NF-κB interaction long non-coding RNA inhibits migration, invasion and epithelial-mesenchymal transition of cervical cancer cells through inhibiting NF-κB signaling pathways

FEIPENG WANG, XIAOCHUN JIANG and PEI WANG

Department of Gynaecology, Xianyang Central Hospital, Xianyang, Shaanxi 712000, P.R. China

Received June 18, 2019; Accepted March 17, 2020

DOI: 10.3892/etm.2020.8752

Abstract. The long non-coding RNA (IncRNA) NF-κB interaction IncRNA (NKILA) has been found to exert tumor suppressive effects in numerous types of carcinoma; however, the relationship between NKILA and cervical cancer (CC) remains largely unclear. The present study aimed to investigate the effects of NKILA on the proliferation and metastasis of CC cell lines, in addition to the related molecular mechanisms. Reverse transcription-quantitative PCR was used to detect the expression levels of NKILA in cancer tissues and cell lines. The constructed overexpression vector, pcDNA3.1NKILA, and its corresponding negative control sequence were transfected into CaSki cells and short hairpin RNA targeting NKILA and the corresponding negative control sequence were transfected into C-33A cells. Subsequently, the proliferative, migratory and invasive ability, as well as the process of epithelial-mesenchymal transition (EMT) of C-33 A and CaSki cells were analyzed by performing Cell Counting Kit-8, wound healing, Matrigel invasion and western blot assays, respectively. The expression levels of proteins were detected using western blot analysis. The expression levels of NKILA were decreased in CC tissues and CC cell lines (SiHa, C-33A, CaSki and HeLa) and the downregulation of NKILA expression using shRNA was observed to significantly increase the proliferation of CC cells. Conversely, the upregulation of NKILA inhibited the proliferation of CC cells, in addition to significantly inhibiting the migration and invasion of CaSki cells, whereas the knockdown of NKILA promoted the invasion of C-33A cells. Thus, it was hypothesized that NKILA may inhibit the migration and invasion of CC cells via regulation of EMT processes, which was reflected by the expression of ZO-1, E-cadherin, N-cadherin and Vimentin. Furthermore, the overexpression of NKILA significantly inhibited the activation of NF-κB in CaSki cells, whereas the knockdown of NKILA expression promoted the degradation of inhibitory protein-κB and promoted the transfer of p65 into the nucleus in C-33 A cells. In conclusion, the results from the present study suggested that NKILA may be involved in the inhibition of migration and invasion in CC cells through regulating EMT processes, which may be related to its inhibition of NF-κB activation.

Introduction

Cervical cancer (CC) is one of the most common malignant gynecological tumors worldwide (1). The human papillomavirus infection is the main etiological factor that contributes to the development of the disease (2); however, early detection and treatment can effectively improve the clinical prognosis of patients with CC (2). Thus, investigating the molecular mechanisms of CC development and progression is crucial for the diagnosis and treatment of this disease.

Long non-coding RNAs (lncRNAs) are non-coding RNAs of >200 nucleotides in length that have no protein coding function (3). Having gained an increased understanding of lncRNAs in recent years, a significant amount of evidence suggests that lncRNAs serve important roles in the regulation of tumor-related genes. For example, lncRNAs such as maternally expressed gene 3 (MEG3), highly upregulated in liver cancer (HULC) and HOX transcript antisense intergenic RNA (HOTAIR) have been observed to participate in the occurrence and development of CC (4-6). In addition, the discovery of lncRNAs that exert inhibitory activity over metastatic processes in recent years has introduced a new perspective for the relationship between the tumor microenvironment and metastatic phenotype regulation (7). NF-κB interaction IncRNA (NKILA) is a IncRNA found located on chromosome 20q13, which regulates the inhibitory protein (1)κB kinase (IKK)/NF-κB signaling pathway (8). In a previous study, it was demonstrated that the expression levels of NKILA were negatively correlated with the invasion and metastasis in clinical breast cancer specimens (8). Ke et al (9) reported that NKILA expression was decreased in esophageal squamous cell carcinoma (ESCC) tissues and cancer cells, and that it inhibited the proliferation and migration of ESCC cells by preventing the activation of NF-κB signaling. In inactivated cells, NF-κB binds to its inhibitory protein family member IκB to form a trimer, which causes it to be retained in the cytoplasm in
an inactive state and prevent nuclear translocation (10). Additionally, NF-κB, which is involved in downstream cyto-
kine signaling, was found to induce NKILA expression, which
inhibited NF-κB activation in normal mammary epithelial
cells by forming a NF-κB/NKILA complex that resulted in
a negative feedback loop (11). Thus, the mutual regulation
of NKILA and NF-κB suggested that IncRNAs may bind
to the functional domain of signaling pathway molecules
to participate in the regulation of signal transduction. However,
the role of NKILA in CC remains unclear. Given that chronic
inflammation is an important driver of CC invasion and metas-
tasis (12), and that the NF-κB signaling pathway is a crucial
link between inflammation and tumor development (13), it
was hypothesized that NKILA may serve an important role
in the development of CC. The present study found that NKILA
expression was abnormally low in CC tissue. Therefore, CC cell
lines with relatively low expression levels of NKILA (C-33A
and CaSki) were selected and the effect of NKILA overex-
pression or knockdown on the proliferation and metastasis of
these CC cell lines was analyzed. In addition, the molecular
mechanisms involved in this regulation were investigated to
assess the role of NKILA in the progression of CC.

