

ALOX12B promotes carcinogenesis in cervical cancer by regulating the PI3K/ERK1 signaling pathway

TAO JIANG^{1,2}, BING ZHOU³, YUAN MENG LI², QUI YING YANG², KAI JIA TU⁴ and LONG YU LI^{1,4}

¹Department of Gynecological Oncology, Jiangxi Medical College of Nanchang University, Nanchang, Jiangxi 330031; Departments of ²Gynecology and ³Pathology, The Affiliated Jiujiang Hospital of Nanchang University, Jiujiang, Jiangxi 332000; ⁴Department of Gynecological Oncology, Jiangxi Maternal and Child Health Hospital, Nanchang, Jiangxi 330006, P.R. China

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Abstract. Cervical cancer is a malignant disease and a threat to women's health worldwide. Surgical resection followed by radiotherapy or chemotherapy is the main treatment strategy for cervical cancer; however, patients with cervical cancer, especially those with late-stage disease, may not benefit from these traditional therapies, which results in poor clinical outcome. ALOX12B is a gene encoding lipoxygenase, and a mutation in ALOX12B was detected in lung and breast cancer. Furthermore, ALOX12B is essential to the proliferation of epidermoid carcinoma cells; however, the role of ALOX12B in cervical cancer has not been studied thus far, to the best of our knowledge. In the present study, the expression levels of ALOX12B were reduced in cervical cancer cells by lentiviral transfection, and it was found that both cell proliferation and clone formation ability were significantly reduced, and the cell cycle was blocked at G₁ phase. Tumor growth was also suppressed *in vivo* in a xenograft tumor model, but the migration of tumor cells was not affected by ALOX12B. Subsequently, using western blotting, it was demonstrated that knockdown of ALOX12B decreased the expression levels of PI3K, MEK1, ERK1, C-fos and cdc25. Meanwhile, overexpression of ALOX12B increased the expression levels of these five molecules. Conclusively, ALOX12B promoted cell proliferation in cervical cancer via regulation of the PI3K/ERK1 signaling pathway. The present study may improve our understanding of the molecular mechanisms underlying the function of ALOX12B in cervical cancer and inform new therapeutic strategies.

Introduction

Cervical cancer is a common malignant tumor in women (1). According to a statistical report released in 2019, the number of estimated new cases was 13,170, and the estimated cervical cancer-associated deaths will increase to 4,250 in the USA in 2019 (2). Meanwhile, in China, a total of 98,900 women were predicted to be diagnosed with cervical cancer, and ~30,500 cervical cancer-associated deaths were predicted in 2015 (3). Although the prognosis of cervical cancer is not dismal, the 5-year survival rate for patients with metastasis 17%, and it is ~50% for those with regional cervical cancer (2); however, only 15% of patients are diagnosed with metastasis (2).

Currently, the primary therapy for regional cervical cancer is surgical resection followed by radiotherapy or chemotherapy, while palliative therapy for recurrent or metastatic cancer does not improve clinical survival time (4). The benefit from all these therapies is usually short lived and only effective in some patients (4). Recently, target therapy and immunotherapy have been introduced as new therapies for patients with cancer, particular for patients with late-stage cancer. For example, the chimeric antigen receptor T-cell (CAR-T) therapy drug KYMRIAH[®] improved the prognosis in patients with acute lymphoblastic leukemia (5). However, the major issue regarding this novel therapy is that a critical protein has to be expressed in or on the surface of tumor cells. The single chain antibody fragment targeting CD19 molecule was essential for the clinical outcome as the CAR-T therapy drug recognized and bound to CD19 (5). The antibody drug KEYTRUDA[®] is another successful example for patients with cancer (6). PD-1 serves an inhibitory role in activation of T cells, and KEYTRUDA[®] blocks the inhibitory signaling via targeting PD-1, allowing T-cell activation. Therefore, elucidation of the molecular mechanism and identifying key molecules, such as PD-1, involved in tumor formation and progression is critical and necessary for new therapies.

Genetic mutations are important factors contributing to tumorigenesis (7). ALOX12B encodes a lipoxygenase, and is responsible for the conversion of arachidonic acid to 12R-hydroxyeicosatetraenoic acid (8). The *ALOX12B* gene is located at chromosome 17p13.1, and contains 15 exons (8).

Correspondence to: Mrs. Long Yu Li, Department of Gynecological Oncology, Jiangxi Maternal and Child Health Hospital, 318 Bayi Street, Nanchang, Jiangxi 330006, P.R. China
E-mail: lyli5919@163.com

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Lipoxygenases are reported to be associated with inflammation, skin disorders and tumorigenesis (9). Mutations in the *ALOX12B* gene mainly result in non-bullous congenital ichthyosiform erythroderma (10,11). *ALOX12B* has also been reported to inhibit immune cytolytic activity in breast and renal cell tumors (12), and single nucleotide polymorphisms (SNPs) in *ALOX12B* are associated with increased risk of lung and breast cancer (13,14). Agarwal *et al* (15) reported that inhibition of *ALOX12B* directly reduced the proliferation of the vulvar epidermoid carcinoma A431 cell line. Hence, *ALOX12B* serves important roles in the carcinogenesis of tumors, but, to the best of our knowledge, there are no studies investigating the role of *ALOX12B* in cervical cancer.

