	2.437	1.612-3.329	0.004

HR, hazard ratio; HPV, human papillomavirus; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; DLEU1, long noncoding RNA deleted in lymphocytic leukemia 1.

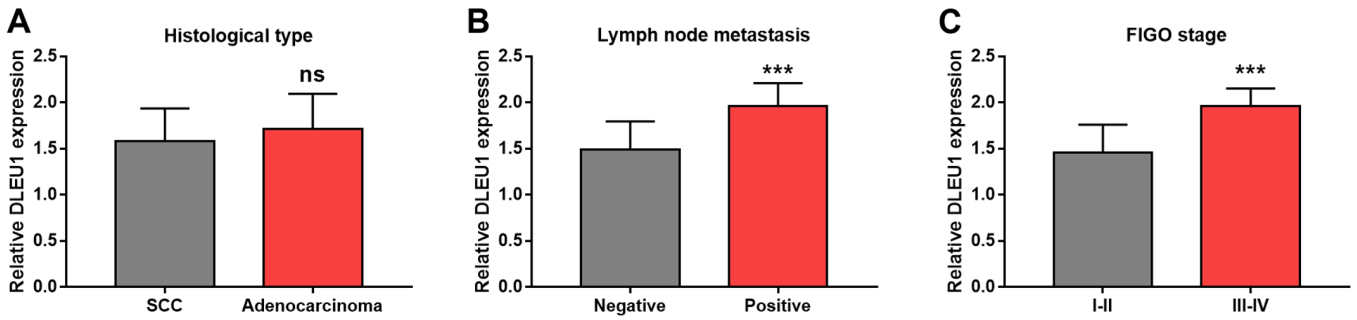


Figure 3. Association between DLEU1 expression and histological type, lymph node metastasis and FIGO stage of patients with CC. (A) DLEU1 expression in patients with CC with SCC and adenocarcinoma. (B) DLEU1 expression in patients with CC with negative and positive lymph node metastasis. (C) DLEU1 expression in patients with CC with FIGO stage I-II and III-IV. ***P<0.001 vs. Negative lymph node metastasis or FIGO stage I-II. ns, no significance; CC, cervical cancer; SCC, squamous cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; DLEU1, long noncoding RNA deleted in lymphocytic leukemia 1.

High DLEU1 predicts poor prognosis of patients with CC. Analysis of the 5-year follow-up data of the present study indicated that patients with CC with high levels of DLEU1 had poor survival prognosis (log-rank P=0.0004; Fig. 4A). Similarly, analysis of the TCGA database indicated that patients with CC with high levels of DLEU1 had poor prognosis (log-rank P=0.0028; Fig. 4B). After dividing the patients into HPV+ and HPV- groups, the relationship between DLEU1 and survival was analyzed separately. The results indicated that after grouping, the relationship between DLEU1 and survival of HPV- patients was not obvious (log-rank P=0.1691; Fig. 4C), but DLEU1 was still associated with survival of HPV+ patients (log-rank P=0.0026; Fig. 4D). As presented in Table III, lymph node metastasis [hazard ratio (HR)=1.692, 95% confidence interval (CI)=1.115-2.388, P=0.042], FIGO stage (HR=2.124, 95% CI=1.560-2.974, P=0.006) and DLEU1 (HR=2.437, 95% CI=1.612-3.329, P=0.004) were independently associated with the survival prognosis of patients with CC.

Predicted molecules interacting with DLEU1. Molecules interacting with DLEU1 were predicted using the RNAInter database, most of which were miRNAs. The top 5 predicted interacting molecules were miR-490-3p, human cytomegalovirus (hcmv)-miR-US25-1-5p, 13q14.3, miR-371a-5p and miR-506-3p (Fig. 5).

