

## Over-expression of EPB41L3 promotes apoptosis of human cervical carcinoma cells through PI3K/AKT signaling

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**Background:** Cervical cancer is a significant malignancy of the female reproductive system. This study aimed to investigate the functions of Erythrocyte Membrane Protein Band 4.1 Like 3 (EPB41L3) in cervical cancer and to explore its underlying mechanisms. **Methods and Results:** Expression of EPB41L3 in cervical cancer was analyzed by in-depth mining in The Cancer Genome Atlas (TCGA) clinical sequencing database. Patients with high expression of EPB41L3 had a good prognosis. EPB41L3 was overexpressed in HeLa and SiHa cells by a chemically synthesized lentivirus; its overexpression significantly inhibited cell proliferation, promoted apoptosis of HeLa and SiHa cells, and suppressed tumorigenesis in nude mice bearing HeLa cells. The results of microarray analysis showed that EPB41L3 overexpression led to marked gene expression changes, including 258 up-regulated genes and 168 down-regulated genes. Furthermore, EPB41L3 overexpression inhibited PI3K and AKT phosphorylation, leading to the apoptosis of cervical cancer cells. **Conclusions:** EPB41L3 overexpression inhibits cell proliferation, promotes apoptosis of cervical cancer cells, and suppresses tumorigenicity *in vivo*. Our findings suggest that EPB41L3 might be a new diagnostic biomarker and a therapeutic target for cervical cancer.

**Keywords:** Cervical cancer, EPB41L3, proliferation, apoptosis, PI3K, AKT

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### INTRODUCTION

Cervical cancer is one of the most common malignancies of the female reproductive system. Recently, the age of onset of cervical cancer has declined (Vu *et al.*, 2018; Fang *et al.*, 2014; Kabekkodu, *et al.*, 2017). Traditional treatments of cervical cancer include radical surgery and chemo-radiotherapy (Liontos *et al.*, 2019). More recent cervical cancer treatments have emerged as neoadjuvant chemotherapy, concurrent chemotherapy, and comprehensive treatment systems, such as targeted therapy, gene therapy, and vaccine prevention (Hu & Ma, 2018). However, cervi-

cal cancer is still a serious threat to women's health. Therefore, personalized treatment programs for cervical cancer patients are important for improving their prognosis and life span.

The development of cervical cancer is a multifactorial process, and mechanism and pathogenesis of which remain unclear. The most relevant factor is a persistent infection by high-risk human papilloma virus (HPV) (Venetianer *et al.*, 2020). Previous studies have shown that multiple or chronic infections activate one or more oncogenes (Wu *et al.*, 2019). For instance, *C-erbB-2* and *c-Myc* serve as oncogenes, *p53* and *Rb* as the tumor suppressor genes, are closely related to the occurrence and development of cervical cancer (Nucci & Crum, 2007; Marquina *et al.*, 2018). High-risk variant genes involved in cervical cancer tumorigenesis were found by high-throughput and whole-genome sequencing of malignant and healthy tissue sections. E6, Beclin1, and LC3 proteins can be primary targets for early tumor diagnosis and for designing targeted drugs (Ojesina, *et al.*, 2014; Khattak, 2017; Grace *et al.*, 2003; Cheng *et al.*, 2012; Zhu *et al.*, 2012; Wang *et al.*, 2007; Sun *et al.*, 2011).

Erythrocyte membrane protein band 4.1-like 3 (EPB41L3) plays an essential role in cell adhesion, cell motility, and cell growth (Tran *et al.*, 1999; Charboneau *et al.*, 2002; Bernkopf *et al.*, 2008). EPB41L3 has been reported to act as a tumor suppressor gene (TSG) in various cancer types, including lung, breast, ovarian and cervical cancer (Tran *et al.*, 1999). Recent evidence suggested that inhibition of EPB41L3 leads to the disruption of the cytoskeletal organization, which increases the metastasis and invasion potential of cancer cells. Furthermore, overexpression of EPB41L3 dramatically decreases cancer cell growth *in vitro* and *in vivo* (Charboneau *et al.*, 2002; Dafou *et al.*, 2010; Wong *et al.*, 2007; Jiang & Newsham, 2006). Hence, the differential expressed EPB41L3 may function as a conditioning agent in tumor process. However, the function of EPB41L3 overexpression in cervical cancer is still unknown.