The aim of the present study was to investigate the role of the *ALOX12B* gene in cervical cancer by knocking down this gene in cervical cancer cells and systemically studying its role both *in vitro* and *in vivo*. Additionally, the underlying molecular mechanism of *ALOX12B* in cervical cancer was explored using western blot analysis.

Materials and methods

Cell culture. Human Ca-Ski and C33A cells were purchased from the American Type Culture Collection and cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere with 5% CO₂ at 37°C. C33A cells are derived from HPV-negative cervical cancer, while Ca-Ski cells are derived from HPV 16-positive cervical cancer (16).

Reverse transcription-quantitative (RT-q) PCR assay. RNA was extracted from tumor cells, including C33A and Ca-Ski cells, using an RNAeasy™ kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. RNA was reverse transcribed into complementary DNA (cDNA) using 1 μg RNA and PrimeScript™ 1st strand cDNA Synthesis kit (Takara Bio, Inc.). qPCR was carried out using SYBR Green mix (Shanghai Yeasen Biotechnology Co., Ltd.) using an ABI 7000 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) and PrimeScript™ RT Master mix (Takara Bio, Inc.). The thermocycling protocol was as follows: 95°C for 2 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 15 sec. GAPDH was used as the internal control. The relative quantity of the target gene was calculated using the 2^{-ΔΔCq} method (17). The primer sequences for each gene are shown in Table I.

Western blotting. Total protein was extracted using a protein extraction kit (Shanghai Yeasen Biotechnology Co., Ltd.) according to the manufacturer's protocol, and the protein concentration was determined using an ultraviolet spectrophotometer (Onedrop1000; Shanghai Genechem Co., Ltd.). A total of 10 μg protein/lane was loaded on a 12% polyacrylamide gel, resolved using SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Sangon Biotech Co., Ltd.). The PVDF membrane was blocked with 5% non-fat milk for 2 h at room temperature. Subsequently, the PVDF membrane was incubated with the corresponding primary antibody against the target protein overnight at 4°C followed by incubation with the secondary antibody at room temperature for 1 h. The protein band was detected using a Beyo ECL Star kit

(Beyotime Institute of Biotechnology). The antibodies used were as follows: Anti-PI3K (ab70912; 1:100), anti-MEK1 (ab32091; 1:1,500), anti-ERK1 (ab32537; 1:600), anti-cdc25 (ab111830; 1:2,000) (all Abcam), anti-C-fos (554C1a; 1:500; Santa Cruz Biotechnology, Inc.), anti-*ALOX12B* (PA5-23608; 1:800; Invitrogen; Thermo Fisher Scientific, Inc.), anti-GAPDH (ab181602; 1:10,000; Abcam), goat anti-mouse IgG antibody (ab97035; 1:2,000) and goat anti-rabbit IgG antibody (ab7090; 1:5,000) (both Abcam).

Package of lentiviral vector carrying small hairpin RNA (shRNA) targeting *ALOX12B* and transduction. Three shRNA fragments targeting the *ALOX12B* gene were designed, synthesized and transferred into the expression vector pPLK-GFP (pPLK-sh*ALOX12B*-GFP). The sequences of each shRNA fragment are shown in Table II. In parallel, a non-targeting sequence was used as the negative control (NC). Subsequently, a lentiviral vector carrying sh*ALOX12B* (Lvsh*ALOX12B*) was prepared as follows: 10 μg pspax2 plasmid (Invitrogen; Thermo Fisher Scientific, Inc.), 5 μg pMD2.G plasmid (Invitrogen; Thermo Fisher Scientific, Inc.) and 15 μg pPLK-sh*ALOX12B*-GFP were transferred into 293T cells (RanYanBio Co., Ltd.) and cultured in DMEM containing 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂ for 72 h. Subsequently, the supernatant was collected, and the lentiviral particles were concentrated from the supernatant by centrifugation at 14,000 x g at 4°C for 40 min.

Cervical cancer cells were treated with Lvsh*ALOX12B* or NC (scrambled) for 6 h and then cultured for 72 h. The transduction efficiency was detected under a fluorescence microscope (magnification, x100; Nikon Corporation). The knockdown efficiency of *ALOX12B* was determined by qPCR assay as aforementioned.

Construction of plasmid expressing *ALOX12B* and transfection. The coding sequence for *ALOX12B* (NM_001139.3) was synthesized and cloned into the pcDNA3.1 vector (Addgene, Inc.). The recombinant plasmid pcDNA3.1-*ALOX12B* (to over-express *ALOX12B*) was confirmed by DNA sequencing. Subsequently, the pcDNA3.1-*ALOX12B* plasmid was transfected into tumor cells using Lipofectamine reagent (40802ES02; Shanghai Yeasen Biotechnology Co., Ltd.). The transfection efficiency was detected using western blotting for detection of *ALOX12B* expression levels, as aforementioned. The time interval between transfection and subsequent experimentation was 48 h.

Cell proliferation assay using Cell Counting Kit-8 (CCK-8). A total of 6,000 lentiviral-infected C33A or Ca-Ski cells/well were seeded into a 96-well plate. After 24, 48, 72 or 96 h of culture, 10 μl CCK-8 (Beyotime Institute of Biotechnology) was added into each well and cells were cultured at 37°C with 5% CO₂ for another 4 h according to the manufacturer's protocol. Subsequently, the absorbance at 450 nm was determined using a microplate reader. Each assay was repeated independently at least three times.

