

# MicroRNA-125a-5p modulates human cervical carcinoma proliferation and migration by targeting ABL2

Xian Qin<sup>1</sup>  
Yajun Wan<sup>1</sup>  
Saiying Wang<sup>2</sup>  
Min Xue<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, <sup>2</sup>Department of Anesthesiology, Third Xiangya Hospital, Central South University, Changsha, People's Republic of China

**Background:** In this study, we intended to understand the regulatory mechanisms of microRNA-125a-5p (miR-125a-5p) in human cervical carcinoma.

**Methods:** The gene expressions of miR-125a-5p in seven cervical carcinoma cell lines and 12 human cervical carcinoma samples were evaluated by quantitative real-time reverse transcription polymerase chain reaction. Ca-Ski and HeLa cells were transduced with lentivirus carrying miR-125a-5p mimics, and the effects of lentivirus-induced miR-125a-5p upregulation on cervical carcinoma proliferation and migration were examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and transwell assays, respectively. In addition, HeLa cells were inoculated into null mice to evaluate the effect of miR-125a-5p upregulation on in vivo cervical carcinoma growth. The direct regulation of miR-125a-5p on its target gene, ABL proto-oncogene 2 (ABL2), in cervical carcinoma was evaluated by quantitative real-time reverse transcription polymerase chain reaction, Western blotting and luciferase reporter assays, respectively. ABL2 was then downregulated by small interfering RNA to examine its effect on cervical carcinoma proliferation and migration.

**Results:** miR-125a-5p was downregulated in both cervical carcinoma cell lines and human cervical carcinomas. In Ca-Ski and HeLa cells, lentivirus-mediated miR-125a-5p upregulation inhibited cancer proliferation and migration in vitro and cervical carcinoma transplantation in vivo. ABL2 was shown to be directly targeted by miR-125a-5p. In cervical carcinoma, ABL2 gene and protein levels were both downregulated by miR-125a-5p. Small interfering RNA-mediated ABL2 downregulation also had tumor-suppressive effects on cervical carcinoma proliferation and migration.

**Conclusion:** The molecular pathway of miR-125a-5p/ABL2 plays an important role in human cervical carcinoma. Targeting miR-125a-5p/ABL2 pathway may provide a new treatment strategy for patients with cervical carcinoma.

**Keywords:** cervical carcinoma, miR-125a-5p, ABL2, cancer proliferation, cancer migration

## Introduction

Cervical carcinoma is one of the most common malignant cancers in women. In the USA alone, there are approximate 13,000 cases of newly diagnosed cervical carcinomas and 4,000 cases of cervical carcinoma related deaths every year.<sup>1</sup> In the developing countries, such as People's Republic of China, the incident rates of cervical carcinoma are much higher than the rates in the USA or other developed countries.<sup>2-4</sup> In addition, women in developing countries who were diagnosed with cervical carcinoma were very likely in advanced stages already with poor survival rates.<sup>4,5</sup> Therefore, it is critical to better understand the molecular mechanisms of cervical carcinoma and develop efficient methods for early diagnosis, vaccination, and treatment.

Correspondence: Min Xue  
Department of Obstetrics and Gynecology, Third Xiangya Hospital, Central South University, 138 Tongzipo Road, Changsha 410013, People's Republic of China  
Tel +867 318 861 8821  
Email minxue56@aol.com

MicroRNA (miRNAs) are families of 18–22 pair noncoding RNAs that induce gene or protein degradation through the binding on 3'-untranslated regions (3'-UTR) of their target genes.<sup>6</sup> In human cervical carcinoma, studies have shown that miRNAs could act as either oncogenic or tumor-suppressive factors to widely regulate cervical carcinoma proliferation, migration, or apoptosis.<sup>7–9</sup> For example, miR-21 is an oncogene in cervical carcinoma inducing cancer proliferation by suppressing programmed cell death 4 gene.<sup>10</sup> On the other hand, miR-34c is a tumor-suppressive gene, inhibiting cervical carcinoma migration by downregulating Notch1 and Jagged1.<sup>11</sup> Among many of the carcinoma-associated miRNAs, miR-125 family, including miR-125a-3p, miR-125a-5p, and miR-125b, is a group of homologous miRNAs that are highly conserved across various species and play critical roles in cancer regulation.<sup>12</sup> In lung cancer, miR-125a-5p is de-regulated in carcinoma tissue and its upregulation promoted cancer migration.<sup>13</sup> In human hepatocellular carcinoma, miR-125a-5p is shown to suppress cancer development through the regulation of SIRT7.<sup>14</sup> In verrucous carcinoma of the head and neck, miR-125a-5p was found to be downregulated in cancer tissues.<sup>15</sup> In cervical carcinoma, it was shown that miR-125b was downregulated and inversely correlated with HPV production, suggesting a possible tumor-suppressive role of miR-125b.<sup>16</sup> However, the expression and function of other miR-125 family members, including miR-125a-5p, are still unknown.

ABL proto-oncogene 2 (ABL2) is an oncogene that promotes carcinoma proliferation and invasion in various cancers, such as breast cancer,<sup>17</sup> prostate cancer,<sup>18</sup> or pancreatic cancer.<sup>19</sup> Though ABL2 is expressed in human cervical carcinomas, its exact molecular mechanisms remain elusive.