Materials and methods

Patient studies. The present study was approved by the
Ethics Committee of Xianyang Central Hospital and written
informed consent was obtained from each patient. Both CC
tissue and adjacent normal cervical tissue were collected from
60 patients with CC (age: 30–61 years; mean age: 46±6 years)
that underwent CC surgery between January 2016 and January
2019 at Xianyang Central Hospital, Xianyang. Pathological
analysis confirmed that all patients had CC.

Cell lines and reagents. The CC cell lines SiHa (cat.
no. BNCC337881), C-33A (cat. no. BNCC341097), CaSki
(cat. no. BNCC338223) and HeLa (cat. no. BNCC337633),
and the human cervical epithelial cell line HCeRIP (cat.
no. BNCC340374) were obtained from the Cell Bank of the
Shanghai Institute of Biochemistry and Cell Biology (Chinese
Academy of Sciences). All cells were cultured in DMEM
(Gibco; Thermo Fisher Scientific, Inc.), supplemented with
10% FBS (Gibco; Thermo Fisher Scientific, Inc.), and main-
tained in a humidified atmosphere at 37°C and 5% CO₂.

Reverse transcription-quantitative PCR (RT-qPCR). Total
RNA was extracted from SiHa, C-33A, CaSki, HeLa, and
HCeRIP cells (1x10⁶/well in six-well plates) and tissues
(50 mg) using TRIzol® reagent (Thermo Fisher Scientific,
Inc.) according to the manufacturer's protocol. The purity and
concentration of RNA was determined using an ultraviolet
spectrophotometer. Total RNA was reverse transcribed into
cDNA using the BeyoRT cDNA First-strand Synthesis kit
(cat. no. D7168M; Beyotime Institute of Biotechnology)
according to the manufacturer's protocol, and the following RT
temperature protocol: 37°C for 30 min, 85°C for 5 sec and 4°C
to 10 min. qPCR was subsequently performed. The following
primer pairs were used for the qPCR: NKILA forward, 5'-AAC
CAAACCTACCACAACG-3' and reverse, 5'-ACCACTAG
TCAATCCCAGGTG-3'; and GAPDH forward, 5'-CCTGCA
CCACCAACTGCCTTA-3' and reverse, 5'-GGCCATCCACAG
TCTTCTGG-3'. The following thermocycling conditions
were used for the qPCR: 92°C for 3 min; 39 cycles of 92°C
for 30 sec, 58°C for 10 sec and 72°C for 2 min. The relative
expression levels were quantified using the 2⁻ΔΔCq method (14)
and GAPDH was used as the internal reference gene.

Cell grouping and transfection. CaSki cells were divided into
two groups: i) the NC group, which was transfected with the
control pcDNA3.1 sequence (5'-ACGUGACACGUCCG
AGAATT-3'); and ii) the NKILA group, which was transfected
with the pcDNA3.1-NKILA overexpression sequence (5'-AAC
CAAACCTACCACAACG-3'). C-33A cells were divided into the short hairpin RNA (sh) negative control (NC) group,
which was transfected with the NC sequence (5'-UUUCUC
GAACGUGUCAGGT-3') and the shNKILA group, which
were transfected with NKILA shRNA sequences (shNKILA-1,
5'-GGAGAAGTCACAGTTGTT-3'; or shNKILA-2,
5'-GGCGATTGGGAGGAATTG-3'). The NKILA over-
expression and NC vector, as well as the shNKILA and shNC
were all synthesized and purchased from Shanghai GeneChem
Co., Ltd. The transfection of shRNA (50 pmol/ml) and overex-
pression plasmids (2 µg) was performed using Lipofectamine®
2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.),
according to the manufacturer's protocol. The transfection
efficiency was analyzed 48 h after transfection.

Cell Counting Kit-8 (CCK-8) assay. Following 48 h of trans-
fection, the cells were seeded at 2x10⁴ cells/ml into well-
plates in 100 µl DMEM/well, with five duplicate wells/group.
After 48 h of culture at room temperature, 10 µl CCK-8
reagent (Beyotime Institute of Biotechnology) was added/well
for 2 h at 37°C according to the manufacturer's protocol. The
absorbance value at 450 nm was measured using a fluorescent
plate reader.

