Introduction

MicroRNAs (miRNAs) are noncoding RNAs that are 18–23 nucleotides in length. They function as endogenous regulators of gene expression by binding specific sites at the 3’ untranslated region (UTR) of target messenger RNAs (mRNAs) and cause translational repression or transcript degradation [1]. Recent studies have shown that miRNAs are aberrantly expressed in different cell types and at different developmental stages, suggesting that miRNAs play important roles in different biological processes and disease entities, including cell proliferation, differentiation, metabolism, and programmed cell death, metastasis, and tumor angiogenesis [2,3].

MiR-124 (also called miR-124a) is the most abundant miRNA in the brain and plays an important role in brain cancer [4]. Many recent reports have described the functions of miR-124 in different types of cancer. MiR-124 can suppress growth by targeting STAT3 and iASPP in colorectal cancer [5,6], SOS1 and Clock in glioma [7,8], CD151 in breast cancer [9], PIK3CA in hepatocellular carcinoma [10], androgen receptor in prostate cancer [11], ERK in cutaneous squamous cell carcinoma [12], and SPHK1 in gastric cancer [13].

MiR-124 inhibits migration and invasion by targeting CD151 in breast cancer, ROCK1 in glioma [14], and ITGB1 in oral squamous cell carcinoma [15]. MiR-124 inhibits metastasis by targeting Rac1 in pancreatic cancer [16]. MiR-124 targets Slug to regulate epithelial–mesenchymal transition (EMT) and the metastasis of breast cancer [17]. Thus, miR-124 functions to suppress a series of genes involved in cancer growth and metastasis in vitro and in vivo. However, the function of miR-124 in cervical cancer and tumor angiogenesis has not been determined.

Angiogenesis, the formation of new blood vessels, plays an important role in multiple pathological diseases, particularly in the growth and metastasis of solid tumors because tumors require a blood supply to sustain growth [18]. When a tumor grows to approximately 2–3 mm, the formation of new blood vessels is a key factor in supplying nutrients and oxygen and removing waste products [19,20]. Sprouting angiogenesis involves several steps: proliferation of endothelial cells, modification of the extracellular matrix, cell migration, and tube morphogenesis [21]. Previous reports suggest that angiogenesis is a complex process regulated by many factors, including VEGF, Tsp-1, GAX, HOX5, Stat3, IGF-1R [22–24], and miRNAs such as miR-23/miR-27, miR-126, miR-93 and miR-409 [2,25–27].

In the study of angiogenesis, vasculogenic mimicry (VM) is an important method. Maniotis et al. [28] first described VM as a process by which aggressive tumor cells generate non-endothelial cell-lined channels delimited by the extracellular matrix, not de novo formation of endothelial cell-lined vessels, in human malignant uveal melanoma. Among the regulatory factors of angiogenesis, the...
angiomotin-like protein AmotL1 (also referred to as a JEAP, junctionenriched and associated protein) belongs to a novel protein family characterized by a conserved glutamine-rich domain, coiled-coil domains and a PDZ-binding motif [29,30]. The family has three members, Amot, AmotL1, and AmotL2. AmotL1 was initially cloned from mouse endothelial cell line as a tight JEAP [31]. The proteins of the angiomotin family localize to tight junctions and regulate cell growth and motility [32,33]. AmotL1 has been found to control endothelial migration and cell polarity and to regulate sprouting angiogenesis by affecting tip cell migration and controlling cell-cell adhesions in vivo [30]. TNF-α can increase AmotL1 expression in HLMVECs, and this effect can be regulated by the transcription factor NF-κB [34]. Another study reported that Amot/p130 and AmotL1 are negatively regulated by Nedd4-like E3 ubiquitin ligases at the post-transcriptional level [35]. But, YAP1 recruits c-Abl to protect AmotL1 against Nedd4-mediated degradation [36]. However, whether AmotL1 can be regulated by miRNA remains unknown.