In the present study, we investigated the expression pattern and molecular functions of miR-125a-5p in human cervical carcinoma. We also examined the possible association of miR-125a-5p and ABL2 in regulating cervical carcinoma proliferation and migration. The results in this study may help develop new therapeutic targets for treating patients with cervical carcinoma.

## Materials and methods

### Cervical carcinoma cell lines and human carcinoma samples

Seven human cervical carcinoma cell lines, HeLa, SiHa, Ca-Ski, C-33-A, DoTc24510, HT-3, and C-4I, as well as two primary non-carcinoma human cervix epithelial cell lines, GH329 and Ect1/E6E7, were purchased from the American Type Culture Collection (Shanghai, People's Republic of China). All cells were maintained in Dulbecco's Modified

Eagle's Medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific) and 1% antibiotic-antimycotic solution (Thermo Fisher Scientific) in a humidified incubator with 5% CO<sub>2</sub> at 37°C. Human cervical tissues, including carcinoma as well as adjacent non-carcinoma tissues, were surgically retrieved from 12 patients in the Department of Obstetrics and Gynecology at the Third Xiangya Hospital, Changsha, People's Republic of China, between September 2014 and June 2015. Consent forms were signed by all participating patients. Clinical procedures for human tissue processing were approved by the Ethics Committee at the Third Xiangya Hospital of Central South University in Changsha, People's Republic of China.

### RNA extraction and qRT-PCR

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was used to evaluate the expression levels of miR-125a-5p and ABL2. Total RNA was extracted from the cervical carcinoma cells or human samples using a TRIZOL reagent (Thermo Fisher Scientific) according to the manufacturer's protocols. Reverse transcription was conducted using a TaqMan Reverse Transcription Kit (Thermo Fisher Scientific) according to the manufacturer's protocols. qRT-PCR was conducted on an ABI PRISM® 7000 Sequence Detection System (Thermo Fisher Scientific) according to the manufacturer's protocol. To detect miR-125a-5p gene expression level, a TaqMan miRNA qRT-PCR assay was used (Thermo Fisher Scientific) with U6 transcript as an internal control. To detect ABL2 mRNA expression level, a SYBR Green PCR Master Mix kit (Thermo Fisher Scientific) was used with GAPDH as an internal control. The relative gene expression level was calculated using the (2<sup>-ΔΔCT</sup>) method and normalized to control.

### Lentiviral transduction assay

To evaluate the functional effect of miR-125a-5p in cervical carcinoma, lentivirus-mediated miRNA upregulation was used in Ca-Ski and HeLa cells. Lentivirus of human miR-125a-5p mimics (miR-125-mimic) as well as its negative control miRNA lentivirus (miR-C) was commercially obtained from SunBio (SunBio Medical Biotechnology, Guangzhou, People's Republic of China). Lentiviral transduction was conducted by mixing 5 μL Lipofectamine 2000 reagent (Thermo Fisher Scientific) with 200 μL transduction medium containing 100 pmol lentiviruses for 6 hours according to the manufacturer's protocol. After being replenished with fresh culture medium, qRT-PCR and other experiments were then conducted in 24 hours.

## Proliferation assay

To evaluate the proliferation capability of cervical carcinoma, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Thermo Fisher Scientific) was performed in Ca-Ski and HeLa cells according to the manufacturer's protocol. Briefly, Ca-Ski and HeLa cells were maintained in 96-well plates ( $5 \times 10^3$  cells/well). From 1 day to 5 days after lentiviral transduction, 200  $\mu$ L MTT solution (5 mg/mL) was added in the culture for 4 hours. Culture medium was then aspirated and formazan crystals were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich Co., St Louis, MO, USA). The proliferation rate was measured as optical density at an absorbance of 450 nm, and then normalized to the optical density of untreated control.

## Migration assay

To evaluate the migration capability of cervical carcinoma, a Transwell assay (Becton Dickinson, San Diego, CA, USA) was used according to the manufacturer's protocol. Briefly, the upper chamber was coated with 100  $\mu$ L Matrigel (Thermo Fisher Scientific) at 37°C for 30 minutes. After that, lentivirus-transduced Ca-Ski and HeLa cells were resuspended and plated in the upper chamber in 200  $\mu$ L DMEM without FBS. In the lower chamber, 500  $\mu$ L DMEM +10% FBS was added to act as chemoattractant. After 24 hours, the upper chamber and non-migrated cells were removed, and the lower chamber was fixed with ethanol and stained with crystal violet. The transwell was then placed on an Olympus IX-70 inverted microscope fluorescent microscope (Olympus Corporation, Tokyo, Japan). Relative migration capability was measured as the number of migrated cells for each experimental condition, and then normalized to control.

## In vivo transplantation assay

To evaluate the in vivo tumor growth capability of cervical carcinoma, a tumor transplantation assay was conducted. Briefly, 24 hours after lentivirus transduction, a million healthy HeLa cells were subcutaneously inoculated into the left flank of female nude mice (8-week old). The growth of tumor transplantation was evaluated weekly using an equation of  $\text{length} \times \text{width}^2/2$ . Five weeks after in vivo inoculation, mice were killed and tumors were harvested for Ki-67 immunostaining (Santa Cruz Biotechnology Inc., Dallas, TX, USA).