In this study, cervical cancer patients with high expression of EPB41L3 were found to have a good prognosis. Over-expression of EPB41L3 inhibited cell proliferation, promoted cell apoptosis *in vitro*, and suppressed tumorigenesis in a nude mouse model. Furthermore, we discovered that EPB41L3 overexpression inactivated PI3K and AKT phosphorylation and promoted apoptosis of cervical cancer cells.

## MATERIALS AND METHODS

### Editorial Policies and Ethical Considerations

The current study was approved by the Ethics Committee of Xinjiang Medical University Affiliated Tumor Hospital. All patients delivered informed consent. Two hundred cervical tissue specimens were collected from patients who underwent a hysterectomy at the Xinjiang Medical University Affiliated Tumor Hospital. According to the diagnosis of histopathology in our hospital, two hundred cervical tissue specimens were divided into 4 groups of 50. EPB41L3 expression was detected in different stages (Chronic Cervicitis, CIN I, CIN II-III and Cervical Cancer).

### Cell culture

All cell lines were purchased from the American Type Culture Collection. A normal cervical cell line Ect1/E6E7 (CRL-2614), and human cervical cancer cell lines HeLa (CCL-2), C-4I (CRL-1594), SiHa (HTB-35), C33A (CRM-HTB-31), and CaSki (CRM-CRL-1550) were maintained in DMEM (Corning, USA) supplemented with 10% fetal bovine serum (FBS, Ausbian, China) and were incubated at 37°C in 5% CO<sub>2</sub>.

### Synthesis and transfection of shRNA

Lentiviral vectors were designed and synthesized by the Rosy Clouds group (Beijing, China). PCR identified the most efficient construct for further experiments. The homologous sequence was GAGGATCCCCGGGTACCGGTCCCAACATGACGACCGAATCTGGATCAG. HeLa and SiHa cells were digested using trypsin (Sangon Biotech, Shanghai) and seeded into 6-well plates. When the cell density reached 2×10<sup>5</sup>/well, we randomly divided the cells into control and experimental groups for transfection. After 12 hours, we changed the medium and the expression of green fluorescent protein (GFP) was detected to confirm the successful transfection of LvEPB41L3 into HeLa and SiHa cells.

### Proliferation assay

LvEPB41L3-transfected HeLa and SiHa cells were seeded into 96-well plates at cell density of 2×10<sup>3</sup>/well. Ten microliters of CCK-8 (Sigma, USA) was added to the wells 2~4 hours before detection. The microplate reader detected the optical density (OD) (Tecan infinite, Switzerland) at 450 nm.

When the transfected cells grew to 80% confluence, the cells were digested into a single-cell suspension. Then, these cells were stained with PI (Sigma, USA) according to the manufacturer's protocol and analyzed by fluorescent-activated cell sorting (FACS) (Millipore, USA).

Clone formation was detected by crystal violet staining. In brief, 1×10<sup>3</sup> cells per well were seeded into 6-well cell culture plates. 10 d later, the cells were stained with crystal violet solution (Sangon Biotech, China), and the clones were photographed by a digital camera and counted. All experiments were performed in triplicate.

### Apoptosis assay

Transfected HeLa and SiHa cells were digested into a single-cell suspension by trypsin 24 hours after culture. All cells were collected and analyzed using a 7AAD/Annexin V-APC apoptosis detection kit (eBioscience,

USA) according to the manufacturer's protocol and analyzed by FACS. All experiments were performed in triplicate.

As for Terminal deoxyribonucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick end-labeling (TUNEL) assay, a TUNEL kit (KeyGEN BioTECH, China) has been used. Briefly, cells were incubated with TUNEL reaction mixture containing 2 μL TdT enzyme and 48 μL fluorescent labeling solution, and then counterstained with DAPI. Images were acquired on a Nikon microscope (90i, Japan).

### Tumor formation in BALB/c nude mice

All experiments were conducted under the ethical guidelines for animal experiments of Xinjiang Medical University. HeLa cells were transfected with LvCtrl and LvEPB41L3. 1×10<sup>6</sup> transfected HeLa cells were transplanted into BALB/c nude mice (female, four weeks of age, n=5) and subcutaneously injected under the left and right axilla. Bodyweight and tumor volume were examined every day. Length (L) and width (W) of the tumor were measured with calipers to monitor the tumor volume (V). The calculation formula was  $V = (L \times W^2) \times \pi / 6$ . Tumor weight was measured with a tray balance (Sartorius, Germany).