Colony forming assay. A total of 100 C33A or Ca-Ski cells/well were seeded into a 24-well plate. After 15 days, the cells

Table I. Sequence of the primers used for quantitative PCR.

Gene name	Sequence, 5'-3'	Fragment size, bp
ALOX12B		196
Forward	GCCTGTTGGACTGCAAACATT	
Reverse	GTGACGGGGAACCTGTCTGG	
GAPDH		217
Forward	TCATCATCTCTGCCCCCTCT	
Reverse	GGGCCATCCACAGTCTTCTG	

Table II. shRNA target sequences for ALOX12B.

Name	Target sequence, 5'-3'
shALOX12B-1	GATACACCGTCCAGATCAAT
shALOX12B-2	GCACGCGGATCCCAGACAA
shALOX12B-3	CCGATATGTCACTATAGTCAT
NC	GTTCTCCGAACGTGTCACGTT

NC, negative control; sh, small hairpin.

on the plate were fixed with 4% paraformaldehyde at room temperature for 30 min and stained with 0.1% crystal violet at room temperature for 15 min. The colony number was counted manually under a light microscope (magnification, x40). Each assay was repeated independently at least three times.

Wound healing assay. A total of 2×10^4 C33A or Ca-Ski cells/well were seeded into a 24-well plate and cultured to 90% confluence. Next, a 10- μ l sterile tip was used to make a scratch in the middle of each well. The debris was washed with PBS, and fresh DMEM without FBS was added. After 24 h, the plate was observed, and images were captured under a light microscope (magnification, x100), and the width of each scratch was recorded. Each assay was repeated independently at least three times.

Cell cycle analysis. A total of 1×10^5 C33A or Ca-Ski cells treated with lentiviral vector for 72 h were harvested and washed with cold PBS followed by fixing in 70% ethyl alcohol. Next, 0.5 ml 1X staining buffer (Beyotime Institute of Biotechnology) with 10 μ l propidium iodide (PI) reagent (Beyotime Institute of Biotechnology) was used to resuspend the tumor cells, which were then cultured for 30 min at 37°C. The cell cycle distribution was analyzed using fluorescence-activated cell sorting (Beckman Coulter, Inc.).

In vivo tumor growth assay. Nanchang Royo Biotech Co., Ltd. performed the following experiments: 20 female BALB/c-nu mice (6 weeks old; 20-30 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. and divided equally into NC and shALOX12B groups. Mice were kept in groups of 4 or 5 in individual cages and provided with sterilized food and water *ad libitum*. Mice were maintained in conditions

with a 12-h light-dark cycle at 22°C and 55% humidity. After *in vitro* culture, $\sim 2 \times 10^7$ C33A cancer cells were inoculated subcutaneously into the right flank. Tumor growth was monitored consecutively for 35 days. Subcutaneous tumor volume (V) was measured twice a week and calculated as follows: $V = (L \times W^2) / 2$, where L is the length and W is the width of the tumor. At the 35th day, mice were anesthetized according to the guidelines involving the use of diethyl ether approved by the Laboratory Animal Ethics Committee of Nanchang Royo Biotech Co., Ltd. (approval no. RYE2019011104); a sterile gauze soaked in 99.5% diethyl ether was placed in a 500 ml beaker and mice were placed into the beaker for 5 min. Subsequently, the mice were sacrificed by cervical dislocation. If the tumor volume was $>1,500 \text{ mm}^3$ or if ulcers occurred, the study was terminated prematurely.

Statistical analysis. All data are displayed as the mean \pm standard deviation, and were analyzed using SPSS version 16.0 (SPSS Inc.). The difference between two groups was compared using an unpaired Student's t-test. The differences among multiple groups were compared using a one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effective ALOX12B-knockdown in cervical cancer C33A cells. LvshALOX12B was used to transfect C33A cells, and the transfection efficiency was monitored by GFP expression. As shown in Fig. 1A, the transfection efficiency was high after transduction for 72 h, according to GFP expression. Subsequently, qPCR technology was used to determine the expression levels of the *ALOX12B* gene in C33A cells. qPCR analysis demonstrated that the knockdown efficiency of each shRNA was $>75\%$ compared with that of the NC (Fig. 1B). The knockdown efficiency of shALOX12B-3 was $\sim 90\%$; therefore, shALOX12B-3 was chosen for functional analysis.

Knockdown of ALOX12B inhibits the proliferation and colony formation of C33A cells. A CCK-8 assay was designed to detect the effect of shALOX12B-3 on the proliferation of C33A and Ca-Ski cells. As shown in Fig. 2A and B, the proliferation rate of C33A treated with shALOX12B-3 was 69% compared with that of the NC group, while the proliferation rate was 76% in Ca-Ski cells after transfection for 96 h. Colony forming ability also reflects the proliferative potential. The relative colony formation rate in the NC group was significantly higher compared with that of the shALOX12B-3 group in C33A cells ($P < 0.05$; Fig. 2C and D). For Ca-Ski cells, the relative colony formation rate in the NC group was also significantly higher compared with that of the shALOX12B-3 group ($P < 0.05$; Fig. 2C and D). These results suggested that ALOX12B participates in the proliferation and growth of cervical cancer cells.