Wound healing assay. CaSki and C33A cells (5x10⁴/well)
were plated into six-well plates 48 h after transfection. Upon
reaching 100% confluence, wounds were created by scratching
the cell monolayers with a 3-µl pipette tip. The medium was
discarded, the cells were washed twice with PBS to remove
the detached cells and cells were then replenished with fresh
serum-free DMEM. Cells were subsequently incubated at 37°C
in a 5% CO₂ incubator. Images of each well were obtained at
0 and 24 h and the wound closure was observed using alight
microscope (magnification, x200). The relative migration
width was calculated as the relative distance divided by the
scratch width at 0 h. Three independent experimental repeats
were performed for each group.

Matrigel invasion assay. After transfection for 48 h, CaSki
and C33A cells were suspended into serum-free DMEM.
Subsequently, 1x10⁵ cells were plated into the upper chambers
of Transwell plates (Corning Inc.) pre-coated with 100 µl
Matrigel (Corning Inc.) overnight at 4°C. A total of 600 µl
DMEM supplemented with 10% FBS was plated in the lower
chambers. Following incubation at 37°C for 24 h, the non-inv-
asive cells remaining in the upper chamber were removed and
the invasive cells were fixed for 4 min in 4% paraformalde-
hyde at room temperature and subsequently stained with 0.1%
crystal violet for 20 min at room temperature. Stained cells were counted in five randomly selected fields using a light microscope (magnification, ×200). The number of cells represents the invasive ability of the tumor cells.

**Western blot analysis.** Total protein was extracted from the nucleus and cytoplasm of CaSki and C33Acells (1x10⁶/well in six-well plates) using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.). Total protein was quantified using a bicinchoninic acid assay and 40 µg protein/lane was separated by 12% SDS-PAGE. The separated proteins were transferred subsequently onto a PVDF membrane and blocked with 5% non-fat dry milk for 1 h at room temperature. The membranes were then incubated with the following primary antibodies at 4°C overnight: Anti-zonula occludens-1 (ZO-1; 1:500; cat. no. ab96587; Abcam), anti-E-cadherin (1:10,000; cat. no. ab40772; Abcam), anti-N-cadherin (1:500; cat. no. ab202030; Abcam), anti-Vimentin (1:1,000; cat. no. ab92547; Abcam), anti-p65 (1:1,000; cat. no. 8242; Cell Signaling Technology, Inc.), anti-phosphorylated (p)-p65 (1:1,000; 3039; Cell Signaling Technology, Inc.), anti-IkB (1:1,000; cat. no. 9242; Cell Signaling Technology, Inc.), anti-p-IkB (1:1,000; cat. no. 2859; Cell Signaling Technology, Inc.), anti-GAPDH (1:10,000; cat. no. ab8245; Abcam) or anti-Lamin B (1:1,000; cat. no. ab16048; Abcam). Following the primary antibody incubation, the membranes were washed and incubated with goat anti-mouse (1:3,000; cat. no. A0216; Beyotime Institute of Biotechnology) and goat anti-rabbit (1:1,000; cat. no. A0208; Beyotime Institute of Biotechnology) horseradish peroxidase-conjugated secondary antibodies for 1 h at 37°C. Protein bands were visualized using an enhanced chemiluminescence kit (Cell Signaling Technology, Inc.) and protein expression was semi-quantified using Image Lab 3.0 software (Bio-Rad Laboratories, Inc.).

**Statistical analysis.** Statistical analysis was performed using SPSS 17.0 software (SPSS Inc.) and data are presented as the mean ± SD of three experimental repeats. Statistical differences between two groups were analyzed using Student’s t-test, whereas statistical differences between multiple groups were determined using one-way ANOVA and Tukey’s post hoc test. 
P<0.05 was considered to indicate a statistically significant difference.

**Results**

**NKILA expression levels are decreased in CC tissues and cell lines.** The expression levels of NKILA in CC tissues and cell lines (SiHa, C-33A, CaSki, HeLa and HCEpIC) were determined using RT-qPCR. Compared with adjacent normal tissues, the expression levels of the IncRNA NKILA were significantly decreased in CC tissues (P 0.05; Fig. 1A). Consistent with the data found in CC tumor tissue, the expression levels of NKILA in CC cell lines were also significantly decreased compared with those in HCEpIC cells (P<0.05; Fig. 1B). C-33A and CaSki cells were selected for use in subsequent studies to investigate the effects of NKILA on the proliferation and metastasis of CC cells, as NKILA expression was highest in the C33A cells and lowest in CaSki cells.