In this study, we found that miR-124 repressed VM, migration and invasion of the human cervical cancer in HeLa and C33A cell lines. Furthermore, miR-124 down- regulated the expression of AmotL1 by binding its 3′UTR to modulate the EMT process and VM. Together, our findings may contribute to the characterization of the molecular mechanisms of cancerogenesis in cervical cancer and may provide new potential therapeutic targets for cervical cancer.

**Methods and materials**

**Clinical human cervical carcinoma specimens**

Eleven paired human cervical carcinoma and adjacent non-tumorous cervical tissues were collected from the cancer center of Sun Yat-Sen University. Informed consent was obtained from each patient, and ethics approval was granted by the Ethics Committee of Sun Yat-Sen University. The category of the cervical samples was confirmed by pathological analysis. The large RNA and small RNA of tissue samples were isolated using the mirVanaTM miRNA Isolation Kit (Ambion) according to the manufacturer’s instructions.

**Quantitation of miRNAs**

A cDNA sequence containing one pre-miR-124 unit was inserted into a mammalian expression vector pcDNA3 in the enzyme sites BamHI and EcoRI (Promega, Madison, WI, USA). The sequence of pre-miR-124 is identical to the endogenous sequence. Two strands were annealed to clone a fragment of the AmotL1 3′UTR containing the target site of miR-124 with BamHI and XhoI sites. This construct was inserted into a BamHI-XhoI digested pcDNA3-EGFP reporter vector. To generate a mutant containing a mutation in the miR-124 target site, two strands were annealed and inserted into a BamHI-XhoI digested pcDNA3-EGFP reporter vector. The AmotL1 shRNA was designed with a shRNA designer tool (Invitrogen, Carlsbad, CA, USA). Two strands were annealed, followed by insertion into a HindIII-BamHI digested pSilencer vector. The AmotL1 cDNA containing the coding sequence was cloned by PCR, and the PCR product was cloned into the pcDNA3-Myc (C terminal) vector between the HindIII and EcoRI sites. The insert was confirmed by DNA sequencing.

**Cell culture, transfection, and RNA extraction**

The human cervical cancer cell lines C33A and HeLa were cultured in MEM-α and RPMI-1640 medium (Gibco, Gaithersburg, MD, USA), respectively, supplemented with 10% fetal bovine serum (MimHai Bio-Engineering, Lanzhou, China), 20 mM HEPES, 2 mM glutamine and antibiotics in a humidified atmosphere at 37°C with 5% CO2.

**Table 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5′–3′)</th>
</tr>
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<tbody>
<tr>
<td>Pri-miR-124-S</td>
<td>5′-ACGGGATCTCTTACATTCCATCTCTTACCTACC-3′</td>
</tr>
<tr>
<td>Pri-miR-124-A</td>
<td>5′-CGAAATTTCTGGCTGTCGTCGTCGTCC-3′</td>
</tr>
<tr>
<td>ASO-NC</td>
<td>5′-GGCAUUCACCCGCGCCUCUAA-3′</td>
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<tr>
<td>AmotL1-3′UTR-S</td>
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</tr>
<tr>
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<tr>
<td>AmotL1-3′UTR-MS</td>
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<tr>
<td>AmotL1-3′UTR-MA</td>
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<tr>
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<tr>
<td>U6 forward primer</td>
<td>5′-GATCCGGTACTCTGCTTAAACCTTGCTTAAATGCTATAATG</td>
</tr>
</tbody>
</table>

The primers and oligonucleotides used in this work.
Western blotting

Cell lysates were prepared from cells seeded on six-well plates at 10^6 cells per well using RIPA buffer (10 mM Tris-HCl, pH 7.4, 1% Triton X-100, 0.1% SDS, 1% NP-40, 1 mM MgCl₂) containing protease inhibitors at 48 h post-transfection. Protein expression was analyzed by western blot. The separated proteins were transferred to the nitrocellulose membranes followed by immunostaining with the primary antibodies overnight at 4 °C. On the following day, the membranes were washed and incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibody for 2 h at room temperature followed by ECL detection. After detection of protein bands, the blot was re-probed with anti-GAPDH antibody to confirm equal loading of the samples. The following antibodies were used: AmotL1 (1:300), GAPDH (1:500), E-cadherin (1:300), Vimentin (1:300), and ICAM-1 (1:100) (Tianjin Saier Biotech, Tianjin, China).

miRNA target prediction

The analysis of the predicted targets for miR-124 was performed using the TargetScan, PicTar and miRanda algorithms. The related functions of the targets were also considered.