### Microarray analysis

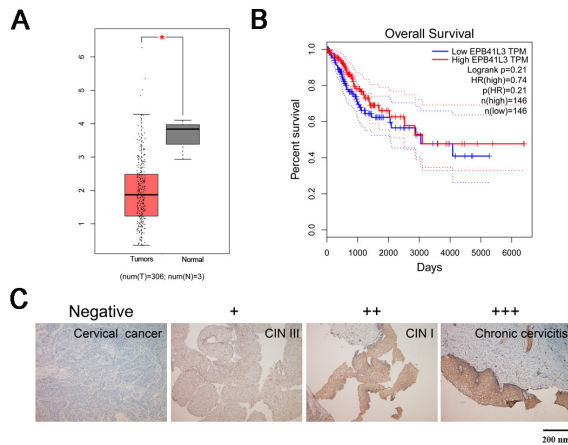
Total RNA of HeLa cells transfected with LvCtrl and LvEPB41L3 was isolated with an RNA 6000 Nano Kit (Agilent Technologies, USA). The Limma13 (version 3.83, linear models for microarray data, [www.bioconductor.org/packages/2.8/bioc/html/limma.html](http://www.bioconductor.org/packages/2.8/bioc/html/limma.html)) package in the Bioconductor Affymetrix annotation files of Brain Array Lab was used to preprocess and calculate the gene expression profile. Differentially expressed genes in the original path analysis were analyzed by Qiagen's original pathway analysis algorithm ([www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity), Qiagen, Redwood City, CA, USA). Typical path analysis, upstream analysis and interaction network analysis were used.

### Western Blot Analysis

Cells were lysed in RIPA buffer (Beyotime, China) containing a protease inhibitor cocktail without EDTA (Roche, USA). The protein samples were electrophoresed on 6–10% SDS-PAGE, transferred to polyvinylidene fluoride membranes (Millipore, Billerica, USA), blocked with 5% bovine serum albumin for one hour and then incubated overnight with the primary antibodies at 4°C. The antibodies were directed against EPB41L3 (Proteintech, USA), β-actin (Abcam, England), PI3K (Abcam, England), p-PI3K(phospho Y458, Abcam, England), AKT (Abcam, England), p-AKT (phospho T308, Abcam, England), BAX (Abcam, England), cleaved caspase-3 (Abcam, England), Bcl2 (Abcam, England), and GAPDH (Santa Cruz, USA). Horseradish peroxidase (HRP)-conjugated monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Kangchen, Shanghai, China) was used as the internal control. After incubation with HRP-conjugated secondary anti-mouse or anti-rabbit antibodies (Santa Cruz, USA), images were captured by medical X-ray film (Carestream, Canada).

### RNA Isolation and Real-Time PCR

Total RNA were extracted by TRIzol kit (Pufei, Shanghai, China) under the guidance of the manufacturer's protocol. cDNA was then synthesized by an M-



**Figure 1.** Expressions of EPB41L3 in human cervical cancers and cell lines.

(A) expression of EPB41L3 in human cervical cancer (red) and normal cervical tissue (gray) was analyzed in the cervical cancer clinical sequencing database by TCGA. (B) analysis of prognostic survival curve in cervical cancer with high expression and low expression of EPB41L3. (C) different expression levels of EPB41L3 in different stages of cervical diseases analyzed by immunohistochemical staining, including chronic cervicitis, CIN I, CIN III, and cervical cancer; the expression level of EPB41L3 were divided into four levels, such as negative, +, ++ and +++.

MLV-reverse transcriptase kit (Promega, USA). qPCR was performed using SYBR<sup>®</sup> Green reagents and the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). The primer sequences of EPB41L3 were as follows: forward AGGAGGAGCAGCAGCAGGCC and reverse GCTGTTTTGCAGCCCTGGCA; that of GAPDH were: forward CCACATCGCTCAGACACCAT and reverse GCGCCAAATACGACCAAAT. Sequence Detection System

software (Applied Biosystems) was used to analyze the data.