**NKILA inhibits CC proliferation.** The role of NKILA in CaSki and C-33A cells was further investigated by overexpressing or knocking down the expression of NKILA in the cells using a NKILA overexpression plasmid or shNKILA, respectively. Since NKILA expression was the highest in the C-33A cell line and the lowest in the CaSki cell line, the expression levels of NKILA were knocked down in C-33A cells and overexpressed in CaSki cells. RT-qPCR results demonstrated that the over-expression of NKILA significantly increased the expression levels of NKILA in CaSki cells compared with the NC group, whereas shNKILA-1- and shNKILA-2-transfected cells had significantly reduced expression levels of NKILA compared with the shNC-transfected cells (Fig. 2A). shNKILA-1 was chosen for subsequent experiments. As the abnormal proliferation of tumor cells is required for tumor initiation, the effects of NKALA overexpression and knockdown on cell proliferation was investigated. The overexpression of NKALA significantly reduced the proliferation of CaSki cells compared with the NC group at 72 h, whereas the genetic knockdown of NKILA significantly promoted the proliferation of C-33Acells compared with the shNC group at 72 h (Fig. 2B). These results suggested that the downregulation of NKILA expression may promote the proliferation of CC cells, while the upregulation of NKILA expression may inhibit the proliferation of CC cells.

**NKILA inhibited the migratory and invasive ability of CC cells.** The wound healing assay was used to determine the effects of NKILA overexpression or knockdown on cell migration. It was found that the overexpression of NKILA significantly inhibited wound closure in CaSki cells compared with the NC group (Fig. 3A). By contrast, NKILA knockdown with shNKILA significantly enhanced the wound healing ability of C-33A cells compared with the shNC group (Fig. 3A). Moreover, Transwell assays were also used to detect the effects of NKILA expression on the invasive ability of CC cells (Fig. 3B). The overexpression of NKILA significantly inhibited the invasive ability of CaSki cells compared with the NC group, whereas shNKILA-transfected C-33 A cells demonstrated significantly increased invasive abilities compared with shNC-transfected cells (Fig. 3B), revealing that the downregulation of NKILA may promote the migration and invasion of CC cells, whereas the upregulation of NKILA may inhibit the migration and invasion of CC cells.

**NKILA inhibits epithelial-mesenchymal transition (EMT) in CC cells.** EMT is considered to be an important mechanism for cancer cell migration and invasion (15). Changes in EMT and invasive properties in vitro can be used to hypothesize the metastatic potential of cancer cells in vivo (16). To further investigate the inhibitory effect of NKILA on the migration and invasion of CC cells, western blotting was used to examine the expression levels of EMT-related markers. Compared with the NC group, the expression levels of E-cadherin and ZO-1 protein in the NKILA overexpression group were significantly increased, whereas the expression levels of interstitial markers, N-cadherin and Vimentin were significantly reduced (P<0.05; Fig. 4A). Conversely, in C-33A cells, NKILA knockdown significantly reduced the expression levels of E-cadherin and ZO-1 proteins, whilst it significantly increased the expression levels of N-cadherin and Vimentin compared with the
Effects of NKILA on the NF-κB signaling pathway. NF-κB is a broad-range nuclear transcriptional regulatory element that, when combined with the corresponding gene promoter, promotes the expression of multiple genes, including EMT-related genes (17). p65 is an important subunit of NF-κB and activated NF-κB serves a regulatory role by controlling the translocation of p65 into the nucleus (18). The expression levels of p65 in the nuclei of cells in the NKILA overexpression group were significantly lower compared with the NC group in Ca Ski cells (P<0.05; Fig. 5A). Furthermore, shNKILA-transfected C-33A cells stimulated the transfer of p65 into the nucleus; which was observed through significantly increased p65 nuclear expression levels compared with the shNC group (Fig. 5A). In addition, the overexpression of NKILA was found to inhibit the migratory and invasive ability of CC cells by regulating EMT processes.
to significantly inhibit the phosphorylation of \( \text{I} \kappa \text{B} \alpha \) and p65 in CaSki cells compared with the NC group, whereas NKILA knockdown significantly increased the expression levels of p-I\( \kappa \)B\( \alpha \) and p-p65 in C-33A cells compared with the shNC group (Fig. 5B).

Discussion

CC poses a serious threat to the health of women worldwide; although radiotherapy, chemotherapy and molecular-targeted therapies have made great progress in recent years, the therapeutic effect of these treatments remains unsatisfactory, which is largely due to the occurrence of postoperative metastasis and recurrence (2). Therefore, investigating more effective therapeutic interventions is the primary focus of current research in CC. Following our improved understanding of tumor biology, the important roles of a large number of non-coding RNAs in tumor growth has been gradually recognized, especially the role of lncRNAs in cancer, which have received increasing attention (19). In the present study, it was discovered that the expression of the lncRNA NKILA is decreased in CC tissues and cell lines, which was observed to subsequently inhibit the migratory and invasive ability of CC cells by regulating the EMT process. In addition, this inhibitory effect of NKILA on the migration and invasion of CC cells may be related to the inhibition of NF-\( \kappa \)B activation, which was also reported in this study. Thus, it was hypothesized that NKILA may serve a potential role in the development of CC, which may provide novel ideas for the diagnosis and treatment of CC.