## Western blot

Total protein was extracted using a cell lysis buffer (Promega Corporation, Fitchburg, WI, USA). Protein (50  $\mu$ g) of each sample was then diluted in 10% of SDS-PAGE gel and transferred to nitrocellulose membranes (Bio-Rad Laboratories Inc.,

Hercules, CA, USA). The membranes were then incubated with primary antibodies of ABL2 and beta-actin (1:200, Sigma-Aldrich) at 4°C overnight, followed by HRP-conjugated secondary antibodies (1:1,000 dilution) at RT for 1 hour. The blots were incubated with enhanced chemiluminescence substrate (Amersham Pharmacia Biotechnology, Princeton, NJ, USA) for 5 minutes, and then visualized using an enhanced chemiluminescence film system (Amersham) according to the manufacturer's protocol.

## Luciferase assay

Human 3'-UTR of ABL2 was cloned into the pMir-Report vector (Ambion, Austin, TX, USA) to generate a wild-type luciferase reporter vector (ABL2 WT 3'-UTR). The miR-125a-5p binding sequences on ABL2 3'-UTR were mutated using a Site-Directed Mutagenesis Kit (SBS Genetech, Beijing, People's Republic of China), and verified by sequencing. It was also cloned into pMir-Report vector to generate a mutated luciferase reporter vector (ABL2 Mu 3'-UTR). An empty luciferase reporter vector (vector) was used as the control. HEK293T cells were transiently co-transfected with miR-125-mimic and 3  $\mu$ g luciferase reporter vectors using Lipofectamine 2000 for 48 hours. The relative luciferase activities were evaluated by a dual-luciferase reporter assay (Promega) according to the manufacturer's protocol.

## siRNA transfection assay

The small interfering RNA (siRNA) targeting human ABL2 (siRNA-ABL2) and its negative control siRNA (siRNA-C) were commercially obtained from SunBio. Ca-Ski and HeLa cells were plated in 96-well plates ( $5 \times 10^3$  cells/well) and transfected with siRNAs (50 nM) using Lipofectamine 2000 (Thermo Fisher Scientific) for 12 hours. After that, the culture medium was replenished and cells were continuously cultured for 24 hours before the commencement of other experiments.

## Statistical analyses

The data in the present study were presented as mean  $\pm$  standard error. To evaluate the statistical significance between compared data, Student's *t*-test was conducted using GraphPad Prism6 software (Graphpad Software, Inc., La Jolla, CA, USA). A *P*-value of  $<0.05$  was considered significant. All experiments were performed in triplicates.

## Results

### miR-125a-5p is downregulated in cervical carcinoma

The gene expression of miR-125a-5p was examined by qRT-PCR in both cervical carcinoma cell lines and the human

cervical carcinoma. In cell lines, we found that the gene expression levels of miR-125a-5p in seven cervical carcinoma cells, HeLa, SiHa, Ca-Ski, C-33-A, DoTc24510, HT-3, and C-4I, were significantly lower than the gene expression levels of miR-125a-5p in two non-carcinoma human cervix epithelial cell lines, GH329 and Ect1/E6E7 cells (Figure 1A,  $*P<0.05$ ). With qRT-PCR analysis on paired cervical tissues from 12 patients, we found that, in each patient, miR-125a-5p was significantly downregulated in carcinoma tissue than its adjacent non-carcinoma tissue (Figure 1B,  $*P<0.05$ ).

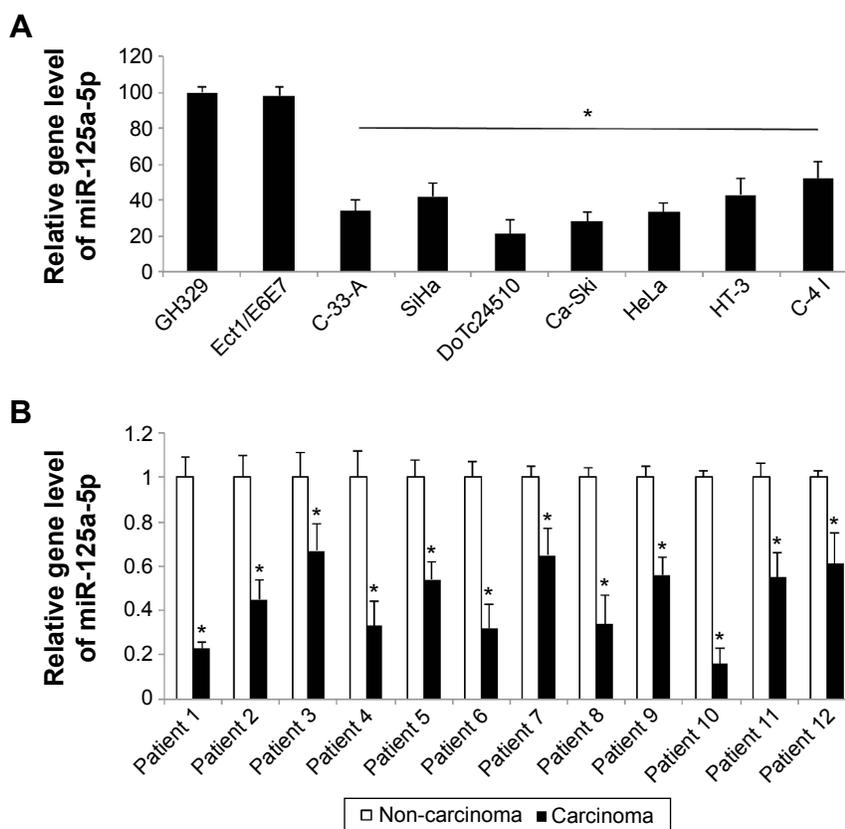
## miR-125a-5p overexpression inhibits cervical carcinoma proliferation and migration in vitro