## Statistical analysis

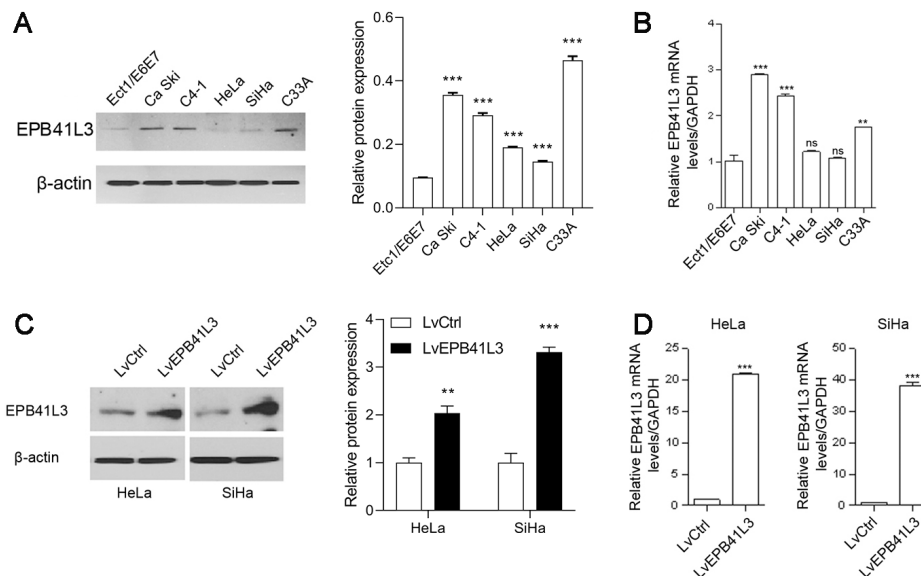
All data represent at least three independent experiments and are presented as the means  $\pm$  S.E.M. in this study. Statistical analysis was performed using SPSS 17.0 software (SPSS, Chicago, IL). The differences were tested with the Student's *t*-test. A *P*-value < 0.05 was considered to be statistically significant.

## RESULTS

### Association of EPB41L3 expression with clinicopathological features in cervical cancer

As shown in Fig. 1A, the expression of EPB41L3 in cervical cancer was significantly suppressed compared with healthy tissue. The survival curves indicated that patients with high expression of EPB41L3 had a good prognosis, while patients with low expression of EPB41L3 had poor survival (Fig. 1B).

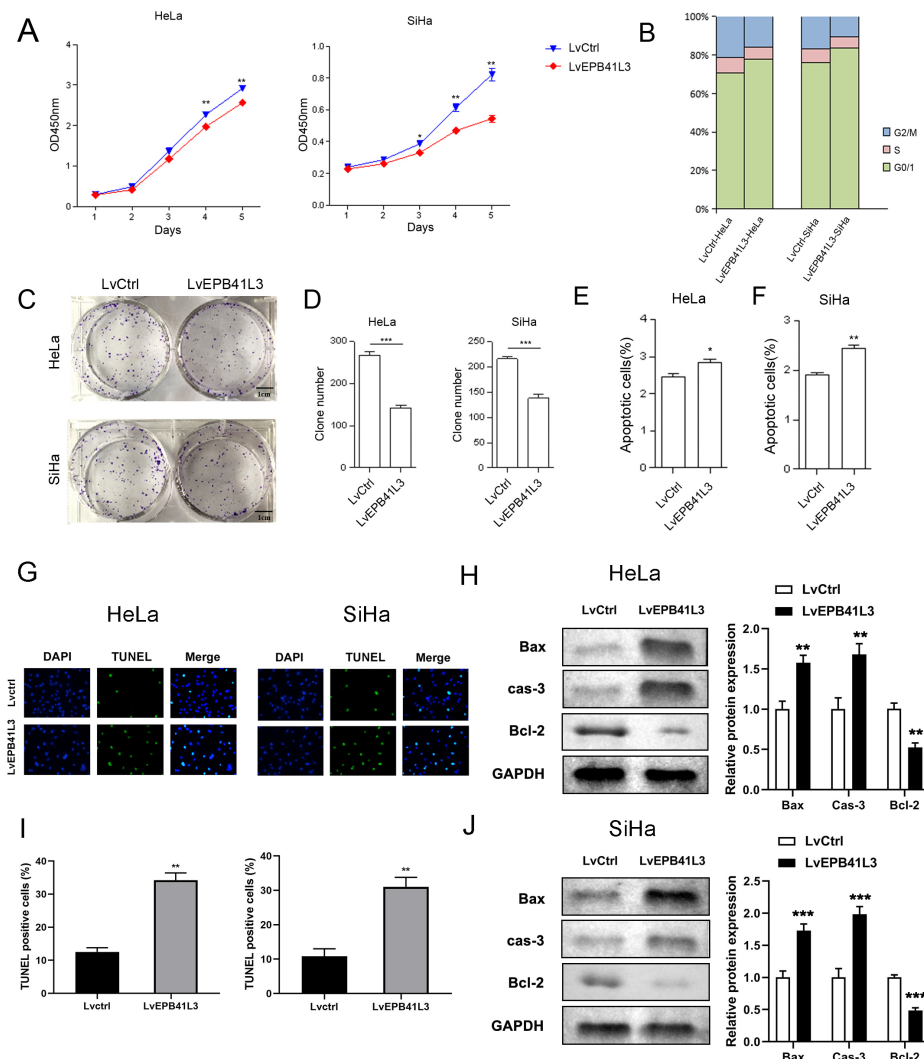
In addition, EPB41L3 expression was detected in different stages (Chronic Cervicitis, CIN I, CIN II-III and Cervical Cancer) of cervical tissues from each group of 50 people. Immunohistochemical staining results demonstrated the aberrant expression of EPB41L3 in different stages (Fig. 1C). Low expression levels of EPB41L3 were negative and +, and the high expression levels of EPB41L3 were ++ and +++. The number of low and high expression in each group was Chronic Cervicitis 10/40, CIN I 19/31, CIN II-III 28/22 and Cervical Cancer 43/7. EPB41L3 expression was negatively correlated with the stages of cervical cancer (Chi-square value=47.520; \*\*\**P*<0.001).