Intriguingly, IncRNAs have been validated to have comprehensive functions in biological processes via various mechanisms, such as coordinating gene expression via gene imprinting and controlling transcription or post-transcriptional processing (20). Studies have since reported that IncRNAs can participate in the regulation of gene expression at the epigenetic, transcriptional and post-transcriptional level through interacting with proteins, DNA and RNA (21,22). IncRNAs serve an important role in tumorigenesis and prognosis, and multiple IncRNAs have been found to be abnormally expressed in colon cancer (23), breast cancer (24) and CC (25). IncRNAs that have been reported to be associated with CC include metastasis associated lung adenocarcinoma transcript (MALAT-1) (26), MEG3 (27) and small nucleolar RNA host gene 1 (25), among others. Of note, previous studies have demonstrated that NKILA expression is decreased in breast cancer (28), tongue squamous cell carcinoma (TSCC) (11) and non-small cell lung cancer (NSCLC) (29), where it was suggested to be involved in tumor progression by serving as a tumor suppressor gene. Consistent with these studies, the present study also observed decreased expression levels of NKILA in CC tissues and cell lines. Additionally, the overexpression of NKILA significantly inhibited the proliferation of the CC cell line CaSki, whereas the genetic knockdown increased the proliferative rate of the CC cell line C-33A. Thus, these findings suggested that NKILA might be involved in the development of CC as a tumor suppressor gene.

Invasion and metastasis are basic biological characteristics of malignant tumors, including CC, and are the...
main cause of poor prognoses in patients with cancer (30); however, the full mechanism of tumor metastasis and invasion is not fully understood. Previous research has found that the ability of tumors to invade and metastasize occurs when tumors gain the ability to escape from the primary site, and EMT serves an important role in regulating this process (31). EMT is defined as the process by which epithelial cells lose cell polarity and tight junctions between cells to form spindle-opening mesenchymal cells with migratory abilities (32). EMT is closely associated with the invasion and metastasis of a variety of malignancies, including CC (32). Epithelial cell malignancies can invade and metastasize to surrounding and distant tissue sand organs due to the decreased expression levels of epithelial protein markers, such as E-cadherin and ZO-1, and the increased expression of stromal cell protein markers, such as N-cadherin and Vimentin (33). Notably, numerous studies have confirmed that lncRNAs can participate in tumor metastasis and invasion by regulating EMT processes (34). For example, enhancer of zeste homolog2 (EZH2)-binding lncRNA in cervical cancer (EBIC) can bind to EZH2 to prevent E-cadherin from promoting CC invasion (35); HOTAIR promoted EMT through regulating the expression levels of Snail, and the genetic knockdown of HOTAIR in esophageal cancer cells significantly inhibited tumor invasion and EMT (36); MALAT-1, which is upregulated in bladder cancer, promoted cellular migration through the Wnt signaling pathway and induced EMT (37); and in CC, MALAT-1 (38) and taurine upregulated gene 1 (39) were also found to promote the invasion and metastasis of CC cells through inducing EMT. In addition, Lu et al (29) demonstrated that NKILA could inhibit the migration and invasion of NSCLC through regulating the NF-κB/Snail signal pathway. Similarly, the results of the current study demonstrated that the overexpression of NKILA significantly inhibited the migration and invasion of the CC cell line CaSki, whereas the short hairpin RNA; ZO-1, zonula occludens-1.

Figure 4. NKILA inhibits epithelial-mesenchymal transition in CC cells. (A and B) Effects of NKILA on the expression levels of E-cadherin, ZO-1, N-cadherin and Vimentin proteins were detected using western blotting in (A) CaSki cells transfected with NKILA overexpression plasmid and (B) C-33A cells transfected with shNKILA. *P<0.01 vs. NC or shNC. NKILA, NF-κB interaction long non-coding RNA; NC, negative control; sh, short hairpin RNA; ZO-1, zonula occludens-1.
classical pathway, NF-κB is located in the cytoplasm in a non-activated dimeric form (mainly p65/p50) in combination with IκB (41). When stimulated by extracellular signals, IκB is rapidly degraded by phosphorylation or ubiquitination of the IκB kinase (IKK) complex; this releases the p65 subunit from the NF-κB dimer and subsequently allows p65 to translocate to the nucleus, where it is phosphorylated to exert its various biological effects (42,43). The constitutive activation