To identify the functional mechanisms of miR-125a-5p in cervical carcinoma, we transduced two cervical carcinoma cell lines, Ca-Ski and HeLa cells, with lentivirus expressing miR-125-mimic. In the control, Ca-Ski and HeLa cells were transduced with a lentivirus expressing negative control miRNA (miR-C). One day after transduction, efficiency of lentiviral transduction

was confirmed by qRT-PCR (Figure 2A,  $*P<0.05$ ). Then, we used an MTT assay to evaluate the effect of miR-125a-5p overexpression on cancer proliferation. We found that in miR-125a-5p overexpressed Ca-Ski and HeLa cells, cancer proliferation was significantly slowed 3–5 days after lentiviral transduction (Figure 2B and C,  $*P<0.05$ ). We also examined the effect of miR-125a-5p overexpression on cervical carcinoma migration since metastasis is one of the signature characteristics in cervical cancer. Using a transwell assay, we found that, in miR-125a-5p overexpressed Ca-Ski and HeLa cells, significantly less migrated cancer cells were seen in the lower chambers (Figure 2D and E, left panel). Quantitative analysis confirmed that miR-125a-5p overexpression reduced the migration capabilities by 66% in Ca-Ski cells and 79% in HeLa cells, respectively (Figure 2D and E, right panel,  $*P<0.05$ ).

## miR-125a-5p overexpression inhibits cervical carcinoma growth in vivo

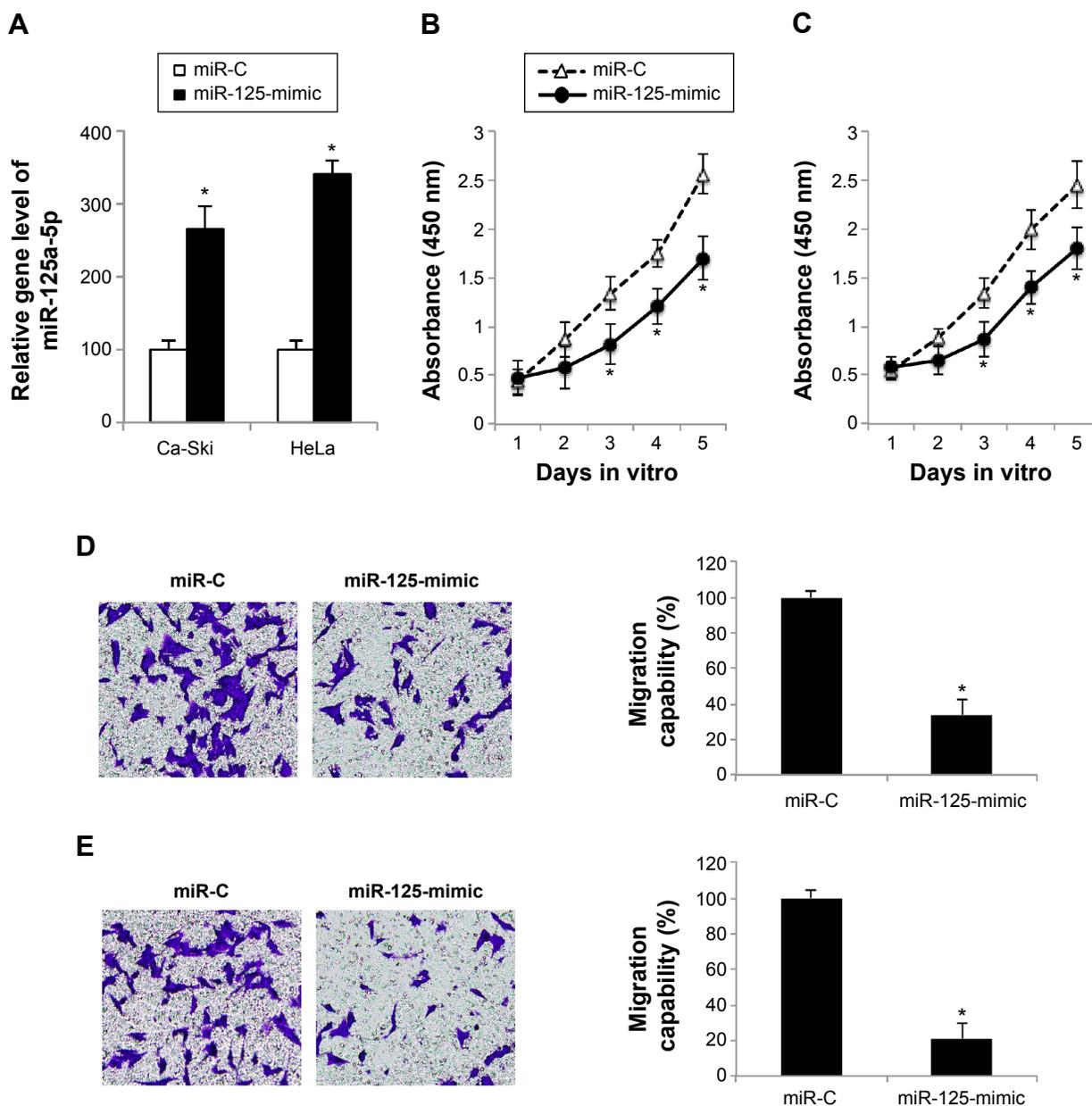
Next, we investigated whether miR-125a-5p would also have tumor-suppressive effect on in vivo carcinoma growth.