ALOX12B is not essential for cell migration in cervical cancer. In general, cancer cells have a marked migration ability in the majority of cancers (18). A wound healing assay was performed to determine the effect of shALOX12B-3 on the migration of cervical cancer cells. However, no significant

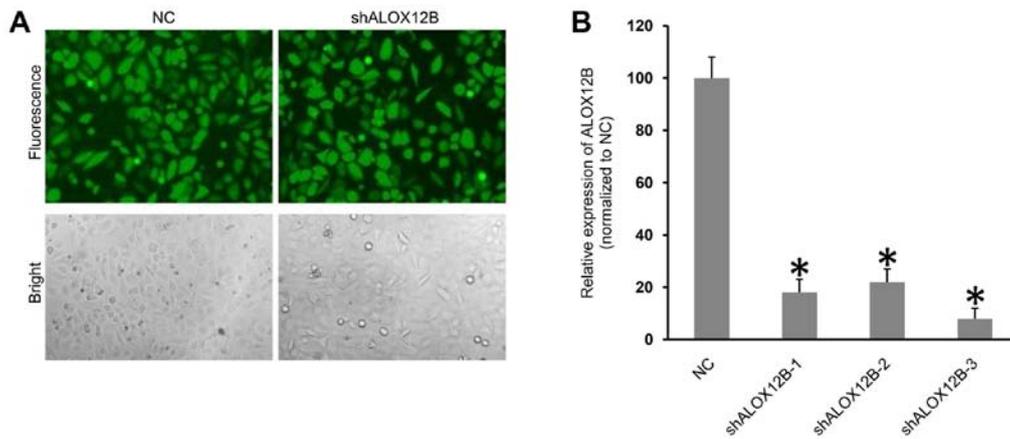


Figure 1. ALOX12B-knockdown in cervical cancer cells. (A) GFP expression of C33A transfected with the lentiviral expression vector carrying shALO12B or NC after 72 h. Magnification, x100. (B) Quantitative PCR analysis of ALOX12B expression levels after lentiviral vector transfection for 72 h. * $P < 0.05$. sh, small hairpin; NC, negative control; GFP, green fluorescent protein.

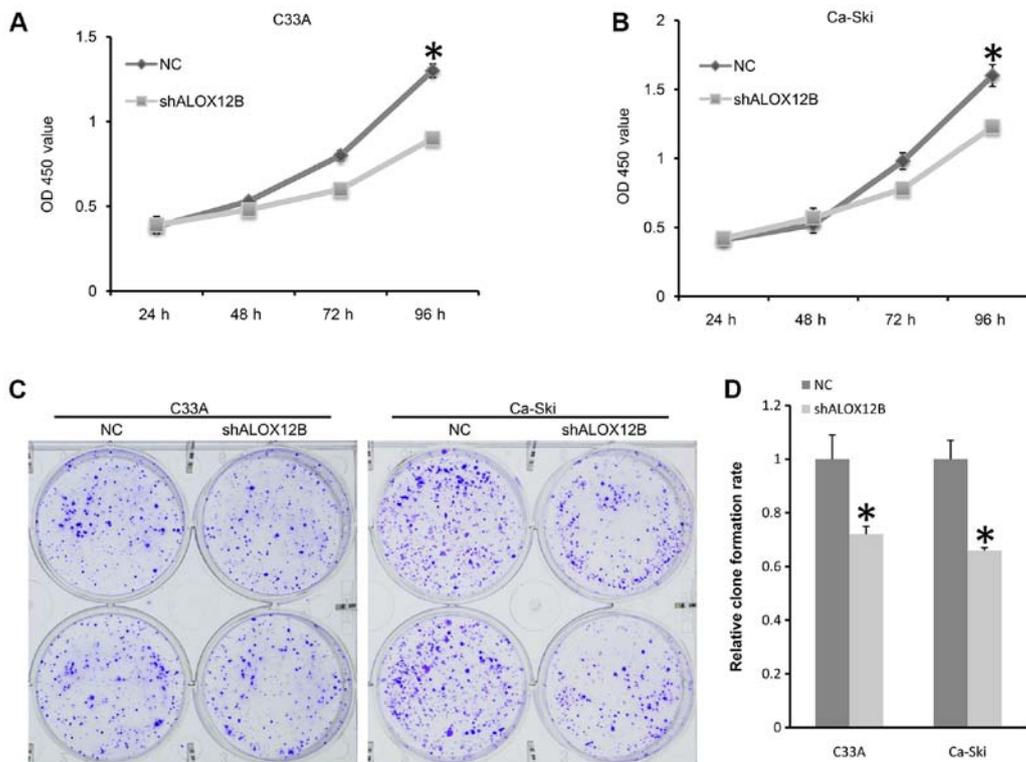


Figure 2. Knockdown of ALOX12B inhibits cell proliferation and colony formation. The proliferation of (A) C33A and (B) cells Ca-Ski was inhibited when the ALOX12B levels were decreased following knockdown. (C) The colony forming ability of C33A cells and Ca-Ski cells was inhibited after ALOX12B-knockdown. The top plates are replicates of the bottom plates. (D) Quantitative analysis of colony formation in C33A and Ca-Ski cells upon ALOX12B-knockdown or transfection with NC. * $P < 0.05$. sh, small hairpin; NC, negative control; OD, optical density.

difference was observed at 24 h between the NC group and the shALO12B-3 group in C33A or Ca-Ski cells (Fig. 3A and B). The 48-h time point was also assessed in the wound healing assay, but no significant differences were observed, as the relative migration rate in the NC group was equivalent to that in the shALO12B group (data not shown). Therefore, it is possible that ALOX12B may not regulate cell migration in cervical cancer.