Fluorescence assays

HeLa cells were cultured on 24-well tissue culture plates at a density of 3 × 10^4 cells per well in RPMI-1640 medium containing 10% fetal bovine serum (FBS) the day before transfection. The culture were maintained at 37 °C for 24h, followed by co-transfection with the EGFP reporter constructs and miRNA using Lipofectamine 2000 as described in the manufacturer’s instructions. The cells were then collected and lysed with RIPA after 48 h. The RFP expression vector, pDsRed2-N1, was used as the internal control. The intensities of EGFP and RFP fluorescence were detected with an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). All of the experiments were repeated three times.

Invasion and migration assays, morphologic analysis

The 24-well Boyden chamber with an 8-μm-pore-size polycarbonate membrane (Corning, Cambridge, MA, USA) was used to analyze the migration and invasion of tumor cells. Cells transfected with miR-124 expression plasmid or a control vector were seeded on the transwells coated with or without Matrigel for the invasion assays. Overexpression of miR-124 reduced invasion by 46–55% of the tube-like channel formation assays were performed. Overexpression of miR-124 decreased approximately 48% and 25% of the tube-like channels in HeLa and C33A cells, respectively (Fig. 1C and D). ASO-miR-124 transfection caused 4.18- and 1.31-fold increases in MMP-2 expression level in HeLa and C33A cells, respectively, compared to scramble ASO-NC (Fig. 1E and F).

Degradation of basal membranes and the extracellular matrix (ECM) is essential for angiogenesis, invasion and metastasis. Matrix metalloproteinase 2 (MMP-2) (gelatinase A, 72 kDa) is an enzyme that can cleave type IV collagen and gelatin, which are the main structural components of the basal membrane. So, we determined the MMP2 protein expression level in cells with overexpressed miR-124 and in blocked cells. Western blot assays showed that when miR-124 was overexpressed, the MMP2 expression level decreased by approximately 75% in HeLa cells (Fig. 1G). When miR-124 was blocked, the MMP2 expression level increased 2.48 fold in HeLa cells (Fig. 1G). These results together indicate that miR-124 could repress VM and the expression level of MMP2 in cervical cancer cells.

miR-124 inhibits migration and invasion in cervical cancer cells

To investigate the migration and invasion function of miR-124 in cervical cancer cells, the cells with overexpressed miR-124 and control vector were cultured in transwells with (for invasion) or without (for migration) Matrigel. The overexpression of miR-124 reduced migration approximately 46% and 42% in HeLa and C33A cells, respectively (Fig. 2A and B). Blocking miR-124 by ASO increased migration 1.4 and 1.25 fold in HeLa and C33A cells, respectively (Fig. 2C and D). Overexpression of miR-124 reduced invasion by 46% and 60% in HeLa and C33A cells, respectively (Fig. 2E and F). Blocking miR-124 by ASO-miR-124 increased invasion by 1.4 and 1.3 fold in HeLa and C33A cells, respectively (Fig. 2G and H). These data indicates that miR-124 can repress the migration and invasion ability of cervical cancer cells.

miR-124 targets AmotL1-3′UTR directly

To determine the effect of miR-124 on formation of VM, tube-like channel formation assays were performed. Overexpression of miR-124 decreased approximately 48% and 25% of the tube-like channels in HeLa and C33A cells, respectively (Fig. 1C and D). ASO-miR-124 transfection caused 4.18- and 1.31-fold increases in HeLa and C33A cells, respectively, compared to scramble ASO-NC (Fig. 1E and F).

