Int J Clin Exp Pathol 2020;13(3):607-615
www.ijcep.com /ISSN:1936-2625/IJCEP0107052

Original Article

Kin17 knockdown suppresses the migration and invasion of cervical cancer cells through NF-κB-Snail pathway

Meifeng Zhong[1,2*], Zhenping Liu[3*], Kunhe Wu[4], Ziyang Hong[1,2], Yuzhao Zhang[5], Jing Qu[2], Chuiyu Zhu[1], Zhiyu Ou[1,2], Tao Zeng[1,2]

[1]Department of Medical Laboratory, School of Laboratory Medicine and Biotechnology, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China; [2]Laboratory Medicine Center, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China; [3]Department of Laboratory Medicine, The People's Hospital of Shajing, Shenzhen 518104, Guangdong, P. R. China; [4]Department of Pathology, Guangdong Women and Children's Hospital, Guangzhou 511400, Guangdong, P. R. China; [5]Department of Laboratory Medicine, The First Affiliated Hospital, Guangzhou University of Chinese Medicine, Guangzhou 510405, Guangdong, P. R. China. [*]Equal contributors.

Received December 27, 2019; Accepted February 6, 2020; Epub March 1, 2020; Published March 15, 2020

Abstract: Cervical cancer is one of the most common cancers in women worldwide. Metastasis in cancer has been a Gordian knot due to unsatisfactory clinical treatments. *KIN17,* a highly conserved gene from yeast to human, up-regulation is associated with the pathogenesis and development of several common cancers. Our previous works revealed that elevated expression of kin17 observed in cervical cancer tissues showed a close association with lymph node metastasis. This study aimed to explore roles and mechanisms of kin17 in the migration and invasion of cervical cancer cells. Cervical cancer cell lines HeLa and SiHa with kin17 knockdown were constructed by using recombinant lentiviral vector that carry specific siRNA targeting *KIN17* gene. The mRNA and protein levels of kin17 in cells were determined by RT-qPCR and western blotting, respectively. Wound healing assay and transwell assays were performed to assess the migration and invasion abilities of the cancer cells, respectively. The expression of signaling proteins involved in the NF-κB-Snail pathway was analyzed by western blotting. As our results showed, the mRNA and protein levels of kin17 in HeLa cells and SiHa cells showed a significant decrease by transfection with recombinant lentiviral vector carrying specific siRNA. Compared with control group, the migration rates were decreased in the kin17 knockdown group in both HeLa and SiHa cell lines in wound healing assay as well as transwell assay without matrigel. Kin17 knockdown also reduced the cell invasion number of both HeLa and SiHa cells. In addition, the phosphorylation of nuclear factor Kαppa B (NF-κB) p65, IKαppa B kinase α (IKKα), and IKαppa B α (IκBα) in NF-κB pathway and the expression of Snail were decreased in HeLa cells and SiHa cells by kin17 knockdown. Our results demonstrated that knockdown of kin17 in cervical cancer cells suppressed cell migration and invasion, and inhibited the activity of NF-κB signaling pathway and the expression of Snail. These findings suggested kin17 as an essential regulator of the cell migration and invasion and the underlying molecular mechanism involved NF-κB-Snail pathway in cervical cancer. This might serve as a novel molecular therapeutic target for treating cervical cancer metastasis.

Keywords: Kin17, migration, invasion, cervical cancer, NF-κB-Snail pathway

Introduction

Cervical cancer is one of the most malignancies in women worldwide, and is still posing a serious threat to women, showing high incidence in the younger generations [1-4]. Although ultrasonography, cervical smear cytological examination and human papillomavirus (HPV) genotyping are routinely used for clinical screening of cervical cancer, many patients are often detected in advanced stages [5]. Moreover, the treatment outcomes of cancer metastasis still remained unsatisfactory. Thus, it is necessary to elucidate the molecular mechanisms for cancer metastasis in order to develop new therapeutic targets for cervical cancer.