**Figure 2.** Title for whole figure

(A) relative expression of EPB41L3 in Ect/E6E7 and 5 cervical cancer cell lines including HeLa, C-41, SiHa, C33A, and CaSki, by western blot. Densitometric analysis of Western blot from three independent experiments; bars represent means  $\pm$  S.E.M. of EPB41L3 level normalized to  $\beta$ -Actin. \*\*\**P*<0.001. (B) relative expression of EPB41L3 in cervical cancer cell lines by RT-PCR. All data were normalized relative to the mRNA concentration for GAPDH and are presented as the mean  $\pm$  S.E.M. (n=3). \*\**P*<0.01, \*\*\**P*<0.001, ns, no significant differences, compared with the control. (C) expression of EPB41L3 in HeLa and SiHa after transfection with LvCtrl or LvEPB41L3. Densitometric analysis of western blot from three independent experiments; bars represent means  $\pm$  S.E.M. of EPB41L3 level normalized to  $\beta$ -Actin. \*\**P*<0.01, \*\*\**P*<0.001, compared with the control. (D) expression of EPB41L3 in HeLa and SiHa after transfection with LvCtrl or LvEPB41L3 by western blot.





**Figure 3. Effects of LvEPB41L3 on the proliferation and apoptosis of HeLa and SiHa cells after transfection.**

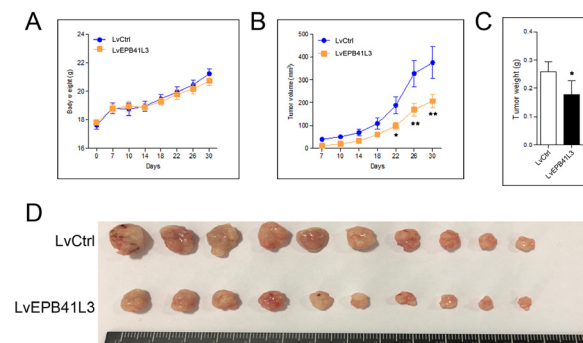
(A) MTT was used to detect the proliferation of HeLa and SiHa cells at 1, 2, 3, 4, and 5 days after LvCtrl or LvEPB41L3 transfection.  $**P < 0.01$ , compared with the control. (B) cell cycle analysis of HeLa and SiHa cells with transfection by flow assay. (C) crystal violet staining was used to detect the formation of HeLa and SiHa clones after transfection. (D) statistical analysis of clone numbers of LvCtrl or LvEPB41L3 HeLa and SiHa cells cultured for 15 days. Data shown are the mean results  $\pm$  S.E.M. ( $n=3$ ),  $***P < 0.001$ , compared with the control. (E, F) annexin V-APC flow assay was used to analyze the apoptosis of HeLa and SiHa cells transfected with LvCtrl or LvEPB41L3. Statistical analysis of the apoptotic percentage of LvCtrl or LvEPB41L3 HeLa transfected and SiHa cells. Data shown are the mean results  $\pm$  S.E.M. ( $n=3$ ),  $*P < 0.05$ ,  $**P < 0.01$ , compared with the control. (G) images of TUNEL-positive cells. (I) quantification of TUNEL-positive cells. (H, J) apoptosis-related proteins were measured by western blot. All data were normalized relatively to the protein concentration for GAPDH and are presented as the means  $\pm$  S.E.M. ( $n=3$ ).  $**P < 0.01$ ,  $***P < 0.001$ , compared with the control.