Cell cycle transition is suppressed by ALOX12B-knockdown.
To detect the effect of shALO12B-3 on cell cycle in

cervical cancer, C33A and Ca-Ski cells were stained with PI dye after treatment with shALO12B-3 and NC. As shown in Fig. 4A and B, the cell cycle was arrested at the G₁ phase after shALO12B was knocked down. The ratio of C33A cells in the G₁ phase increased from 37.4 to 55.7% (NC vs. shALO12B). By contrast, no significant difference was observed in Ca-Ski cells. The phase distribution in Ca-Ski cells showed no significant difference between the shALO12B-3 and NC groups (Fig. 4C and D). This might be attributed to biological variation between different cells.

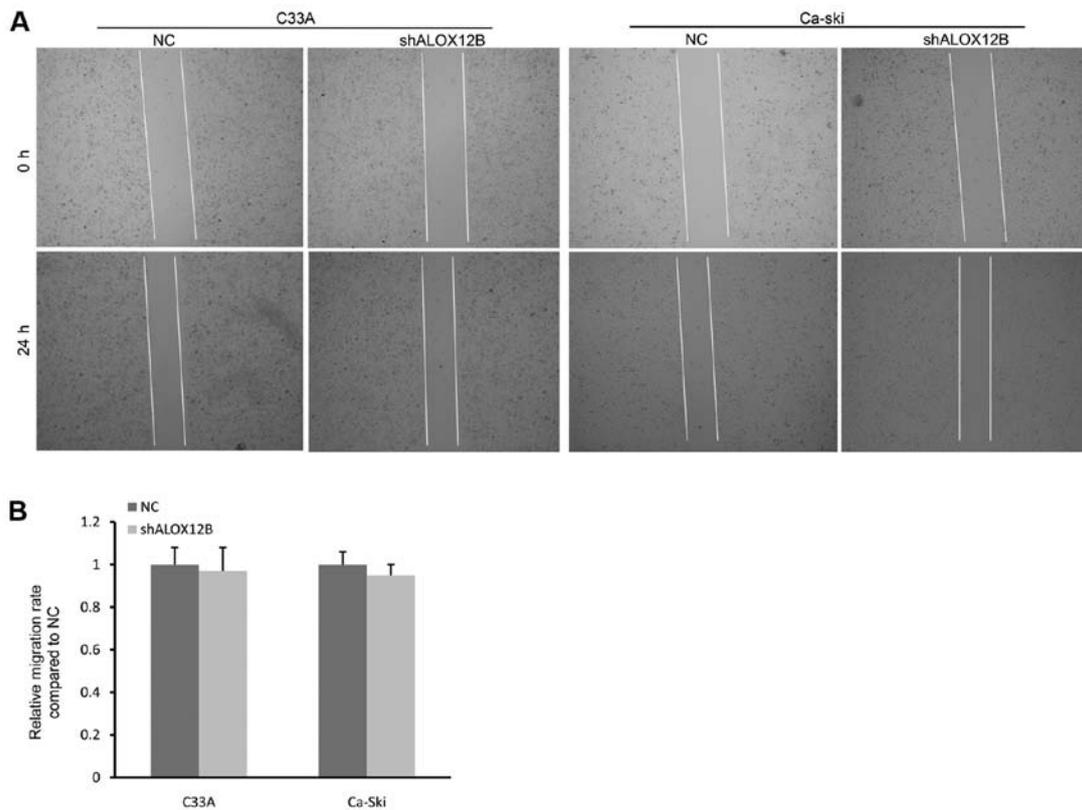


Figure 3. ALOX12B does not affect cell migration in cervical cancer. (A) Wound healing assay demonstrated that the knockdown of ALOX12B had no obvious impact on cell migration. (B) Data analysis of the relative cell migration rate of C33A and Ca-Ski cells transfected with shALOX12B or NC. sh, small hairpin NC, negative control.