KIN17 is a highly conserved gene from yeast to humans, and encodes a protein kin17 with a molecular weight of 45 KDa. According to previous studies, kin17 has been reported to participate in DNA replication [6], DNA damage response [7] and cell cycle progression [8]. Recently, kin17 has been found to be up-regulated in several common cancers including breast cancer [9], colorectal cancer [10], and lung cancer [11], and is related to the pathogenesis and development of these cancers. Our previous study demonstrated that kin17 played an important role in the invasion and metastasis of non-small cells lung cancer (NSCLC) [11]. Elevated expression of kin17 is also observed in cervical cancer samples, showing a close association with lymph node metastasis [12]. However, the association of kin17 with metastasis of cervical cancer remained unclear. Therefore, this study aimed to explore the roles and the relevant mechanisms of kin17 in the migration and invasion of cervical cancer cells in this study.

Materials and methods

Cell culture

Human cervical cancer cell lines HeLa and SiHa were obtained from GeneChem Company (Shanghai, China) and were cultured in Dulbecco Modified Eagle Medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, VA, USA) supplemented with 10% fetal bovine serum (FBS, Tianjin Kαngyuan, Biotechnology Co., Ltd.), 60 μg/mL penicillin and 100 μg/mL streptomycin (Hyclone, USA). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Lentiviral vector construction and cell lines screening

The gene-silencing lentiviral vector GV248-KD with specific siRNA targeting *KIN17* gene sequence and the normal controlled lentiviral vector GV248-NC were successfully constructed, as described previously [11]. Lentiviral vector GV248-KD contained a reporter gene enhanced green fluorescent protein (EGFP). After gene transfection, virus particles transfected and screened with puromycin, HeLa cells transfected with gene-silencing lentiviral vector ($HeLa^{KD}$ cells) or the controlled vector ($HeLa^{NC}$ cells), together with SiHa cells transfected with gene-silencing lentiviral vector ($SiHa^{KD}$ cells) or the controlled vector ($SiHa^{NC}$ cells), were cultured with puromycin until the cells reach ~90% confluence with positive EGFP expression. The cells with stable transfection were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% FBS and puromycin. HeLa cells or SiHa cells without transfection with vector ($HeLa^{Mock}$ cells or $SiHa^{Mock}$ cells) were used as blank control.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the cells and was prepared for cDNAs synthesis using a reverse transcription kit (Promega Corporation, Madison, WI, USA). The primers for *KIN17* gene were as follows: forward: 5'-CCATGATTCCTTCATATTTGC-3', reverse: 5'-GTAATACGGTTATCCACGCG-3'. The primers for GAPDH were as follows: forward, 5'-GGAGCGAGATCCCTCCAAAAT-3'; reverse, 5'-GGCTGTTGTCATACTTCTCATGG-3'. The cDNA was then used for PCR amplification with SYBR Premix Ex Taq (cat. no., DRR420A, TaKαra Bio, Inc., Otsu, Japan) in a thermal cycler (GeneAmp 2400; PE Applied Biosystems, Foster City, CA, USA). All samples were run in triplicate and the relative mRNA levels were calculated using the $2^{-\Delta\Delta Cq}$ method provided by the System software (Applied Biosystems).

Western blot analysis

Total proteins from the cells were prepared for western blot analysis. RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) containing a complete protease inhibitor cocktail tablet (Roche Applied Science, Penzberg, Germany) was used to extract the total proteins as described previously [9]. A 12% SDS-PAGE were used to separate 100 μg of protein per lane and then transferred onto Immobilon®-P PVDF Transfer Membranes (EMD Millipore, Billerica, MA, USA). Next, the protein sample was blocked with non-fat milk at room temperature for 1 hour, and the membranes were incubated with monoclonal primary antibodies using anti-kin17 (dilution, 1:500; cat. no. sc-32769; Santa Cruz Biotechnology, Inc.), anti-Snail (dilution, 1:1000; cat. no. #3879; Cell Signaling TECHNOLOGY, Inc.), anti-IKKα (dilution, 1:1000; cat. no. #11930; Cell Signaling TECHNOLOGY, Inc.), anti-NF-κB p65