### Overexpression of EPB41L3 in human cervical cancer cell lines

The expression levels of EPB41L3 mRNA in 5 cervical cancer cell lines (HeLa, C-4I, SiHa, C33A, and CaSki), and one normal human cervix cell line (Ect/E6E7) were analyzed. Western blot results indicated that EPB41L3 protein expression was up-regulated in C-4I, C33A, and CaSki cell lines, while down-regulated in Ect1/E6E7, HeLa, and SiHa cells (Fig. 2A). Consistently, mRNA level of EPB41L3 was suppressed in HeLa and SiHa cells by RT-PCR analyses (Fig. 2B). Therefore, HeLa and SiHa cells were selected to detect the role of EPB41L3 in cervical cancer cells by transfection with the lentivirus Ctrl and EPB41L3, respectively. As shown in Fig. 2C and 2D, the expression of EPB41L3 was significantly higher than that of the control groups after transfection.

### Overexpression of EPB41L3 inhibited cell proliferation and apoptosis of human cervical cancer cells

To further evaluate the biological effects of EPB41L3 expression in human cervical cancer cells, CCK-8 assays, cell cycle assays, and colony formation assays were used to detect cell proliferation. Over-expression of EPB41L3 inhibited cell proliferation of HeLa and SiHa cells (Fig. 3A). The cell cycle assay also indicated that up-regulated EPB41L3 remarkably increased the percentage of G0 stage cells and reduced the percentage of G2 stage cells in both HeLa and SiHa cells (Fig. 3B). Furthermore, the number of clones in the LvEPB41L3 group was significantly lower than that in the control group ( $***P < 0.001$ ) (Fig. 3C, D). These results suggested that overexpression of EPB41L3 suppressed the proliferation of human cervical cancer cells.



**Figure 4. Effects of LvEPB41L3 on the tumorigenicity of HeLa cells in BALB/c nude mice.**

(A, B) body weight and tumor volume of LvCtrl and LvEPB41L3-HeLa cells transplanted mice were measured at the indicated days after treatment (each group with ten injected sites in five mice). \* $P < 0.05$ , \*\* $P < 0.01$ , compared with the control. (C) tumor weights of LvCtrl and LvEPB41L3-HeLa cells-treated mice were measured at 30 days after treatment (each group with ten injected sites in five mice). \* $P < 0.05$ , compared with the control. (D) images of tumors from LvCtrl and LvEPB41L3-HeLa cells transplanted mice 30 days after injection.

Annexin V-APC flow analysis and TUNEL method were then applied to measure the apoptosis of cervical cancer cells. The results demonstrated that the apoptosis rate of LvEPB41L3-transfected HeLa and SiHa cells was significantly higher than that of the control groups (\* $P < 0.05$ , \*\* $P < 0.01$ ) (Fig. 3E, F, G, I). Moreover, protein levels of apoptosis-related proteins were measured to verify the results of Annexin V-APC flow analysis and TUNEL method (Fig. 3H, J). These results suggested that overexpression of EPB41L3 promoted the apoptosis of human cervical cancer cells.

#### Overexpression of EPB41L3 inhibited the tumorigenicity of human cervical cancer cells *in vivo*

There were no significant differences in body weights between the LvEPB41L3-HeLa group and the LvCtrl group (Fig. 4A). The tumor growth trend of EPB41L3 overexpression group was more obvious than that of the control group (\* $P < 0.05$ , \*\* $P < 0.01$ ) (Fig. 4B), so did the weights of the isolated tumors (Fig. 4C). These results demonstrated that overexpression of EPB41L3 could inhibit the tumorigenicity of human cervical cancer cells *in vivo*.

#### EPB41L3 promoted apoptosis through the PI3K/AKT pathway

To explain the mechanism of EPB41L3 in human cervical cancer cells, we conducted microarray analysis on LvCtrl-HeLa and LvEPB41L3-HeLa cells. As shown in Fig. 5A, over-expression of EPB41L3 resulted in marked changes in gene expression. Compared with LvCtrl HeLa cells, 258 genes were significantly upregulated in LvEPB41L3 HeLa cells, and 168 genes were significantly downregulated (Fig. 5B). Gene Ontology (GO) analysis showed that these differentially expressed genes (DEGs) of LvEPB41L3 HeLa were assigned to three categories, including cellular components, biological processes and molecular functions. In the GO category related to biological process, DEGs were involved in cellular process and biological regulation. Among the DEGs related to cellular component, the most significant terms were cell part, organelle, and membrane. For molecular function

annotation more than half of DEGs were linked to cell binding. (Fig. 5D).