Vasculogenic mimicry formation assays

To test the effect of miR-124 on the VM of cervical cancer cells, the cells were transfected with miR-124 expression plasmid or a control vector. Matrigel (40 μL) was plated on the 24-well plate, and 4 × 10^5 or 5 × 10^5 cells were seeded on it. Twenty-four hours after seeding, the tube-like structures were examined by light microscopy.

Statistical analysis

The data are presented as the mean ± standard deviation (SD). The statistical analyses for the data comparisons were performed using a paired t-test. p ≤ 0.05 was considered statistically significant (* p < 0.05, ** p < 0.01).

Results

miR-124 represses VM in vitro

To study the role of miR-124, we generated a construct expressing miR-124 precursor (pri-miR-124). Antisense 2-O-methoxy-modified oligomer miR-124 (ASO-miR-124) and a negative control nucleotide (ASO-NC) were synthesized. miR-124 expression was examined by qRT-PCR. The levels of miR-124 increased approximately 114 and 137 fold in HeLa and C33A cells transfected with pri-miR-124, respectively, compared with the control vector (Fig. 1A). Transfection with ASO-miR-124 resulted in 60% and 55% reduction in miR-124 expression in HeLa and C33A cells, respectively, compared with scramble ASO-NC in both cells (Fig. 1B).
protein level with a 73% decrease in the pri-miR-124 transfected HeLa cells and a 3.35-fold increase in the ASO-miR-124 transfected HeLa cells (Fig. 3F).

To determine whether AmotL1 expression is inversely correlated with miR-124 levels in clinical specimens, 11 pairs of human cervical cancer tissues and their adjacent non-cancerous tissues were analyzed for AmotL1 and miR-124 expression levels by qRT-PCR. The results showed that miR-124 expression levels were generally lower (approximate 24%) in cervical cancer tissues than in the matched normal cervical tissues (Fig. 3G). But, the AmotL1 mRNA level in cer-
Fig. 2. The effect of miR-124 on cell migration and invasion. (A and B) The effects of the overexpression of miR-124 on migration after 48 h in HeLa and C33A cells, respectively. Photographed at 20 × magnification. (C and D) The effects of knockdown miR-124 on migration after 48 h in HeLa and C33A cells, respectively. Photographed at 20 × magnification. (E and F) The effects of the overexpression of miR-124 on invasion after 48 h in HeLa and C33A cells, respectively. Photographed at 20 × magnification. (G and H) The effects of knockdown miR-124 on invasion after 48 h in HeLa and C33A cells, respectively. Photographed at 20 × magnification.
Fig. 3. AmotL1 is the target gene for miR-124. (A) The target sites for miR-124 in the 3'UTR of AmotL1, and the mutant target sites. (B) The miR-124 target sites at AmotL1 3'UTR nucleotides 5832–5839 were highly conserved across different species. In all of the species obtained, the seed regions that were critical for miR-124 targeting were 95% homologous. (C and D) The EGFP fluorescence reporter assay and qRT-PCR assay: AmotL1-3'UTR and AmotL1-3'UTR-mut co-transfected with miR-124 or the control. (E) The effect of miR-124 on AmotL1 mRNA level as determined by real-time PCR in HeLa cells. (F) The effect of miR-124 on the AmotL1 protein level by western blot in HeLa cells. (G) The expression level of miR-124 in cervical cancer tissues as determined by real-time PCR. (H) The expression level of AmotL1 mRNA in cervical cancer tissues by real-time PCR.
Ectopic expression of AmotL1 and SphK1 in cervical cancers was average approximately 6.13-fold higher in the matched normal cervical tissues (Fig. 3H). These data indicate that there is a negative correlation between miR-124 and AmotL1 mRNA level in cervical cancer. These results indicate that miR-124 targets AmotL1 3′UTR and down-regulates its expression.