(dilution, 1:1000; cat. no. #8242; Cell Signaling TECHNOLOGY, Inc.), anti-IκBα (dilution, 1:1000; cat. no. #4814; Cell Signaling TECHNOLOGY, Inc.), anti-phospho-IKKα (dilution, 1:1000; cat. no. #2697; Cell Signaling TECHNOLOGY, Inc.), anti-phospho-NF-κB p65 (dilution, 1:1000; cat. no. #3033; Cell Signaling TECHNOLOGY, Inc.), anti-phospho-IκBα (dilution, 1:1000; cat. no. #2859; Cell Signaling TECHNOLOGY, Inc.) and anti-GAPDH (dilution, 1:25000; cat. no. 60004-1-Ig; proteintech, Inc.) for overnight at 4°C. After washing with TBST, HRP-conjugated secondary antibodies (dilution, 1:1000; cat. no. #7074/7076; Cell Signaling TECHNOLOGY, Inc.) were used for developing immunoblots at room temperature for 1 hour, and were processed using ECL enhanced chemilu-minescence substrate (Thermo Fisher Scientific, Inc.). Images were captured using the ImageQuant RT ECL™ imager (GE Healthcare Life Sciences, Shanghai, China). Band intensities were quantified by using ImageJ software (GE Healthcare Life Sciences, Shanghai, China).

Wound healing assay

Cells were seeded into 6-well plates (3×10^5 cells/well). Next, a sterile 200 μL micropipette tip was used to make the wound when the cells reached to 90%. The wounded monolayers were washed with phosphate buffer solution (PBS, Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) three times to remove the cell debris. The gap between the two edges of the wound was measured after incubation at 0, 24 and 48 hours.

Transwell assay without matrigel

Transwell assay without matrigel was performed to evaluate the migration ability of HeLa and SiHa cells [13]. A cell density of 5×10^4 cells were seeded in serum-free medium at a volume of 200 μL/well in the upper chamber. The lower chambers were filled with 600 μL medium supplemented with 30% FBS as chemoattractants. After incubation for 24 hours, the cells in the upper chambers were removed by wet cotton swabs. Migrated cells on the lower side of the filter were fixed with 100% methanol (Guangdong Guanghua Sci-Tech Co., Ltd.) and stained with 0.25% crystal violet solution (Shanghai GeneChem Co.) for 30 minutes. After washing with PBS for three times, the migrated cells in the lower chamber were observed and then photographed using an inverted microscope. The numbers of migrated cells was determined by photographing in five random fields per chamber at 200× magnification.

Transwell assay with matrigel

Transwell assay with matrigel was performed to evaluate the invasive capacity of the cancer cells [14]. Briefly, transwell chambers (Costar®, Corning Incorporated, Corning, NY, USA) with 8 μm pore polycarbonate filters were coated with Matrigel (BD Biosciences, San Jose, CA, USA) at 37°C for 1 hour for solidification. A cell density of 5×10^4 cells were seeded in the upper chamber at a volume of 200 μL and incubated for 48 hours. Invasive cells were stained and counted, which followed a protocol that was similar to transwell assay without matrigel.

Statistical analysis

All experiments were repeated three times and the data were presented as mean ± SD. SPSS software (version 16.0; SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. All statistical results and corresponding *p* values reported were two-tailed. $P<0.05$ was considered to be statistically significant difference.

Results

Cervical cells lines HeLa and SiHa with kin17 knockdown were established

To investigate the function of kin17 in cervical cancer cells, the recombinant lentiviral vector with or without *KIN17* siRNA were used to transfect HeLa and SiHa cells and then the stable expression cell colonies were screened by puromycin for nearly a month. The fluorescent positive rates of $HeLa^{NC}$, $HeLa^{KD}$, $SiHa^{NC}$ and $SiHa^{KD}$ cells were more than 90%, and nearly no fluorescence was observed in both $HeLa^{Mock}$ and $SiHa^{Mock}$ cells (**Figure 1A** and **1B**).

The results of RT-qPCR assay revealed that the mRNA levels of Kin17 in $HeLa^{KD}$ cells (**Figure 1C**) and $SiHa^{KD}$ cells (**Figure 1D**) were significantly decreased when compared with levels in $HeLa^{NC}$ cells or $SiHa^{NC}$ cells ($P<0.05$, n=3), respectively. Furthermore, western blot analy-