Discussion

DLEU1 has been indicated to have a promoting role in CC progression (20). Consistently, the present study demonstrated that the expression of DLEU1 was increased in CC tissues from the present cohort and TCGA database. In addition, in patients with CC, DLEU1 expression was related to tumor size, HPV infection, lymph node metastasis and FIGO stage. Furthermore, DLEU1 has been reported to be involved in the progression of other cancer types, such as glioma (22), endometrial cancer (23) and oral squamous cell carcinoma (OSCC) (24). Thus, DLEU1 is involved in the progression of patients with CC.

It is known that infection by HPV is closely related to the progression of patients with CC. Of note, abnormal lncRNAs have been reported to be associated with HPV infection in cancers, including CC. For instance, lncRNA psoriasis-susceptibility-related RNA gene induced by stress (PRINS) has been indicated to be markedly higher in HPV+ patients with head and neck squamous cell carcinoma (HNSCC) than that in HPV- patients with HNSCC, and the expression of PRINS was significantly related to HPV-infected HNSCC (25). Zhou *et al* (26) reported that lncRNA oncogene-induced senescence 1 (lncRNA-OIS1) is decreased in HPV+ patients with cervical squamous cell carcinoma (CSCC) and HPV+

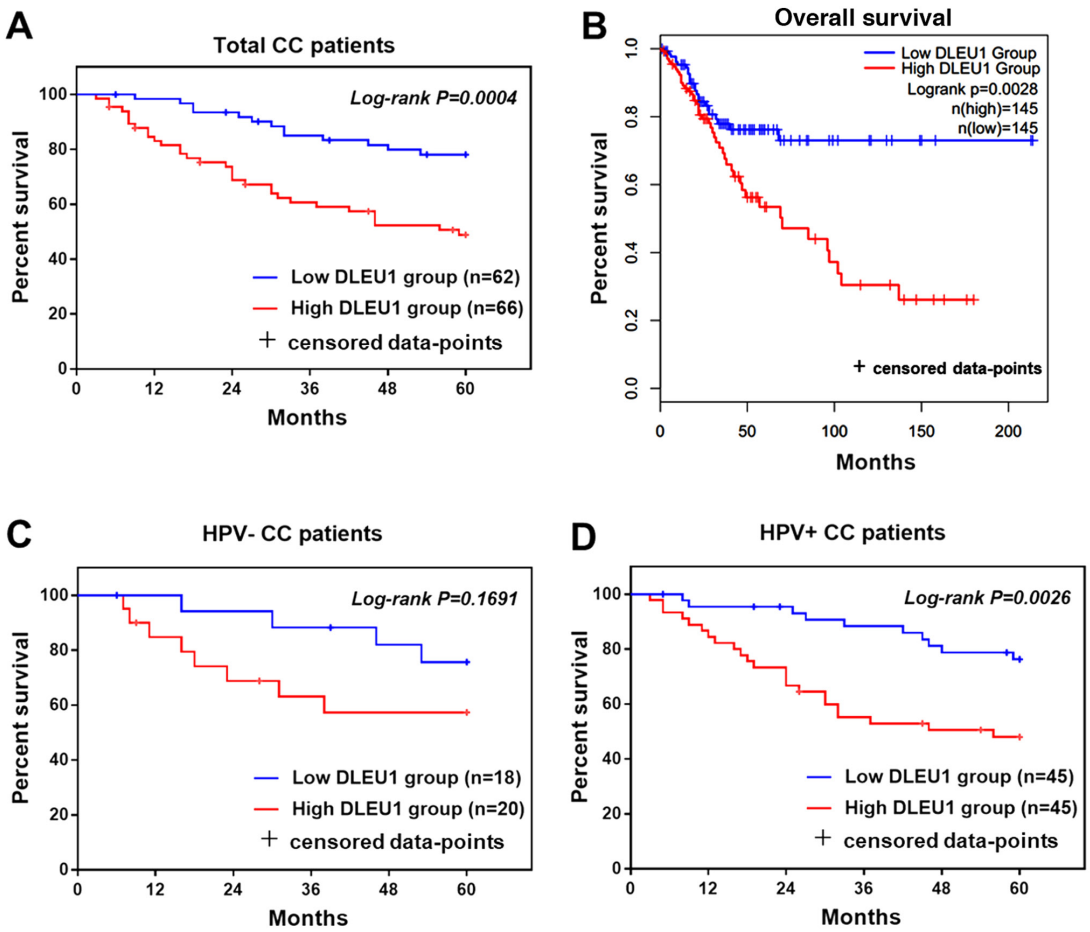
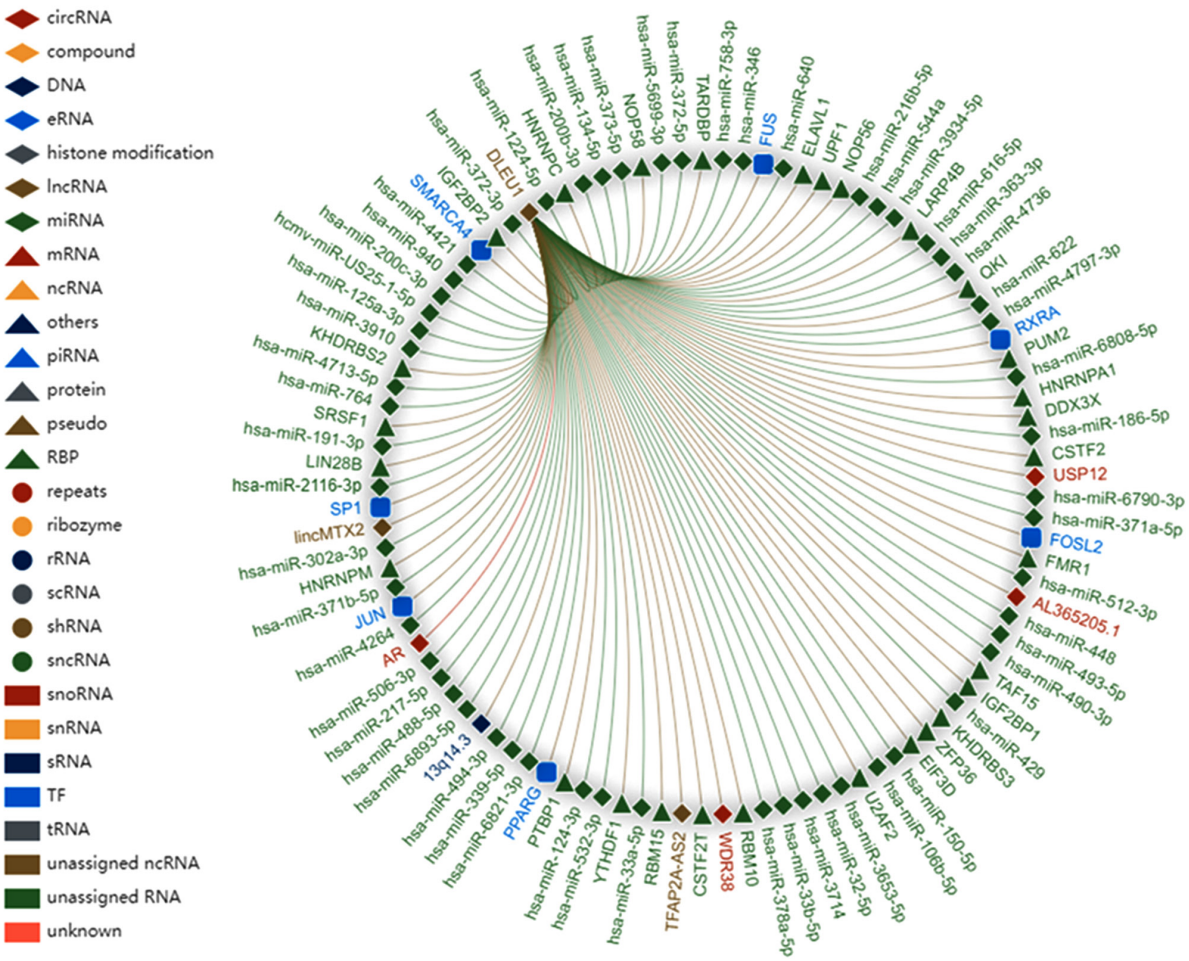