AmotL1 facilitates the formation of VM and migration/invasion of cervical cancer cells

To demonstrate that AmotL1 has an essential role in mediating miR-124 function, a construct to silence AmotL1 expression (pSilencer/sh-AmotL1) and a vector to express AmotL1 (pcDNA-/AmotL1) were generated. Down-regulation of AmotL1 and overexpression of AmotL1 were confirmed by western blot. pSilencer/sh-AmotL1 led to a 41% reduction, and pcDNA-/AmotL1 resulted in a 37-fold increase (Fig. 4A).

To examine the effect of AmotL1 on VM, tube-like channel assays on Matrigel were performed. The results showed that down-regulation of AmotL1 suppressed tube-like channel formation by approximately 39% and 48% and overexpression of AmotL1 enhanced it by 1.78 and 1.4 fold in HeLa and C33A cells, respectively, (Fig. 4B and C). These findings indicate that AmotL1 is involved in VM.

We further examined the effect of AmotL1 on migration and invasion. When AmotL1 was down-regulated, the migration of HeLa and C33A cells was decreased by approximately 84% and 60%, respectively. Overexpression of AmotL1 resulted in the migration of HeLa and C33A cells increasing by 3.43 and 1.91 fold, respectively (Fig. 4D and E). Similar results were obtained with invasion assays, which showed decreases of approximately 64% or 58% when AmotL1 was down-regulated and increased of approximately 2.52 or 1.85 fold when AmotL1 was overexpressed, respectively (Fig. 4F and G).

Restoration of AmotL1 abolishes the effects induced by miR-124 in cervical cancer cells

To confirm that miR-124 represses tube-like channel formation by targeting AmotL1, rescue experiments were performed. Overexpression of miR-124 represses tube-like channel formation in cervical cancer cells (Fig. 5A and B). Ectopic expression of AmotL1 in the miR-124-overexpressed cells reversed the effect of miR-124 on tube-like formation.

Similar results were obtained with the migration and invasion assay. Overexpression of miR-124 could repress the migration and invasion ability of cervical cancer cells. Re-expression of AmotL1 was sufficient to restore migration (Fig. 5C and D), invasion (Fig. 5E and F) and the ability induced by miR-124 in cervical cancer cells. These results indicate that miR-124 repression of tube-like channel formation, migration and invasion was at least partly through repression of AmotL1 expression.

miR-124 and AmotL1 affect cervical cancer cell migration and invasion by regulating the EMT process

Because miR-124 can repress cervical cancer cells migration and invasion and AmotL1 was a direct target of miR-124, we investigated the pathway by which miR-124 and AmotL1 mediated the regulation of migration and invasion of cervical cancer cells in vitro. Furthermore, we found that the blocking miR-124 expression or AmotL1-overexpressing HeLa cells displayed a more elongated spindle-like morphology and induced losing cell–cell contacts (Fig. 6A), one of the main characteristic features of EMT.

To determine whether the typical molecular alterations of EMT occurred, we examined the expression level of epithelial marker E-cadherin in HeLa cells. Western blot analysis showed that E-cadherin was up-regulated by 2.45 fold when miR-124 was overexpressed or when AmotL1 was blocked and that E-cadherin was clearly down-regulated when either miR-124 was blocked or AmotL1 was overexpressed (Fig. 6B). The protein levels of two mesenchymal markers, Vimentin and the intercellular adhesion molecule 1 (ICAM-1) were also assessed. Vimentin and ICAM-1 were down-regulated by approximately 46% and 33%, respectively, following the overexpression of miR-124 (Fig. 6B). However, E-cadherin protein expression was down-regulated by 48%, whereas Vimentin and ICAM-1 were up-regulated by 3.04 and 2.15 fold, respectively, following the knockdown of miR-124 (Fig. 6B). In addition, similar results were observed when AmotL1 was knocked down. E-cadherin protein expression was up-regulated by 1.87 fold, whereas Vimentin and ICAM-1 were down-regulated by 31% and 62%, respectively. Overexpression of AmotL1 decreased the E-cadherin protein level by 52%, whereas Vimentin and ICAM-1 were increased by 2.48 and 2.13 fold, respectively (Fig. 6B). These data suggest that miR-124 and AmotL1 influence the migratory and invasive behavior of cervical cancer cells by way regulating the EMT process.