Figure 5. Effects of NKILA on the NF-κB signaling pathway. (A) The expression of nuclear was analyzed by western blotting in CaSki cells transfected with NKILA, or C33A cells transfected with shNKILA. Lamin B was used as an internal reference. *P<0.05 vs NC or shNC. (B) The expression of p-IκB, IκB, p65 and p-p65 was analyzed by western blotting in CaSki cells transfected with NKILA, or C33A cells transfected with shNKILA. GAPDH was used as an internal reference. *P<0.05 and **P<0.01 vs. NC or shNC. C, cytoplasm; N, nuclear; NKILA, NF-κB interaction long non-coding RNA; CC, cervical cancer; NC, negative control; sh, short hairpin RNA; IκB, inhibitory protein-κB; sh, short hairpin RNA.
of NF-κB has been found to be an important milestone in the malignant transformation of tumors (44), thus investigating the role of the NF-κB pathway in the regulation of tumorigenesis is of great significance. Studies have confirmed that NF-κB promotes the transcription of Snail (45), zinc-finger E-box binding homeobox (ZEB-1) and ZEB-2 (46), which subsequently inhibits the expression of E-cadherin and desmoplakin, and promotes Vimentin expression, to culminate in the induction of EMT. For example, Li et al (47) found that in human proximal tubular HK-2 cells co-cultured with monocytes, the activation of NF-κB increased intercellular adhesion molecule expression levels to induce EMT and Wang et al (48) demonstrated that the activation of NF-κB induced EMT phenotypic changes in human CC stem cells. Recently, a novel mechanism of action for IncRNAs has been reported, in which NKILA was observed to directly inhibit the NK-κB-mediated apoptosis and invasion of breast cancer cells through blocking the phosphorylation site of IκB that directly interacts with NF-κB (28). Huang et al (11) reported that NKILA inhibited the migration and invasion of TSCC cells in vitro, as well as inhibiting lung metastasis in NOD/SCID mice with TSCC tumors in vivo; the NF-κB pathway was found to mediate this effect. In addition, NKILA was also demonstrated to inhibit IκB phosphorylation and the activation of the IKK/NF-κB signaling pathway by interacting with the NF-κB:IKK complex (49). In the present study, it was also observed that increased expression levels of NKILA in CaSki cells inhibited the phosphorylation of IκB and the subsequent nuclear translocation of NF-κB p65, which affected cell migration and invasion. Meanwhile, NKILA knockdown promoted the activation of NF-κB in C-33A cells. However, studies have indicated that the levels of IKK phosphorylation are not affected by changes in NKILA expression in cell lines (49). Mathy et al (50) found that IKK kinase inhibitors could abolish the effects of NKILA on the phosphorylation of IκB and the activation of NF-κB, which suggested that IKK might be an upstream signaling protein of NKILA. In conclusion, the present study observed that NKILA expression was decreased in CC tissues and cell lines, and NKILA was involved in inhibiting the migration and invasion of CC cells by regulating EMT processes, which may be related to its ability to inhibit NF-κB activation. These studies and our current findings suggested that experimental studies on the upstream pathways involved in the inhibition of NF-κB activation by NKILA need to be further investigated.

Acknowledgements
Not applicable.

Funding
No funding was received.

Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions
FPW designed the study, conducted most of the experiments and wrote the manuscript; XCJ and PW conducted the experiments and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of Xianyang Central Hospital and written informed consent was obtained from each patient.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Small W Jr, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, Jingran A, Kitchener HC, Mileshkin LR, Viswanathan AN and Gaffney DK. Cervical cancer: A global health crisis. Cancer 123: 2404-2412, 2017.
2. Jeronimo J, Castle PE, Temin S and Shastri SS: Secondary prevention of cervical cancer: American Society of Clinical Oncology resource-stratified clinical practice guideline summary. J Oncol Pract 13: 129-133, 2017.
3. Gao P and Wei GH: Genomic insight into the role of IncRNA in cancer susceptibility. Int J Mol Sci 18: E1239, 2017.
4. Zhang J, Yao T, Lin Z and Gao Y: Aberrant methylation of MEG3 functions as a potential plasma-based biomarker for cervical cancer. Sci Rep 7: 6271, 2017.
5. Wang YF, Zhang S, Li XQ and Wang Y: Expression of IncRNA HULC in cervical cancer and its correlation with tumor progression and patient survival. Eur Rev Med Pharmacol Sci 20: 3977-3991, 2016.
6. Kim HJ, Lee DW, Yim GW, Nam EJ, Kim S, Kim SW and Kim YT: Long non-coding RNA HOTAIR is associated with human cervical cancer progression. Int J Oncol 46: 521-530, 2015.
7. Chen G, Wang Z, Wang D, Qiu C, Liu M, Chen X, Zhang Q, Yan G and Cui Q: LncRNA Disease: A database for long-non-coding RNA-associated diseases. Nucleic Acids Res 41 (Database Issue): D983-D986, 2013.
8. Wu W, Chen F, Cui X, Yang L, Chen J, Zhao J, Huang D, Liu J, Yang L, Zeng J, et al: LncRNA NKILA suppresses TGF-β-induced epithelial-mesenchymal transition by blocking NF-κB signaling in breast cancer. Int J Cancer 143: 2213-2224, 2018.
9. Ke S, Li RC, Meng FK and Fang MH: NKILA inhibits NF-κB signaling and suppresses tumor metastasis. Aging (Albany NY) 10: 56-71, 2018.
10. Zandi E, Rothwarf DM, Delhase M, Hayakawa M and Karin M: The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell 91: 243-252, 1997.
11. Huang W, Cui X, Chen J, Feng Y, Song E, Li J and Liu Y: Long non-coding RNA NKILA inhibits migration and invasion of tongue squamous cell carcinoma cells via suppressing epithelial-mesenchymal transition. Oncotarget 7: 62530-62532, 2016.
12. Ayre JE. Cervical cancer: Chronic inflammation, stress and adaptation factors. Acta Unio Int Contra Cancrum 12: 20-27, 1956.
13. Makarov SS: NF-kappaB as a therapeutic target in chronic inflammation: Recent advances. Mol Med Today 6: 441-448, 2000.
14. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
15. Brabletz T, Kalluri R, Nieto MA and Weinberg RA: EMT in cancer. Nat Rev Cancer 18: 128-134, 2018.
16. De Craene B and Berx G: Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 13: 97-110, 2013.
17. Shyamsunder P, Verma RS and Lyakhovich A: ROM1 regulates RedOx states and serves as an inducer of NF-xB-driven EMT factors in Fanconia anemia. Cancer Lett 361: 33-38, 2015.
18. Wang B, Parobchak N, Martin A, Rosen M, Yu LJ, Nguyen M, Gololobova K and Rosen T: Screening a small molecule library to identify inhibitors of NF-xB inducing kinase and pro-lab genes in human placenta. Sci Rep 8: 1657, 2018.
19. Li J, Meng H, Bai Y and Wang K: Regulation of lncRNA and its role in cancer metastasis. Oncol Res 23: 205-217, 2016.
20. Yu G, Yao W, Wang J, Ma M, Xiao W, Li H, Xia D, Yang Y, Deng K, Xiao H, et al: LncRNAs expression signatures of renal clear cell carcinoma revealed by microarray. PLoS One 7: e42377, 2012.
21. Yang G, Lu X and Yuan L: LncRNA: A link between RNA and cancer. Biochim Biophys Acta 1839: 1097-1109, 2014.
22. Goyal N, Kesharwani D and Datta M: Lnc-ing non-coding RNAs with metabolism and diabetes: Roles of IncRNAs. Cell Mol Life Sci 75: 1827-1837, 2018.
23. Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, et al: The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/beta-catenin signaling. Mol Cancer 16: 9, 2017.
24. Gooding AJ, Zhang B, Jahanbani FK, Gilmore HL, Chang JC, Valadkhani S and Schiemann WP. The IncRNA BORG Drives Breast Cancer Metastasis and Disease Recurrence. Sci Rep 7: 12698, 2017.
25. Liu Y, Yang Y, Li L, Liu Y, Geng P, Li G and Song H: LncRNA SNHG1 enhances cell proliferation, migration, and invasion in cervical cancer. Biochem Cell Biol 96: 38-43, 2018.