Figure 4. High DLEU1 predicts poor prognosis in patients with CC. (A) High DLEU1 was associated with poor survival in total patients with CC (log-rank P=0.0004). (B) Analysis of the TCGA database data indicated an association between high DLEU1 and poor survival (log-rank P=0.0028). (C) There was no obvious relationship between DLEU1 and the survival of HPV- patients with CC (log-rank P=0.1691). (D) DLEU1 expression was still associated with survival of HPV+ patients with CC (log-rank P=0.0026). CC, cervical cancer; TCGA, the Cancer Genome Atlas; HPV, human papillomavirus; DLEU1, long noncoding RNA deleted in lymphocytic leukemia 1.

CSCC cells and may enhance the proliferation of HPV+ CSCC cells. A study by Zhang *et al* (27) determined upregulated expression of lncRNA human ovarian cancer-specific transcript 2 (lncRNA HOST2) in HPV+ CC tissues and cells and the promoting effect of HOST2 on HPV+ CC cell function. The levels of long intergenic non-protein coding RNA (LINC)01101 and LINC00277 have been indicated to be decreased in HR-HPV+ samples (28). In the present study, DLEU1 expression was higher in HPV+ controls than that in HPV- controls and it was higher in HPV+ patients with CC than that in HPV- patients with CC. Furthermore, DLEU1 expression in HPV+ CC cells was significantly higher when compared with that in HPV- CC cells. In addition, by using the χ^2 test, DLEU1 was indicated to be associated with HPV infection in patients with CC. Thus, DLEU1 is associated with HPV infection and may be involved in the progression of HPV-infected CC.

Accumulating evidence has indicated that lncRNAs may serve as diagnostic and prognostic biomarkers in different types of cancer (29-31). In addition, certain lncRNAs, such as lncRNA SOX21 antisense divergent transcript 1 (32) and lncRNA LIPE antisense RNA 1 (LIPE-AS1) (33), have been indicated to be of use as diagnostic and prognostic biomarkers for patients with CC. The present study also suggested that DLEU1 had the ability to screen patients with CC from normal controls. In addition, DLEU1 was closely related to survival of patients with CC according to analyses of the present study cohort and TCGA database, and was able to independently predict the prognosis of patients with CC. Thus, DLEU1 may be used as a biomarker for the diagnosis of CC and prognostic survival prediction for affected patients.

It has been indicated that lncRNA loc285194 (34), lncRNA heart and neural crest derivatives expressed 2-antisense RNA 1 (35) and lncRNA-OIS1 (26) were able to screen HPV+ patients with CSCC from normal controls. In addition, lncRNA steroid receptor RNA activator 1 was indicated to have high diagnostic value in screening HPV+ patients with CSCC from HPV- CSCC patients (36). Furthermore, PRINS expression was reported to correlate with overall survival in HPV+ HNSCC patients (25). In the present study, DLEU1 had a high diagnostic value in screening HPV+ patients with CC and had a certain ability to screen HPV+ controls. In addition, there was a significant correlation between DLEU1 and survival of HPV+ patients with CC. However, there was no significance regarding the Kaplan-Meier curve results in HPV- patients with CC. This nonsignificant result may be due to the small number of HPV- patients with CC in the present study. Of note, DLEU1 may be used to diagnose and predict the prognosis



Interactor1	Category1	Species1	Interactor2	Category2	Species2	Score
*hsa-miR-490-3p	miRNA	Homo sapiens	DLEU1	lncRNA	Homo sapiens	0.7704
hcmv-miR-US25-1...	miRNA	Human betaherpesv...	DLEU1	lncRNA	Homo sapiens	0.7311
DLEU1	lncRNA	Homo sapiens	13q14.3	DNA	Homo sapiens	0.7311
hsa-miR-371a-5p	miRNA	Homo sapiens	DLEU1	lncRNA	Homo sapiens	0.6841
hsa-miR-506-3p	miRNA	Homo sapiens	DLEU1	lncRNA	Homo sapiens	0.6726