Discussion

Although miR-124 has been shown to regulate multi-step tumor metastasis, including cell migration and invasion, by targeting several target genes, such as Fliotillin-1 in breast cancer [37] and SphK1 in ovarian cancer [38]. Whether it affects the malignant behavior of human cervical cancer cells and is involved in the process of angiogenesis remains unclear. To address these questions, a gain-of and loss-of function approach was used. We revealed that miR-124 represses the migration and invasion ability of cervical cancer cells, and it suppresses VM in cervical cancer cells. An interesting finding in this study is that miR-124 represses VM, an important step in cancer metastasis.

Usually, miRNA exerts its effect by down-regulating its target genes. We combined bioinformatic and functional knowledge of miR-124 to predict and identify AmotL1 as a new target gene. And we confirm the interaction using an EGFP reporter assay. In addition, miR-124 regulated endogenous AmotL1 RNA and protein expression negatively. Furthermore, qRT-PCR analysis showed that miR-124 expression has a negative relationship in clinical cervical cancer tissues. Thus, miR-124 targets AmotL1 by binding its 3′UTR and negatively regulates its expression.

AmotL1 is an angiogenesis-related protein that is expressed on the cell surface and can be shed in the culture medium. AmotL1 is a positive regulator of angiogenesis [32,33,39]. Our results also show that AmotL1 can promote the formation of VM in cervical cancer cells in vitro. There were a lot of reports about the function of AmotL1. It was reported that AmotL1 can regulate YAP1 cytoplasm-to-nucleus translocation through direct protein–protein interaction, which can occur independent of YAP1 phosphorylation status [36]. AmotL1 also activates LATS2 through a novel conserved domain that binds and activates LATS2. LATS2 is activated by the MST2 kinase. The MST2 and LATS2 kinases as well as the transcriptional coactivator YAP form the core of the mammalian Hippo pathway. Another study reported that AmotL1 regulates YAP1/p130 and AmotL1 are negatively regulated by Nedd4-like E3 ubiquitin ligases at the post-transcriptional level [35]. These data suggest that AmotL1 exerts its effect on VM in the Hippo pathway. On the other hand, AmotL1 controls endothelial migration and cell polarity, regulates sprouting angiogenesis by affecting tip cell migration and controls cell–cell adhesions in vivo [30]. The reduction of intercellular cohesion in mesenchymal cells is mainly the result of alterations in desmosomes composed by adherens junctions and tight junctions which function as the key regulator of the entire junctional complex [40]. Activation of EMT is considered essential to make carcinoma cells dissociate from each other by losing cell–cell junctions for single-cell migration and invasion [41]. In our study, E-cadherin protein expression was negatively regulated whereas...
Fig. 4. The effects of AmotL1 on VM, migration and invasion. (A) The effect of overexpression of the AmotL1 vector and knockdown AmotL1 vector. AmotL1, the overexpression AmotL1 vector; sh-AmotL1, the knockdown AmotL1 vector. (B and C) The function of AmotL1 on vasculogenic mimicry in HeLa and C33A cells, respectively. (D and E) The effect of AmotL1 on migration in HeLa and C33A cells, respectively. (F and G) The effect of AmotL1 on invasion in HeLa and C33A cells, respectively. Photographed at 20 × magnification.
Fig. 5. The rescue assay. (A and B) The overexpression of AmotL1 rescues the effect of miR-124 on vasculogenic mimicry in HeLa and C33A cells, respectively. (C and D) Overexpression of AmotL1 rescues the effect of miR-124 on migration in HeLa and C33A cells, respectively. (E and F) The overexpression of AmotL1 rescues the effect of miR-124 on the invasion of HeLa and C33A cells, respectively. Photographed at 20× magnification.
Vimentin and ICAM-1 were positive regulated by AmotL1. And AmotL1 promotes migration and invasion in cervical cancer cells. All of these made us to believe that AmotL1 can promote angiogenesis via EMT partly.