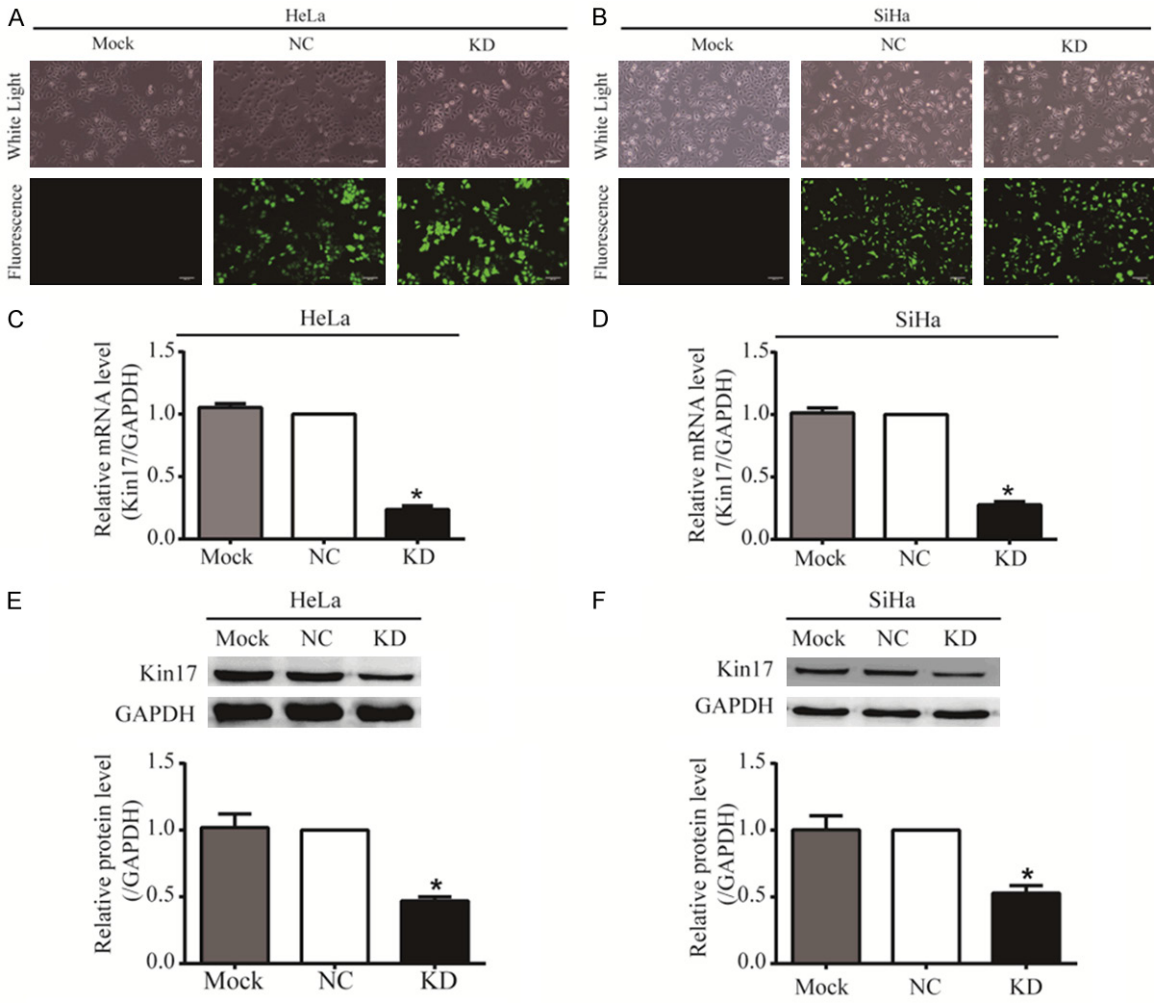

Figure 1. Establishment and determination of cervical cells lines HeLa and SiHa with kin17 knockdown. Morphological features and fluorescence-indicated infection of $HeLa^{Mock}$, $HeLa^{NC}$, $HeLa^{KD}$ cells (A), $SiHa^{Mock}$, $SiHa^{NC}$ and $SiHa^{KD}$ cells (B), ×100. mRNA and protein levels of kin17 in HeLa cells (C, RT-qPCR; E, Western blotting) and SiHa cells (D, RT-qPCR; F, Western blotting) transfected with lentivirus were identified. $HeLa^{KD}$, HeLa cells transfected with recombinant lentiviral vectors carrying the siRNA targeting *KIN17* gene; $HeLa^{NC}$, and HeLa cells were transfected with the control vector; $HeLa^{Mock}$, HeLa cells without transfection of vector. $SiHa^{KD}$, SiHa cells infected with recombinant lentiviral vectors carrying the siRNA targeting *KIN17* gene; $SiHa^{NC}$, SiHa cells transfected with the controlled vector; $SiHa^{Mock}$, SiHa cells without transfection of vector. NC, negative control; KD, knock down; $^{*}P<0.05$, n=3.

sis confirmed that the protein levels of kin17 in $HeLa^{KD}$ cells (**Figure 1E**) and $SiHa^{KD}$ cells (**Figure 1F**) showed a significant decrease when compared with those levels in the controlled cells ($P<0.05$, n=3), respectively.