**Figure 1** Expression of miR-125a-5p in cervical carcinoma.

**Notes:** (A) The gene expression levels of miR-125a-5p in seven cervical carcinoma cells, HeLa, SiHa, Ca-Ski, C-33-A, DoTc24510, HT-3, and C-4I, were measured by qRT-PCR and compared with the gene expression level of miR-125a-5p in two non-carcinoma human cervix epithelial cell lines, GH329 and Ect1/E6E7 cells ( $*P<0.05$ ). (B) In human cervical carcinomas, the gene expression level of miR-125a-5p was compared with carcinoma and adjacent non-carcinoma tissues in 12 samples ( $*P<0.05$ ).

**Abbreviation:** qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction.



**Figure 2** miR-125a-5p overexpression reduced in vitro proliferation and migration in cervical carcinoma.

**Notes:** Ca-Ski and HeLa cells were transduced with lentivirus miR-125-mimic to overexpress miR-125a-5p for 6 hours. The control cells were transduced with a negative control miRNA lentivirus (miR-C) for 6 hours. **(A)** Twenty-four hours after lentiviral transduction, gene expressions of miR-125a-5p in Ca-Ski and HeLa cells were examined by qRT-PCR,  $*P < 0.05$ . **(B and C)** Twenty-four hours after lentiviral transduction, an MTT assay was performed for 5 days to compare cervical carcinoma proliferation between miR-125a-5p overexpressed cells and control cells; **(B):** Ca-Ski, **(C):** HeLa,  $*P < 0.05$ . **(D and E)** Twenty-four hours after lentiviral transduction, a transwell assay was performed. The representative crystal violet images were shown for miR-125a-5p overexpressed cells and control cells (left panel). The migration capabilities were evaluated by calculating the average migrated cells in 96-well plates and normalized to control (right panel); **(D):** Ca-Ski, **(E):** HeLa,  $*P < 0.05$ .

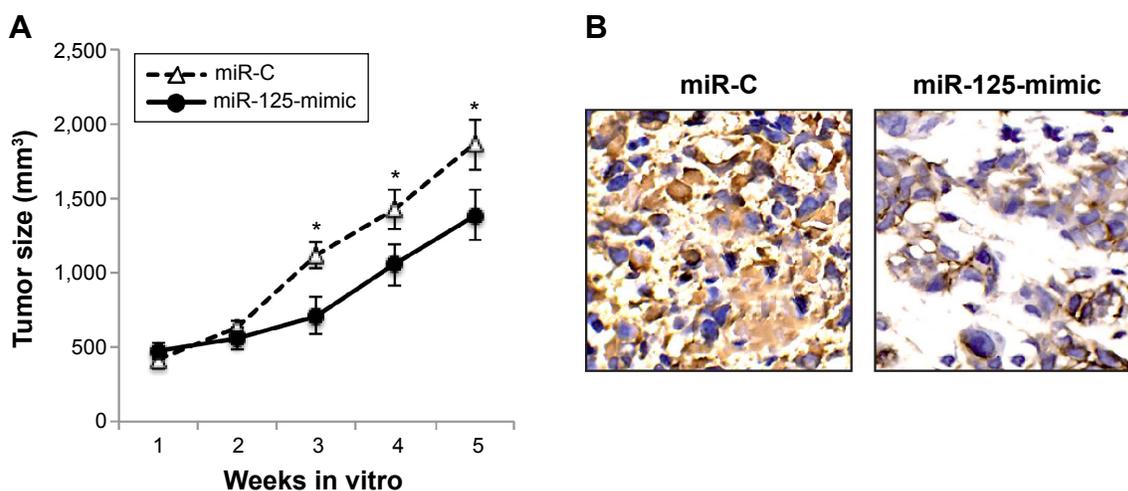
**Abbreviations:** qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Twenty-four hours after lentiviral transduction, HeLa cells were re-suspended and 1 million healthy cells were subcutaneously inoculated into null mice. For 5 weeks, the length and width of the in vivo tumor were measured and the total volumes were calculated. We found that the in vivo growth of cervical carcinoma was markedly reduced while miR-125a-5p was overexpressed (Figure 3A,  $*P < 0.05$ ). In addition, we extracted the tumors and performed Ki-67

staining on paraffin-embedded sections. We found that the expression level of Ki-67 was also significantly reduced in tumors with miR-125a-5p overexpression (Figure 3B).