26. Lu H, He Y, Lin L, Qi Z, Ma L, Li L and Su Y: Long non-coding RNA MALAT1 modulates radiosensitivity of HR-HPV+ cervical cancer via sponging miR-145. Tumour Biol 37: 1683-1691, 2016.
27. Wang X, Wang Z, Wang J, Wang Y, Liu L and Xu X: LncRNA ME3G3 has anti-effect activities of cervical cancer. Biomed Pharmacother 94: 636-643, 2017.
28. Castellanos-Rubio A, Kratchmarov R, Sebastian M, Garcia-Etxebarria K, Garcia L, Irastorza I and Ghosh S: Cytoplasmic form of CARHrRNA facilitates inflammatory gene expression upon NF-xB activation. J Immunol 199: 581-588, 2017.
29. Lu Z, Li Y, Wang J, Che Y, Sun S, Huang J, Chen Z and He J: Long non-coding RNA NKILA inhibits migration and invasion of non-small cell lung cancer via NF-xB/Snail pathway. J Exp Clin Cancer Res 36: 54, 2017.
30. Steeg PS: Tumor metastasis: Mechanistic insights and clinical challenges. Nat Med 12: 935-940, 2006.
31. Thiery JP: Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 15: 740-746, 2003.
32. Qureshi R, Arora H and Rizvi MA: EMT in cervical cancer: Its role in tumour progression and response to therapy. Cancer Lett 356: 321-331, 2015.
33. Tan X, Zhou C, Liang Y, Lai YF and Liang Y: Circ_0001971 regulates oral squamous cell carcinoma progression and chemoresistance by targeting miR-194/miR-204 in vitro and in vivo. Eur Rev Med Pharmacol Sci 24: 2470-2481, 2020.
34. Cao MX, Jiang YP, Tang YL and Liang XH: The crosstalk between lncRNA and microRNA in cancer metastasis: Orchestrating the epithelial-mesenchymal plasticity. Oncotarget 8: 12472-12483, 2017.
35. Sun NX, Ye C, Zhao Q, Zhang Q, Xu C, Wang SB, Jin ZJ, Sun SH, Wang F and Li W: Long noncoding RNA-EBIC promotes tumor cell invasion by binding to EZH2 and repressing E-cadherin in cervical cancer. PLoS One 9: e100340, 2014.
36. Xu F and Zhang J: Long non-coding RNA HOTAIR functions as miRNA sponge to promote the epithelial to mesenchymal transition in esophageal cancer. Biomed Pharmacother 90: 888-896, 2017.
37. Ying L, Chen Q, Wang Y, Zhou Z, Huang Y and Qiu F: Upregulated MALAT-1 contributes to bladder cancer cell migration by inducing epithelial-to-mesenchymal transition. Mol Biosyst 8: 2289-2294, 2012.
38. Sun R, Qin C, Jiang B, Fang S, Pan X, Peng L, Li W, Li Y and Li G: Down-regulation of MALAT1 inhibits cervical cancer cell invasion and metastasis by inhibition of epithelial-mesenchymal transition. Mol Biosyst 12: 952-962, 2016.
39. Hu Y, Sun X, Mao C, Guo G, Ye S, Xu J, Zou R, Chen J, Wang L, Duan P and Xue X: Upregulation of long noncoding RNA TUG1 promotes cervical cancer cell proliferation and migration. Cancer Med 6: 471-482, 2017.
40. Wu Y, Deng J, Rychahou PG, Qiu S, Evers BM and Zhou BP: Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion. Cancer Cell 15: 416-428, 2009.
41. Wang H, Song X, Li M, Wang X, Tao Y, Xiya Y, Liu H, Zhaoy, Chang D and Sha Q: The role of TLR4/NF-xB signaling pathway in activated microglia of rats with chronic high intraocular pressure and vitro scratch injury-induced microglia. Int Immunopharmacol 83: 10085, 2020.
42. Xiao G, Harhaj EW and Sun SC: NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. Mol Cell 7: 401-409, 2001.
43. Hayden MS and Ghosh S: Shared principles in NF-kappaB signaling. Cell 132: 344-362, 2008.
44. Yang Z, Li C, Wang X, Zhai C, Yi Z, Wang L, Liu B, Du B, Wu H, Guo X, et al: Dauricine induces apoptosis, inhibits proliferation and invasion through inhibiting NF-kappaB signaling pathway in colon cancer cells. J Cell Physiol 225: 266-275, 2010.
45. Hsu YL, Chen CY, Lin IP, Tsai EM, Kuo PL and Hou MF: 4-Shogaol, an active constituent of dietary ginger, inhibits metastasis of MDA-MB-231 human breast adenocarcinoma cells by decreasing the repression of NF-xB/Snail on RKIP. J Agric Food Chem 60: 852-861, 2012.
46. Chua HL, Bhat-Nakshatri P, Clare SE, Morimiya A, Badve S and Nakshatri H: NF-kappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: Potential involvement of ZEB-1 and ZEB-2. Oncogene 26: 711-724, 2007.
47. Li Q, Liu BC, Lv LL, Ma KL, Zhang XL and Phillips AO: Monocytes induce proximal tubular epithelial-mesenchymal transition through NF-xB activity and apoptosis induction. Mol Cell Biochem 379: 7-18, 2013.
48. Lu Z, Chen Z, Li Y, Wang J, Zhang Z, Che Y, Huang J, Sun S, Mao S, Lei Y, et al: TGF-β-induced NKILA inhibits ESCC cell migration and invasion through NF-xB/MMPI14 signaling. J Mol Med (Berl) 96: 301-313, 2018.
49. Mathy NW and Chen XM: Long non-coding RNAs (lncRNAs) and their transcriptional control of inflammatory responses. J Biol Chem 292: 12375-12382, 2017.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.