Kin17 knockdown suppressed the migration and invasion of HeLa and SiHa cells

At 24 or 48 hours following the scratch, wound healing assay showed that the rates of migration in $HeLa^{KD}$ cells ($P<0.05$, n=3, **Figure 2A**) and $SiHa^{KD}$ cells ($P<0.05$, n=3, **Figure 2B**) showed a significant decrease when compared with those in $HeLa^{NC}$ cells or $SiHa^{NC}$ cells, respectively. Furthermore, transwell assay without matrigel was used to determine the migration ability of cells. In consistent with the results of wound healing, the migrated cells were also significantly decreased in both $HeLa^{KD}$ cells ($P<0.05$, n=3, **Figure 2C**) and $SiHa^{KD}$ cells ($P<0.05$, n=3, **Figure 2D**) compared with the controlled groups.

In transwell assay with matrigel, an experiment that was performed for testing the invasive ability of cells, the numbers of invaded cells showed a significant reduction in $HeLa^{KD}$ cells

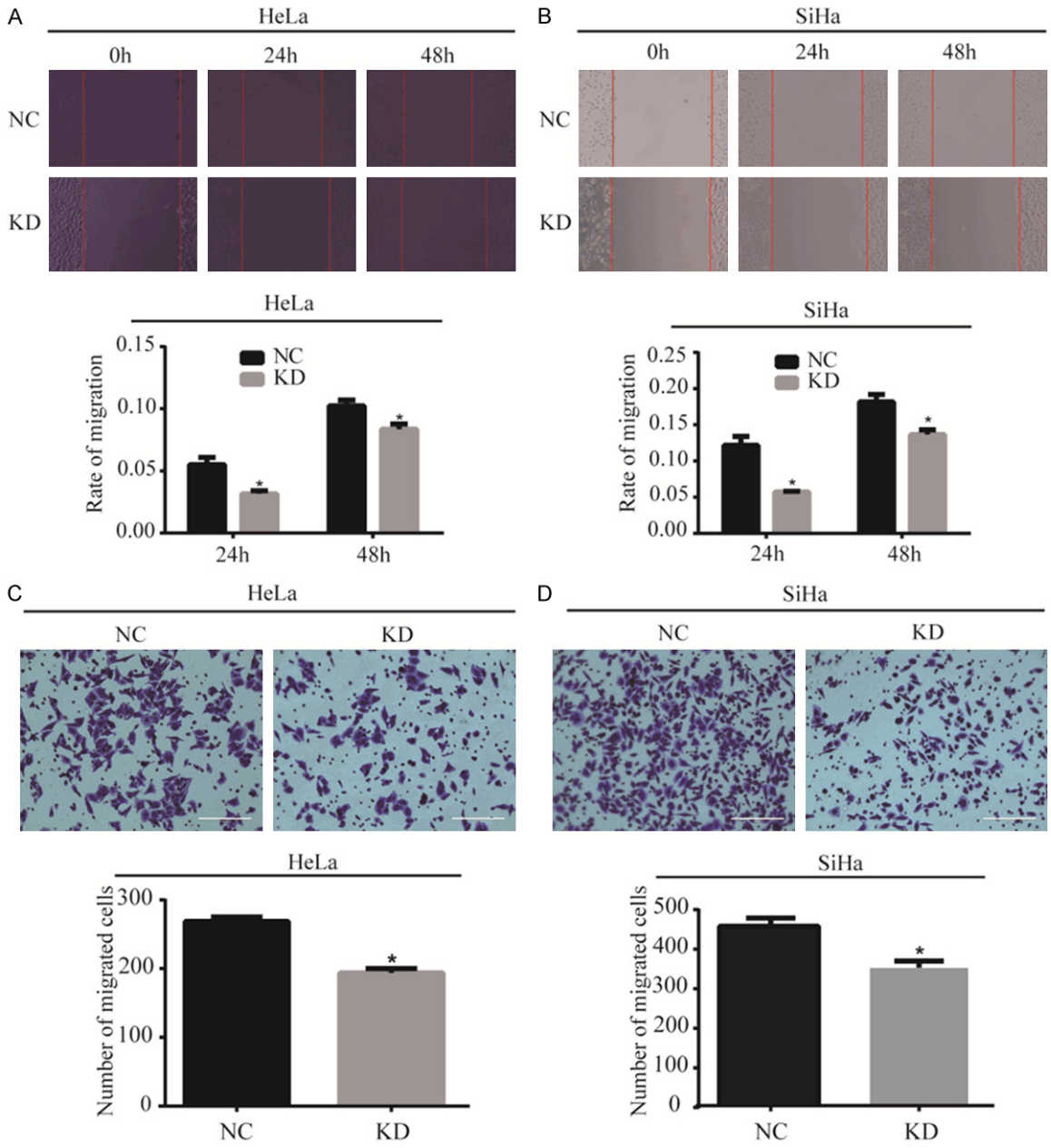

Figure 2. Effect of kin17 knockdown on migration ability of cervical cancer cells. Representative images and quantification of HeLa (A) and SiHa (B) cells in wound healing assay, ×100. Representative images and quantification of HeLa (C) and SiHa (D) cells in transwell assay without matrigel, ×200; $^{*}P<0.05$, n=3.