### ABL2 is regulated by miR-125a-5p overexpression in cervical carcinoma

As we demonstrated miR-125a-5p is generally down-regulated in cervical carcinoma and its upregulation had



**Figure 3** miR-125a-5p overexpression reduced in vivo growth of cervical carcinoma.

**Notes:** HeLa cells were transfected with lentivirus of miR-125-mimic to overexpress miR-125a-5p for 6 hours. The control cells were transfected with a negative control miRNA lentivirus (miR-C) for 6 hours. Twenty-four hours after transfection, cells were re-suspended and 1 million healthy cells were then subcutaneously transplanted into the left side of 8-week-old female null mice. **(A)** The weekly in vivo carcinoma growth was measured by the equation of length  $\times$  width<sup>2</sup>/2 ( $*P < 0.05$ ). **(B)** Five weeks after in vivo transplantation, mice were sacrificed and tumors were extracted for Ki-67 immunostaining.

tumor-suppressive effects on cancer proliferation and migration, we wondered whether miR-125a-5p may regulate cervical carcinoma by negatively associating oncogene in cervical cancer. Through literature search, we noticed that ABL2 was an oncogene in human cancer.<sup>17,18</sup> While ABL2 is reported to be expressed in cervical tissue,<sup>20</sup> its functional mechanisms in cervical development or pathology are unknown. Therefore, to elaborate the molecular pathway associated with miR-125a-5p in human cervical carcinoma, we used qRT-PCR and Western blotting to probe the possible downstream target of miR-125a-5p, ABL2. We found that ABL2 was significantly downregulated in both Ca-Ski and HeLa cells while miR-125a-5p was overexpressed (Figure 4A and B,  $*P < 0.05$ ). In addition, Western blotting analysis showed that the protein levels of ABL2 were also markedly reduced in Ca-Ski or HeLa cells while miR-125a-5p was overexpressed (Figure 4C and D,  $*P < 0.05$ ). Next, we utilized online bioinformatics websites to verify whether ABL2 was the downstream molecular target of miR-125a-5p. We searched TargetScan ([www.targetscan.org](http://www.targetscan.org)) and miRANDA ([www.microRNA.org](http://www.microRNA.org)) and found that ABL2 was very possibly the downstream target of miR-125a-5p (Figure 4E). Furthermore, we performed a luciferase reporter assay and found that miR-125a-5p was indeed directly targeting ABL2 gene (Figure 4F,  $*P < 0.05$ ).

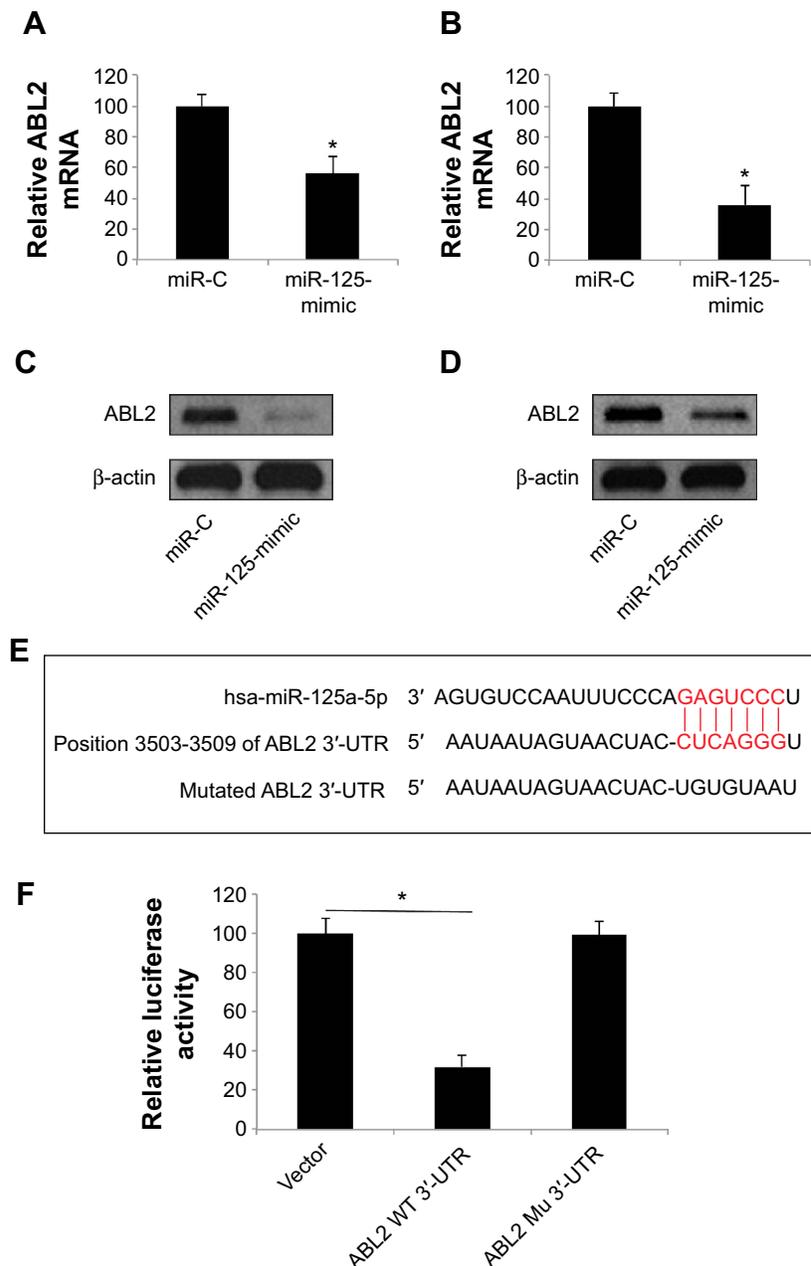
### ABL2 downregulation inhibits cervical carcinoma proliferation and migration in vitro