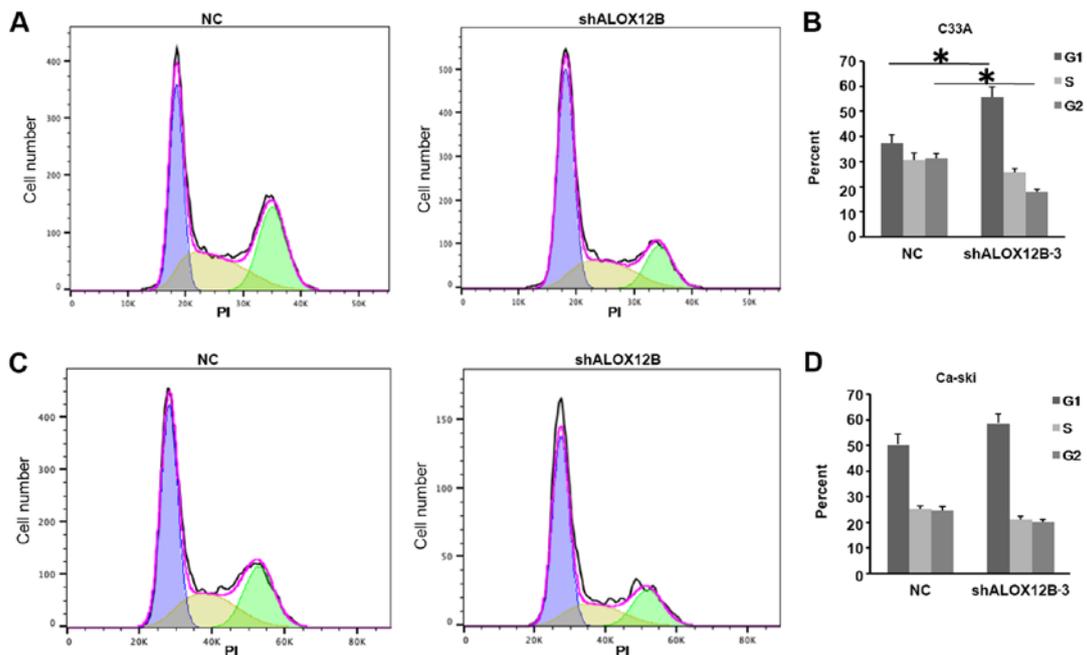


Figure 4. ALOX12B regulates cell cycle transition in cervical cancer. (A) FACS analysis demonstrating that knockdown of ALOX12B arrested cell cycle in the G₁ phase in C33A cells. (B) The difference between cells in G₁ phase in the NC group and cells in G₁ phase in the shALOX12B group was significant. The difference between cells in G₂ phase in the NC group and cells in G₂ phase in the shALOX12B group was also significant. (C) FACS analysis demonstrating that knockdown of ALOX12B did not affect the cell cycle in Ca-Ski cells. (D) No significant differences in the cell cycle distribution in Ca-Ski cells were found after ALOX12B-knockdown. *P<0.05. sh, small hairpin; NC, negative control; FACS, fluorescent-activated cell sorting.

Knockdown of ALOX12B delays tumor growth in a xenograft mouse model. Since ALOX12B contributed to the cell

proliferation and growth of C33A cells, and regulated the cell cycle distribution, it was hypothesized that ALOX12B

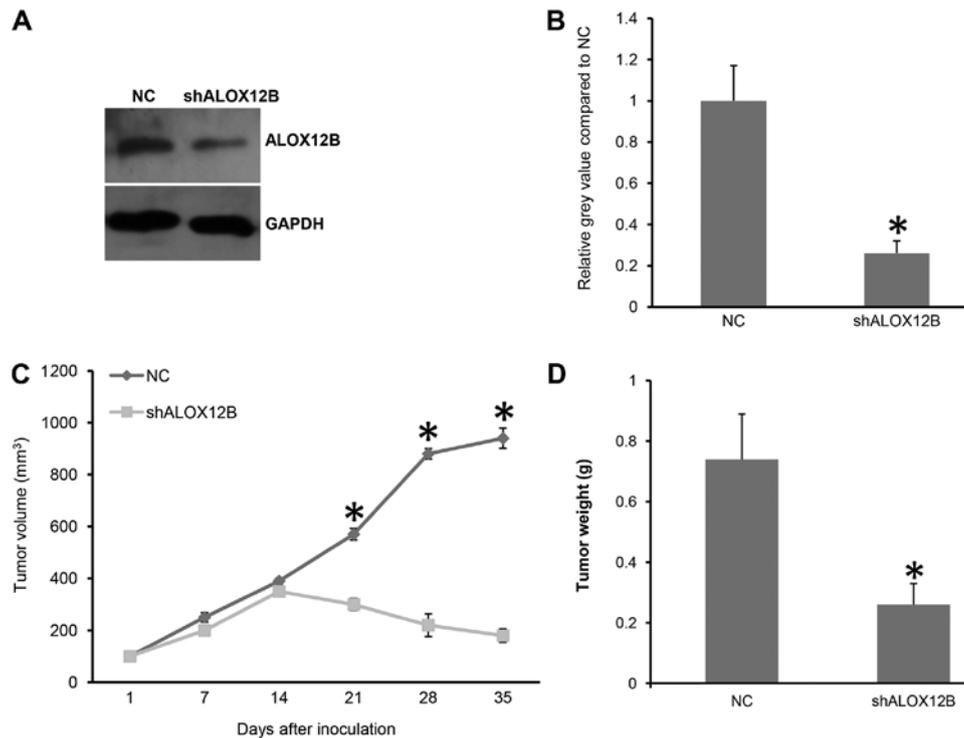


Figure 5. Knockdown of ALOX12B suppresses tumor growth *in vivo*. (A and B) Western blotting confirmed the knockdown of ALOX12B *in vivo*. (C) The tumor volume of mice in the shALOX12B group was significantly smaller than that of mice in the NC group. (D) The tumor weight of mice in shALOX12B group was significantly smaller than that of mice in the NC group. * $P < 0.05$. sh, small hairpin; NC, negative control.

could regulate tumor growth *in vivo*. C33A cells transfected with shALOX12B-3 showed a significant slower growth in a mouse xenograft model ($P < 0.05$; data not shown). As shown in Fig. 5A and B, the knockdown of ALOX12B was confirmed by western blotting. It was demonstrated that the tumor volume in the shALOX12B-3 group was significantly smaller compared with that of the NC group (180 vs. 940 mm³; $P < 0.05$; Fig. 5C). The tumor weight in the shALOX12B-3 group was also significantly lighter compared with that of the NC group (0.26 vs. 0.74g; $P < 0.05$; Fig. 5D). As expected, ALOX12B was essential for *in vivo* tumor growth of cervical cancer.