Figure 5. Predicted molecules interacting with DLEU1 and most of which are miRNAs. *, expression level of hsa-miR-490-3p is lower than hsa-miR-490-5p in the body. miRNA, microRNA; hsa-miR-490-3p, homo sapiens-microRNA-490-3p; hcmv-miR-US25-1-5p, human cytomegalovirus-microRNA-US25-1-5p; hsa-miR-371a-5p, homo sapiens-microRNA-371a-5p; hsa-miR-506-3p, homo sapiens-microRNA-506-3p; DLEU1, lncRNA deleted in lymphocytic leukemia 1; lncRNA, long noncoding RNA.

of other cancer types, such as OSCC (24), glioma (37) and breast cancer (38). The above results indicated that DLEU1 may be a biomarker for the diagnosis and prognosis of HPV+ patients with CC.

Finally, the molecules interacting with DLEU1, which may be involved in DLEU1 exerting its biological function, were predicted. Most of the molecules predicted were miRNAs, illustrating that DLEU1 as an lncRNA may bind miRNAs to regulate key genes to exert its function. Certain studies have reported the role of the lncRNA/miRNA axis in HPV+ CC. For instance, Zhang *et al* (39) revealed that LINC00511 may aggravate HPV+ and HPV- CC by targeting the miR-324-5p/DNA damage regulated autophagy modulator 1 axis. The lncRNA metastasis-associated long adenocarcinoma transcript 1/miR-124/root rake brush grapple 2 axis was reported to be involved in the function of HR-HPV+ CC cells (40). The present study found that hcmv-miR-US25-1-5p is a molecule interacting with DLEU1. In addition, it is worth noting that hcmv-miR-US25-1-5p encoded miRNA and is associated with cytomegalovirus (41). More importantly, a study suggested that human herpesviruses-6 together with cytomegalovirus may promote the development of CC (42). Thus, it was hypothesized that DLEU1 may have a role in HPV-infected CC by regulating hcmv-miR-US25-1-5p, which will be the subject for future research. The prediction of molecules interacting with DLEU1 may serve as a basis for subsequent studies and offers suggestions for future investigations.

The present study was the first, to the best of our knowledge, to explore the association between DLEU1 and HPV infection status and to explore its clinical significance in HPV-infected CC. However, the study sample was small, which is a limitation of the present study, and a large cohort is required in further studies. In addition, as a non-coding RNA, DLUE1 has no ability to code proteins, and thus, it was not possible to measure any protein distribution and levels of DLEU1 in tissue samples. However, the exploration of the downstream miRNAs and target genes may be able to compensate for this deficiency, thus illustrating the expression patterns and function of DLEU1 in CC.

In conclusion, the present study indicated that DLEU1 expression was increased in HPV-infected patients with CC and cells, and was associated with HPV infection in patients with CC. In addition, DLEU1 may be used as a biomarker to distinguish HPV+ patients with CC from HPV- patients with CC and predict the prognosis of HPV-infected patients with CC. The present study may provide novel diagnostic and prognostic biomarkers for the treatment of HPV-infected patients with CC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

XM and BX were responsible for the design and conception of the study. ZW and AD analyzed and interpreted the data. AD and BX performed the cell experiments and the other analyses. ZW and XM contributed essential reagents or tools. All authors wrote and revised the manuscript. All authors read and approved the final manuscript. AD and XM confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The experimental procedures were all in accordance with the guidelines of the Ethics Committee of Weifang People's Hospital (Weifang, China) and were approved by the Ethics Committee of Weifang People's Hospital (Weifang, China; approval no. 0012064). The present study complies with the Declaration of Helsinki. Written informed consent was obtained from each participant.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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