Finally, to investigate whether downregulating ABL2 would also have similar tumor-suppressing functions as

miR-125a-5p upregulation in human cervical carcinoma, we used ABL2-specific siRNA, siRNA-ABL2, to genetically knock down endogenous ABL2 gene expressions in Ca-Ski and HeLa cells. The control cells were transfected with a negative control siRNA, siRNA-C. One day after transfection, the gene expression levels of ABL2 in Ca-Ski and HeLa cells were examined by qRT-PCR. It showed that siRNA-ABL2 significantly downregulated ABL2 mRNAs in Ca-Ski and HeLa cells (Figure 5A,  $*P < 0.05$ ). Next, we used an MTT assay to evaluate the effect of ABL2 downregulation on cervical carcinoma proliferation. We found that in ABL2 downregulated Ca-Ski and HeLa cells, cancer proliferation was significantly inhibited 3–5 days after lentiviral transduction, similar to the effect of miR-125a-5p upregulation (Figure 5B and C,  $*P < 0.05$ ). We then examined cancer migration through a transwell assay. We found that in ABL2 downregulated Ca-Ski and HeLa cells, the migrated cancer cells were significantly reduced in the lower chambers (Figure 5D and E, left panel), and the migration capabilities were reduced by 59% in Ca-Ski cells and 75% in HeLa cells (Figure 5D and E, right panel,  $*P < 0.05$ ). Thus, our data confirmed that ABL2 downregulation had similar tumor-suppressive effects as miR-125a-5p upregulation on cervical carcinoma proliferation and migration.

### Discussion

In the present study, we examined the expression pattern and molecular regulations of miR-125a-5p in human cervical carcinoma. We found that miR-125a-5p was downregulated in cervical carcinoma cell lines as compared to



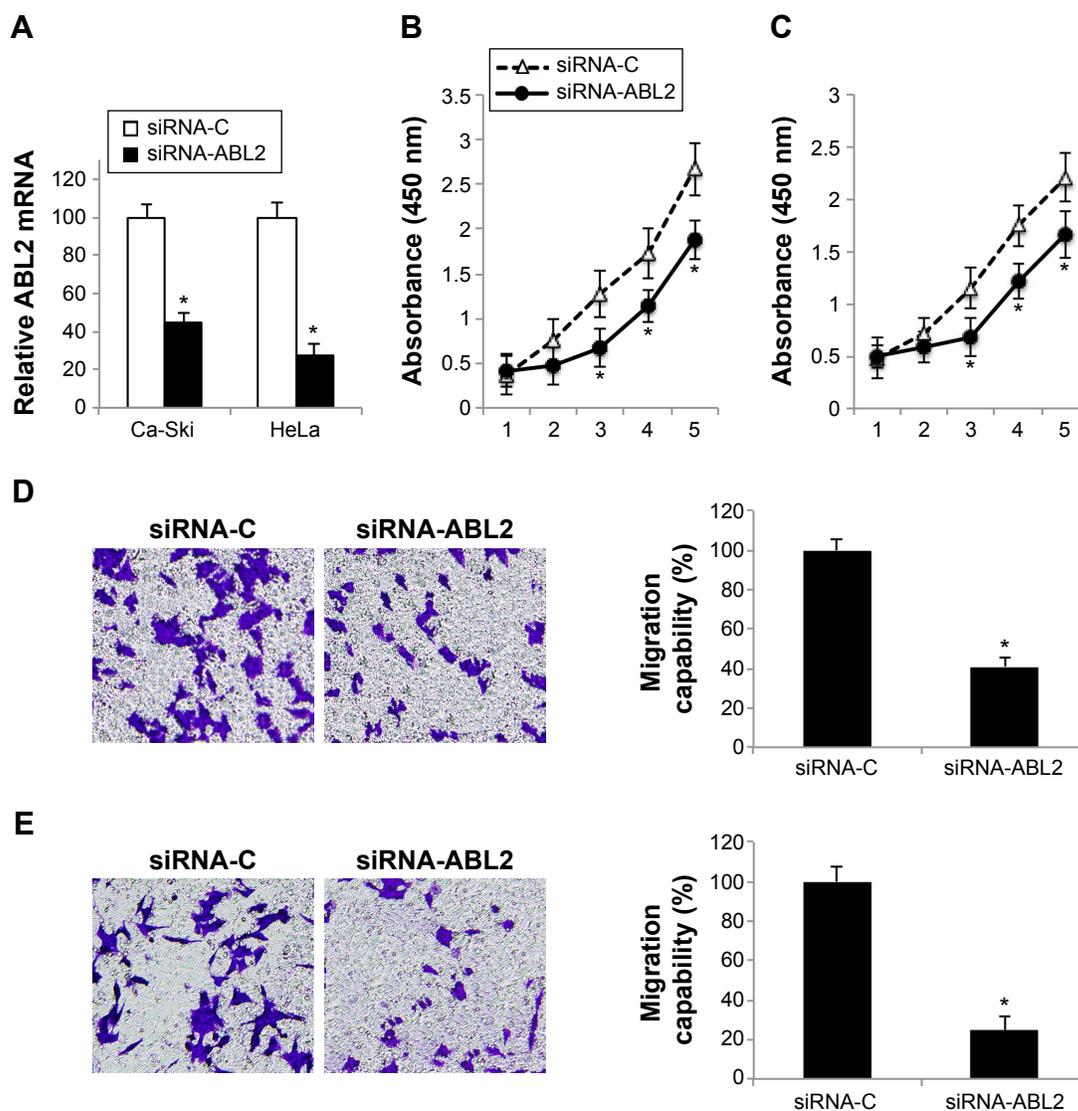
**Figure 4** miR-125a-5p regulates ABL2 in cervical carcinoma.