ALOX12B regulates the PI3K/ERK1 signaling pathway in cervical cancer. As a novel gene, there is little information regarding the signaling pathway regulated by ALOX12B. In several types of cancer, important signaling pathways regulating cell proliferation include PI3K, Wnt, mTOR and MAPK (19-21). The expression levels of these molecules were analyzed using western blotting. As shown in Fig. 6A and B, after knockdown of ALOX12B in C33A cells, the expression levels of PI3K, MEK1, ERK1, C-fos and cdc25 were significantly reduced ($P < 0.05$). By contrast, overexpression of ALOX12B in cells treated with the plasmid pcDNA-ALOX12B significantly increased the expression levels of PI3K, MEK1, ERK1, C-fos and cdc25 ($P < 0.05$; Fig. 6A and C). *In vivo*, knockdown of ALOX12B also significantly reduced the expression of PI3K, MEK1, ERK1, C-fos and cdc25 ($P < 0.05$; Fig. 6A and D). PI3K/ERK1 signaling serves important roles in several types of cancer (19); therefore, it is possible that ALOX12B promotes the proliferation of cervical cancer cells by regulating the PI3K/ERK1 signaling pathway.

Discussion

Cervical cancer is a malignant disease affecting women worldwide, and poses a great threat to life and life quality (2,3). According to the most recent report by the American Association for Cancer Research, both the incidence and death rate of patients with cervical cancer has remained steady in recent years (1). This could be attributed to the failure of traditional therapies such as surgery, chemotherapy and radiotherapy. This high death rate indicates that some patients with cervical cancer, particularly those who have relapsed or have metastasis, do not benefit sufficiently from such therapies (3). In the past decade, a series of new biotherapies have been developed, including immunotherapy and targeted therapy. For example, antibody drugs and cell immunotherapy have greatly improved the survival of patients with cancer (4,5). However, to harness the potential clinical benefit of these therapies, the underlying molecular mechanisms of a particular cancer type need to be clarified to prevent treatment failure.

The majority of cervical cancer cases are caused by HPV infection, which is most commonly transmitted through sexual activity (22). However, genome sequencing technology has revealed that genetic and epigenetic factors also serve important roles during the tumorigenesis of cervical cancer (23). Several genes have been reported to be associated with cervical cancer. For example, Chang *et al* (24) found that high POUF3 expression accelerated the progression of cervical cancer. Heterogeneity is common in almost all types of cancer (25); therefore, it is difficult to determine the function of a single dominant gene in a specific type of cancer.

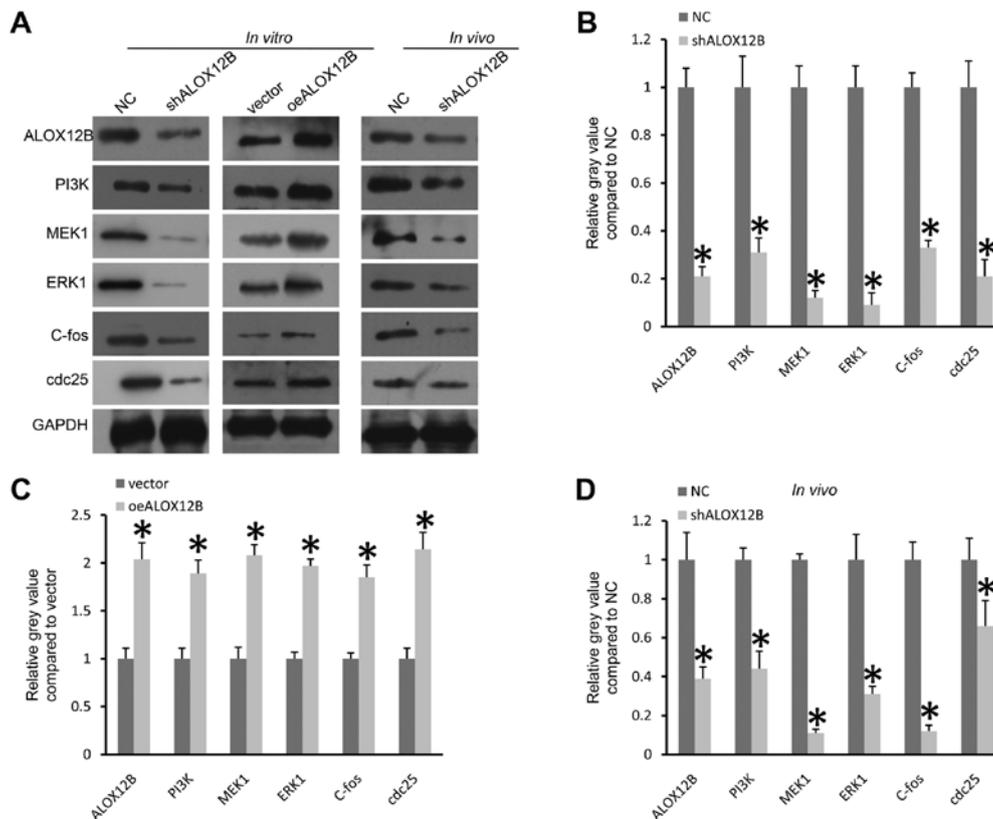


Figure 6. ALOX12B regulates the expression levels of PI3K/ERK1 signaling molecules. (A and B) Knockdown of ALOX12B decreased the expression levels of PI3K, MEK1, ERK1, C-fos and cdc25 in C33A cells. (A and C) Overexpression of ALOX12B in C33A cells increased the expression levels of PI3K, MEK1, ERK1, C-fos and cdc25. (A and D) Knockdown of ALOX12B decreased the expression levels of PI3K, MEK1, ERK1, C-fos and cdc25 in tumor tissues in the xenograft tumor model. GAPDH was used as the internal control. * $P < 0.05$. sh, small hairpin; NC, negative control; oe, overexpressed.