($P<0.05$, n=3, **Figure 3A**) and $SiHa^{KD}$ cells ($P<0.05$, n=3, **Figure 3B**) when compared to control groups.

Kin17 knockdown changed expression of the signaling molecules of NF-κB-Snail pathway in HeLa and SiHa cells

To explore the downstream signaling molecules of kin17 in cervical cancer cells, the expression of the important proteins that are involved in NF-κB signaling pathway and epithelial-mesenchymal transition (EMT) were compared by western blotting. Our results showed that phosphorylation of NF-κB p65, IKKα and IκBα in NF-κB pathway showed a decrease in $HeLa^{KD}$ cells and $SiHa^{KD}$ cells (**Figure 4A** and **4B**). The expression of transcriptional factor Snail in $HeLa^{KD}$ cells and $SiHa^{KD}$ cells was lower than that in $HeLa^{NC}$ cells and $SiHa^{NC}$ cells (**Figure 4C** and **4D**), respectively.

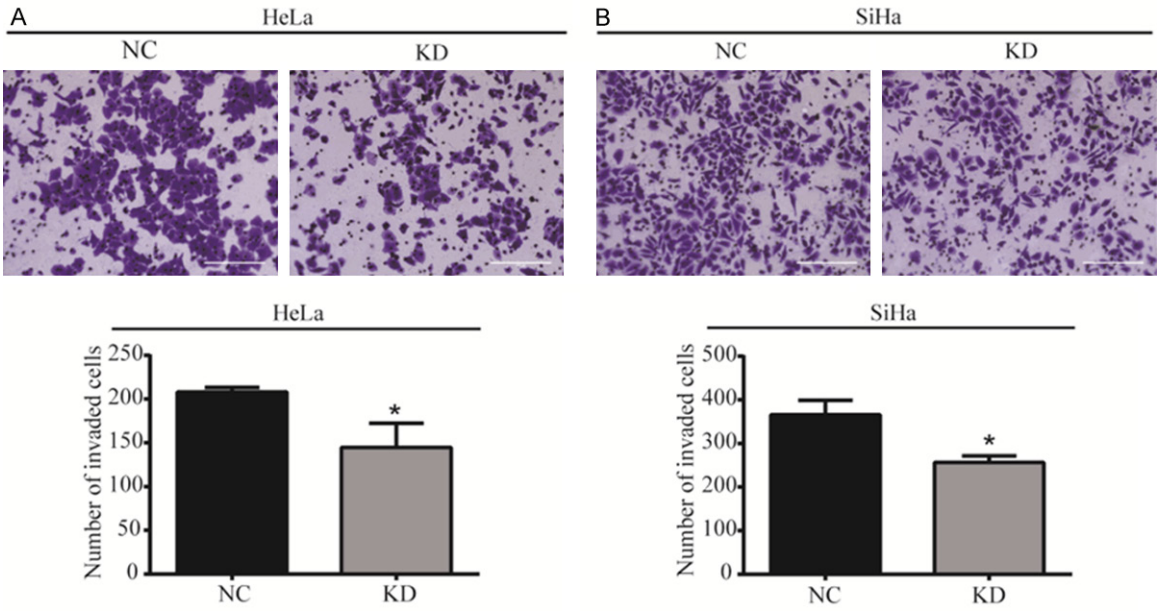

Figure 3. Effect of kin17 knockdown on cervical cancer cells invasiveness. Representative images and quantification of HeLa (A) and SiHa (B) cells in transwell assay with matrigel, ×200; $^{*}P<0.05$, n=3.