**Notes:** Ca-Ski and HeLa cells were transduced with lentivirus miR-125-mimic to overexpress miR-125a-5p for 6 hours. The control cells were transduced with a negative control miRNA lentivirus (miR-C) for 6 hours. Twenty-four hours after lentiviral transduction, the gene expression levels of ABL2 were examined by qRT-PCR in Ca-Ski (**A**) and HeLa (**B**) ( $*P < 0.05$ ). The protein expression levels of ABL2 were examined by Western blotting in Ca-Ski (**C**) and HeLa (**D**). The targeting search result was shown for the binding between miR-125a-5p and ABL2 3'-UTR. A mutated ABL2 3'-UTR with nullified miR-125a-5p binding site was also created (**E**). HEK293T cells were co-transfected with miR-125-mimic, an empty luciferase vector (vector), a luciferase vector inserted with 3'-UTR of wild-type ABL2 (ABL2 WT 3'-UTR), or a luciferase vector inserted with mutated ABL2 3'-UTR (ABL2 Mu 3'-UTR). Forty-eight hours after co-transfection, a dual-luciferase reporter assay was performed. The luciferase activities were measured for all three luciferase vectors and then normalized to the luciferase activity of vector ( $*P < 0.05$ ) (**F**).

**Abbreviations:** qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; ABL2, ABL proto-oncogene 2; 3'-UTR, 3'-untranslated regions.

non-carcinoma human cervix epithelial cell lines. We also found miR-125a-5p was downregulated in human cervical carcinomas as compared to adjacent non-carcinoma cervical tissues. These results suggest that miR-125a-5p is acting as a tumor suppressor in human cervical carcinoma. We then utilized several functional assays to assess the

effects of overexpressing miR-125a-5p in human cervical carcinoma cell lines, Ca-Ski and HeLa cells. We found that lentivirus-induced miR-125a-5p upregulation inhibited *in vitro* cancer proliferation and migration in Ca-Ski and HeLa cells and reduced *in vivo* growth of HeLa transplantation, thus confirming the tumor-suppressive effect of



**Figure 5** ABL2 downregulation reduced in vitro proliferation and migration in cervical carcinoma.

**Notes:** Ca-Ski and HeLa cells were transfected with siRNA-ABL2 or siRNA-C for 12 hours. **(A)** Twenty-four hours after siRNA transfection, gene expressions of ABL2 in Ca-Ski and HeLa cells were examined by qRT-PCR,  $*P < 0.05$ . **(B and C)** Twenty-four hours after siRNA transfection, an MTT assay was performed for 5 days to compare cervical carcinoma proliferation between ABL2 downregulated cells and control cells; **(B):** Ca-Ski, **(C):** HeLa,  $*P < 0.05$ . **(D and E)** Twenty-four hours after siRNA transfection, a transwell assay was performed. The representative crystal violet images were shown for ABL2 downregulated cells and control cells (left panel). The migration capabilities were evaluated by calculating the average migrated cells in 96-well plates and normalized to control (right panel); **(D):** Ca-Ski, **(E):** HeLa,  $*P < 0.05$ .

**Abbreviations:** ABL2, ABL proto-oncogene 2; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; siRNA, small interfering RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

miR-125a-5p in human cervical carcinoma. It was reported that miR-125a-5p was downregulated in non-small-cell lung cancer and inversely correlated with lung cancer invasion and migration.<sup>13</sup> It was also shown that miR-125a-5p was associated with p53 activity and regulated by SIRT7 to suppress the growth human hepatocellular carcinoma.<sup>14</sup> Thus, along with the evidence from our study, it seems like miR-125a-5p is predominantly acting as a tumor suppressor in various types of human carcinomas.

ABL2 was found to be an oncogene in breast and prostate cancer.<sup>17,18</sup> However, there has been no direct evidence regarding the association of miR-125a-5p and

ABL2 in regulating human carcinoma. In the present study, we attempted to explore the possible regulation of miR-125a-5p on ABL2 in human cervical carcinoma. We found that ABL2 was directly downregulated, at both gene and protein levels, by miR-125a-5p upregulation in Ca-Ski and HeLa cells. Most importantly, we discovered that siRNA-mediated ABL2 downregulation had similar tumor-suppressive effects as miR-125a-5p upregulation on cervical carcinoma proliferation and migration. Therefore, our results strongly suggest that the feedback loop of miR-125a-5p and ABL2 may be playing an important role in regulating human cervical carcinoma.

## Conclusion

We present new evidence showing that miR-125a-5p is down-regulated in human cervical carcinoma and miR-125a-5p is acting as a tumor suppressor by inhibiting cancer proliferation and migration. We also showed a novel regulatory mechanism of miR-125a-5p on ABL2 in cervical carcinoma. The results of our study may help to broaden the knowledge of miRNA regulation in cervical carcinoma and develop novel targeted therapy for cervical cancer patients.

## Disclosure

The authors report no conflicts of interest in this work.

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