In addition, we further explored potential signaling pathways by KEGG analysis (Fig. 5C). We found that the differentially expressed genes were enriched in the following pathways: ECM-receptor interaction, PI3K/Akt signaling pathway, beta-alanine metabolism, cancer pathways, etc.

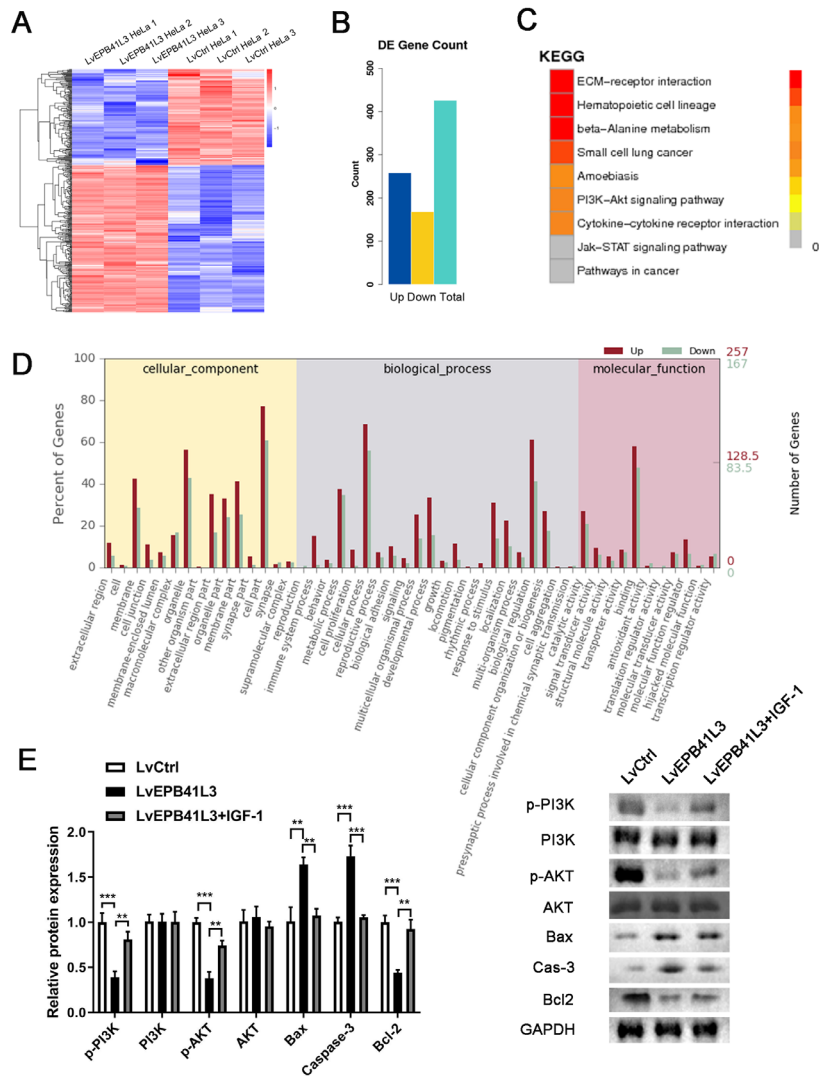
We verified the core factors of the PI3K/Akt pathway by Western blot, which exhibited regulatory effects on the induction of apoptosis. The protein levels of p-PI3K, p-AKT and Bcl2 were significantly down-regulated in the LvEPB41L3 group while BAX and caspase-3 were dramatically up-regulated, which were abrogated by IGF-1, an activator of PI3K/AKT (Kong *et al.*, 2020). These results suggested that over-expressed EPB41L3 promoted apoptosis by inactivating PI3K/AKT signaling pathway (Fig. 5E).

#### DISCUSSION

Cervical cancer is one of the most common gynecological malignancies. The clinical manifestations of early cervical cancer and chronic cervicitis are very similar, and chronic cervicitis with poor treatment is likely to turn into cervical cancer. Therefore, it is very important to find out the factors affecting the transformation of chronic cervicitis into cervical cancer.