Discussion

Several studies have reported that kin17 was involved in the metastasis of several types of cancers. Our previous studies have revealed that up-regulation of kin17 was found in clinical specimens of cervical cancer, and it was associated with lower tumor differentiation, and high risk of lymph node metastasis in a retrospective analysis [11]. To investigate the role of kin17 in the metastasis of cervical cancer, two cell lines with kin17 knockdown by using lentiviral vector transfection were successfully constructed, and the levels of mRNA and protein using RT-qPCR and western blotting, respectively were performed. The results revealed that kin17 knockdown inhibited migration of both HeLa and SiHa cells and suppressed invasion of the two cell lines.

The underlying molecular mechanisms of kin17 in the migration and invasion of cervical cancer cells were further investigated in this study. NF-κB pathway has been identified as one of the key signaling pathways in the metastasis of cancer [15-18]. Activation of NF-κB, a nuclear transcription factor, regulates the expression of downstream oncogenes. Our study revealed that kin17 knockdown in cervical cancer cells decreased the phosphorylation of NF-κB p65, IKKα and IκBα, which are three important components of NF-κB signaling pathway. This suggested that kin17 was elevated in cervical cancer tissues, which contributed to the activation of NF-κB pathway and progression of the cancer. Additionally, NF-κB-Snail pathway in cancer cells showed an association with EMT, and closely related to the stemness, metastasis, and progression in most of the cancers [19-21], including cervical cancer [22]. EMT and phenotype of cancer cells are controlled by the gene regulatory network. As a downstream of NF-κB signaling in NF-κB-Snail pathway, transcriptional factor Snail was involved in the EMT process, contributing to the migration, invasion and even distant metastases of cancer cells through blocking E-cadherin gene and stimulating N-cadherin gene [23, 24]. We found that the phosphorylation of NF-κB signaling factors and the expression of Snail were decreased after kin17 knockdown. This indicated that kin17 might regulate the migration and invasion abilities of cervical cancer cells through NF-κB-Snail pathway, which has been identified as one of the most important signaling pathways for cancer cell metastasis [25, 26].

In conclusion, knockdown of kin17 which was up-regulated in cervical cancer tissues, and inhibited cervical cancer cell migration and invasion via NF-κB-Snail pathway. Kin17 might