To the best of our knowledge, the present study demonstrated for the first time that ALOX12B may serve an oncogenic role in cervical cancer. The *in vitro* data suggested that ALOX12B contributed to cell proliferation in cervical cancer. Normally, cells display an inhibitory effect when they contact each other; however, tumor cells are known to have no contact-inhibitory characteristics and can readily proliferate (16). An accelerated cell cycle is often synchronized with cell proliferation and is common in tumor cells. As expected, knockdown of ALOX12B resulted in cell cycle arrest in the G₁ phase in C33A cells. This was consistent with the proliferation-promoting role of ALOX12B in cervical cancer. However, little difference in cell cycle was observed in Ca-Ski cells. This may be due to the heterogeneity of tumor cells. Infection with HPV was a major cause of cervical cancer, and different strains of HPV caused expression of checkpoint molecules controlling the cell cycle, such as p53 and p105 (16,26,27).

Metastasis is another common phenomenon affecting patients with cancer (18). Generally speaking, wound healing assays are often used to reflect the aggressive ability of tumor cells *in vitro* (28); however, ALOX12B-knockdown did not affect the migration ability of cervical cancer cells in the present study. It is possible that ALOX12B solely regulates cell proliferation in cervical cancer. The mouse xenograft model bearing C33A cells further demonstrated that ALOX12B was essential for tumor cell growth *in vivo*. These data suggested that ALOX12B promoted the progression of cervical cancer. In previous studies, ALOX12B was shown to be involved in

the progression of lung, breast and epidermoid cancer (13-15); thus, the present study may further improve our understanding of the role of ALOX12B in cancer. However, additional experiments are necessary to confirm the role of ALOX12B in promoting the progression of cervical cancer. Although ALOX12B displayed little effect on cell migration *in vitro*, it may be useful to analyze the effect of ALOX12B on the metastasis of cervical cancer cells *in vivo* and to investigate the expression patterns of ALOX12B in tumor versus normal tissues.

ALOX12B is a lipoxygenase that is critical for the synthesis of 12R-hydroxyeicosatetraenoic acid (10). The lipoxygenase family of proteins is large and comprises dozens of members. Different lipoxygenases were shown to be associated with inflammation and tumorigenesis via different signaling pathways (9). For example, ALOX15 suppresses inflammation by inhibiting the IL-6/STAT3 signaling pathway in colorectal cancer (29). Additionally, lipoxygenase 5-LOX and 12-LOX have been revealed to be essential for cell proliferation in pancreatic cancer (30). The most commonly reported alteration concerning ALOX12B was SNP (8-14). Only Agarwal *et al* (15) demonstrated that ALOX12B modulated the ERK and PI3K-Akt signaling pathways in A431 cells. As ALOX12B was shown to regulate cell proliferation in cervical cancer in the present study, the proliferation-related signaling molecules in C33A cells were determined. It was found that ALOX12B could regulate the expression levels of PI3K, MEK1, ERK1, C-fos and cdc25. PI3K is known to be an important

gene in the proliferation of tumor cells, and abnormal activation of PI3K has been shown in a number of cancer types, including cervical cancer (31). MEK1 is a mitogen-activated protein kinase and is often involved in crosstalk with the PI3K signaling pathway. For example, MEK1 transmits signals from PI3K to ERK1, and activates target genes such as C-fos and cdc25 (32). Activation of the PI3K/ERK1 signaling pathway has been observed in several types of cancer and has been shown to promote tumorigenesis (33). C-fos and cdc25 are two common genes that promote tumor progression (27,34). In addition, ALOX12B was shown to regulate PI3K and ERK signaling pathways in epidermoid cancer cells (15); therefore, it is possible that ALOX12B regulates PI3K/ERK1 signaling in cervical cancer.

In summary, the present study demonstrated that ALOX12B promoted cell proliferation via regulation of the PI3K/ERK1 signaling pathway in cervical cancer. These results may provide the basis for future targeting of ALOX12B in cervical cancer treatment. However, the clinical significance of ALOX12B in cervical cancer remains unclear. The association of ALOX12B with survival and with clinicopathological factors in patients was not elucidated in the present study. Additionally, the way in which ALOX12B affects the PI3K signaling pathway requires further investigation. Another limitation is that the present study only used a couple of cell lines, and therefore the role of the ALOX12B gene should be investigated in more cell lines, which may further support the present results. Therefore, more work is required to further support the role of ALOX12B in cervical cancer and to clarify the link between ALOX12B and PI3K/ERK1. The expression pattern of ALOX12B in a large cohort of clinical tumor tissues and its association with clinicopathological factors should be investigated to confirm its clinical significance.

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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 on reasonable request.

Authors' contributions

TJ and LYL conceived and designed the study. QYY and YML conducted the literature review and interpreted the data. BZ and TJ analyzed the data. TJ wrote the manuscript. KJT, TJ, BZ and YML performed the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Laboratory Animal Ethics Committee of Nanchang Royo Biotech Co., Ltd. (approval no. RYE2019011104).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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