EPB41L3, which plays an influential role in various cancers including lung cancer, breast cancer, and ovarian cancer, is an accurate and feasible screening method for CIN2 (Bernkopf & Williams, 2003; Dafou *et al.*, 2010; Wong *et al.*, 2007; Jiang & Newsham, 2006; Kong *et al.*, 2020). Besides, Hou and others (Hou *et al.*, 2020) found that long non-coding RNA (lncRNA) MAGI2-AS3 may sponge miR-233 to upregulate EPB41L3, thereby inhibiting cervical cancer cell invasion and migration. Hence, EPB41L3 may be a potential biomarker of cervical cancer. In this study, by in-depth mining in TCGA's clinical sequencing database, patients with high expression of EPB41L3 were found to have a good prognosis. Moreover, the over-expression of EPB41L3 significantly inhibited the growth of HeLa and SiHa cells, promoted apoptosis, and effectively inhibited the tumorigenicity of the BALB/c nude mouse model. Therefore, over-expression of EPB41L3 may be a novel treatment for cervical cancer.

Previous studies have reported many mechanisms by which EPB41L3 inhibits tumor formation, such as activation of Rac1-dependent c-Jun-NH2-kinase (Gerber, *et al.*, 2006), increased caspase-8 activity (Jiang & Newsham, 2006), mediation of posttranslational protein modifications, and regulation of the methylome (Singh *et al.*, 2004). What is more, abnormalities in PTEN/Akt/mTOR and SOCS1/SOCS3-related pathways lead to cervical cancer treatment failure (Li *et al.*, 2017; Kim *et al.*, 2015). In the present study, we detected whole gene expression in LvEPB41L3 HeLa cells by microarrays. We found that overexpression of EPB41L3 were linked with PI3K/AKT pathway, which has not been discussed before. Our data suggested that inhibition of phosphorylation of PI3K and Akt kinases induced by LvEPB41L3 was reversed by IGF-1 (an activator of PI3K/AKT), demonstrating that overexpressed EPB41L3 suppressed PI3K/AKT signaling pathway in HeLa cells. Furthermore, IGF-1 abrogated the effects of overexpressed EPB41L3 on apoptosis-related proteins, which indicated that overexpression of EPB41L3 suppressed apoptosis



**Figure 5. Global expression analysis in LvCtrl HeLa and LvEPB41L3 HeLa.**

(A) heatmap showing the global expression of LvCtrl HeLa and LvEPB41L3 HeLa. (B) comparison of differentiated expressed genes between LvCtrl HeLa and LvEPB41L3 HeLa. (C) KEGG analysis of differentiated expressed genes of LvEPB41L3 HeLa. (D) gene Ontology analysis of differentiated expressed genes of LvEPB41L3 HeLa. (E) expression of the proteins of PI3K/AKT signaling and apoptosis-related proteins in LvCtrl HeLa and LvEPB41L3 HeLa. All data were normalized relative to the protein concentration for GAPDH and are presented as the means  $\pm$  S.E.M. (n=3). \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

of cervical cancer by inactivating PI3K/AKT signaling pathway *in vitro*.

PI3K/AKT signaling pathway is one of the most important signaling pathways to transmit extracellular signals to the nucleus, and plays a key role in the regulation of cell survival, proliferation, differentiation, apoptosis, metabolism and other functions (Aoki & Fujishita, 2017). Abnormal PI3K/AKT signaling has been reported in breast cancer, lung cancer, liver cancer, and cervical cancer (Costa *et al.*, 2018; Tan, 2020; Kahraman *et al.*, 2019; Bossler *et al.*, 2019). Furthermore, various studies suggest that activating PI3K/AKT promotes cervical cancer process (Wang *et al.*, 2020; Yang & Xie, 2020), which is in line with our data. Hence, the underlying molecular mechanisms of the phosphorylation of PI3K and Akt kinases should be further investigated. PTEN is one of important genes upstream of PI3K target genes, and can dephosphorylate PIP3 to PIP2, reduce the phosphorylation ability of P13K, and then block the phosphorylation of Ser/Thr kinase AKT, and inhibit the activity of downstream target genes of AKT, so as to play a nega-

tive role in the regulation of cell growth (Papa & Pandolfi, 2019; Haddadi *et al.*, 2018). For instance, Apigenin 7-O-glucoside promoted cell apoptosis of HeLa cells by suppressing the PTEN/PI3K/AKT pathway (Liu *et al.*, 2020). Thereby, lack of study on the inactivation of PTEN is a limitation of the present study and should be studied in a deep way in our further research.

In conclusion, our results indicated that the overexpression of EPB41L3 promoted the apoptosis of human cervical cancer cells by inactivating PI3K/AKT signaling pathway *in vitro*, and inhibited the tumorigenicity of human cervical cancer cells *in vivo*. EPB41L3 might be a potential therapeutic target and could be exploited in cervical cancer diagnosis and therapy.

#### Declarations

Competing interests

The authors declare no competing interests.



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