Cell migration is another essential step in angiogenesis that promotes blood vessel extension. The role of miR-124 in repressing VM was also supported by a number of experiments in vitro that demonstrate that miR-124 can repress cell migration and invasion. Previous work has shown that miR-124 inhibits migration and invasion by targeting CD151, ROCK1, ITGB1 and Rac1 [9,14–16]. Our studies confirmed that miR-124 suppresses the migration of cancer cells and their invasion ability by targeting AmotL1 in HeLa and C33A cells. AmotL1 regulates the cytoplasm-to-nucleus translocation of YAP1, and YAP1 recruits c-Abl to protect AmotL1 against Nedd4-2-mediated degradation, which may promote the effect of AmotL1 on migration and invasion.

EMT is the key process that drives cancer metastasis, which characterized by loss of the epithelial marker E-cadherin, increased expression of the mesenchymal marker Vimentin, and enhanced migratory and invasive behaviors [42]. The epithelial cells are closely associated with neighboring cells because they have keratin filaments and regularly spaced membrane-associated specialized junctions. In contrast to epithelial, mesenchymal cells are loosely associated and the source of growth factor production in collaboration with the surrounding stroma [41]. EMT program accomplishment follows several steps. It involves loss of intercellular cohesion, disruption of extracellular matrix, modifications of the cytoskeleton, increased motility and invasion. The first step is to make cell–cell contacts closer at the tumor leading edge [41]. During EMT, a key change is E-cadherin was replaced to modify the adhesive and invasive behaviors [41]. It was reported that miR-124 targets Slug to regulate epithelial–mesenchymal transitions and the metastasis of breast cancer [17]. In our studies, the depletion of miR-214 by ASO repressed expression of the epithelial marker E-cadherin but increased expression of the mesenchymal markers, Vimentin and ICAM-1. Overexpression of miR-124 resulted in opposite phenotypes, which suggest that miR-124 can repress EMT to regulate the motility (migration and invasion) of cervical cancer cells. With rescue experiments, we confirmed that AmotL1 mediated the regulation of miR-124 in the epithelial–mesenchymal transition. Usually, the loss of intercellular junctions and increased cell migration during EMT are correlated with increased expression of Vimentin and decreased expression of E-cadherin. To test our hypothesis, we examined the expression of the epithelial marker E-cadherin and the mesenchymal markers Vimentin and ICAM-1. As expected, up-regulation of miR-124 or down-regulation of AmotL1 suppressed the EMT phenotype. Thus, miR-124 can suppress cervical cancer cell migration/invasion, VM through at least partial regulating AmotL1. But we cannot exclude that other target genes of miR-124 may involved in this process.

In a living organism, it is necessary but not sufficient to activate cell motility via looser cell–cell contacts. Cells must migrate through the extracellular matrix (ECM) and proceed to its proteolysis before reaching a particular location. To degrade the ECM, the cell enhances its protease activity in the pericellular environment mainly by membrane-anchored proteases consisting of the transmembrane matrix metalloproteinases (MMPs) and the endogenous proteolytic urokinase-type plasminogen activator (uPA) system [41]. MMP-2 (gelatinase A, 72 kDa) and matrix metalloproteinase 9 (MMP-9) (gelatinase B, 92 kDa) have an important function on digest type IV collagen and gelatin, which are the main structural components of the extracellular matrix [42,43]. In this study, we describe a negative correlation between miR-124 and MMP2 expression levels and a positive correlation between AmotL1 and MMP2 expression levels. The data showed that the expression level of MMP2 is consistent with the function of miR-124 on VM. Taken together, our data supports the role of miR-124 on repressing VM.

In summary, our findings demonstrate that miR-124 binds AmotL1 3'UTR and down-regulates its expression. AmotL1 mediates the repression of miR-124 on the VM, migration and invasion ability of cervical cancer cells, which may provide new insights into the mechanism of cancerogenesis in cervical cancer and may contribute to the development of diagnostics and treatments for cervical cancer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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