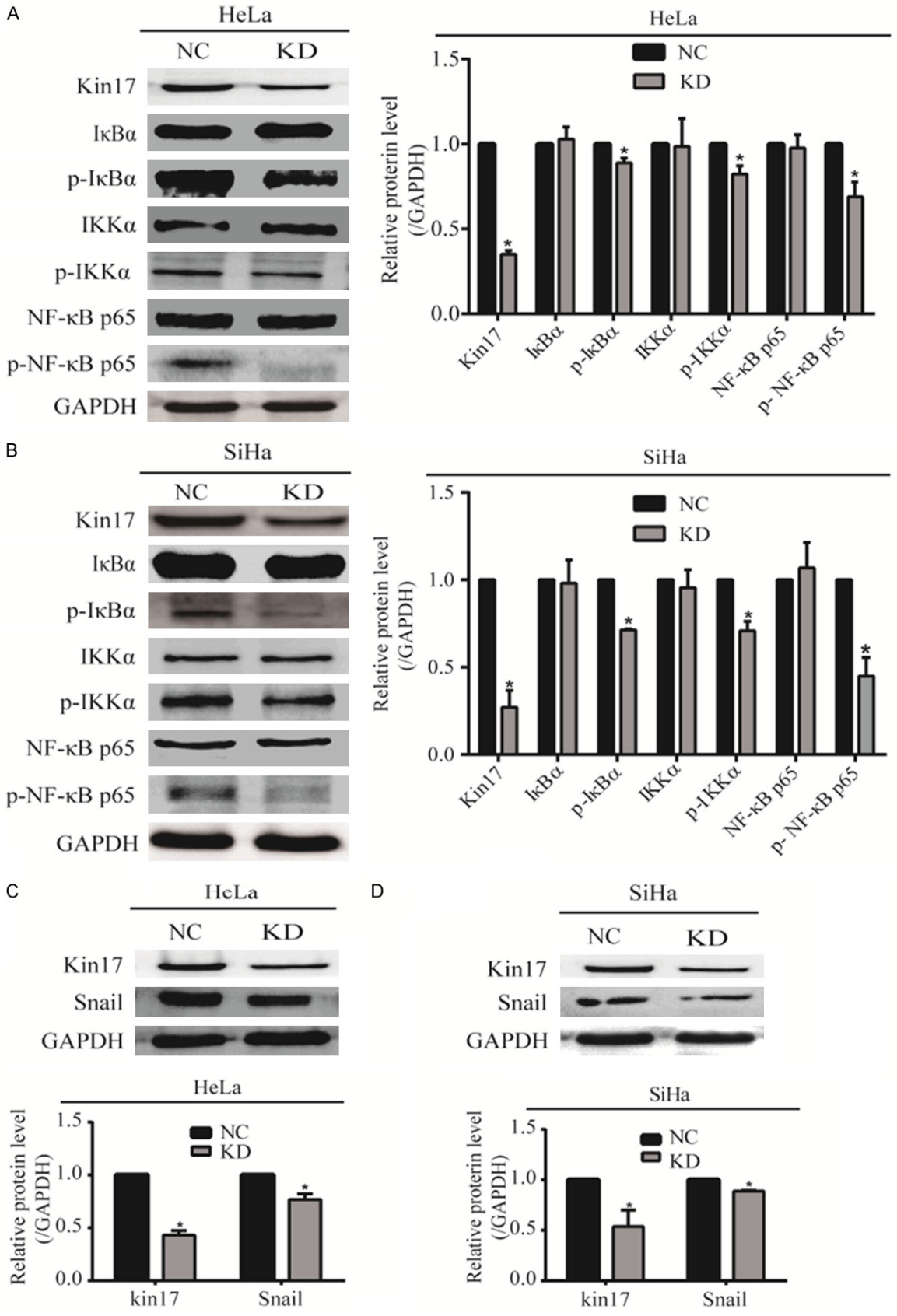
A
HeLa
NC
KD
Kin17
IκBα
p-IκBα
IKKα
p-IKKα
NF-κB p65
p-NF-κB p65
GAPDH
HeLa
NC
KD
Relative proterin level (/GAPDH)
1.5
1.0
0.5
0.0
Kin17
IκBα
p-IκBα
IKKα
p-IKKα
NF-κB p65
p- NF-κB p65
B
SiHa
NC
KD
Kin17
IκBα
p-IκBα
IKKα
p-IKKα
NF-κB p65
p-NF-κB p65
GAPDH
SiHa
NC
KD
Relative protein level (/GAPDH)
1.5
1.0
0.5
0.0
Kin17
IκBα
p-IκBα
IKKα
p-IKKα
NF-κB p65
p- NF-κB p65
C
HeLa
NC
KD
Kin17
Snail
GAPDH
HeLa
NC
KD
Relative protein level (/GAPDH)
1.5
1.0
0.5
0.0
kin17
Snail
D
SiHa
NC
KD
Kin17
Snail
GAPDH
SiHa
NC
KD
Relative proterin level (/GAPDH)
1.5
1.0
0.5
0.0
kin17
Snail

Figure 4. Effect of Kin17 knockdown on the expression of signaling molecules of NF-κB-Snail pathway in cervical cancer cells. Phosphorylation of IKKα, IκBα, NF-κB p65 (A and B) and expression of Snail (C and D) after kin17 knockdown in HeLa and SiHa cells were detected through western blotting analysis. IKKα, Ikappa B kinase α; IκBα, Ikappa Bα; NF-κB, nuclear factor kappa B.

act as a novel molecular therapeutic target for the metastasis of cervical cancer. However, further experiments are required to understand the roles, molecular mechanisms and potential clinical application of kin17 in the metastasis of cervical cancer cells.

Acknowledgements

This work was supported by the Guangdong Planning Project of Science and Technology (grant no. 2016A020215118), the Pearl River S&T Nova Program of Guangzhou (grant no. 201710010015) and the Knowledge Innovation Program of Shenzhen Innovation Committee (grant no. JCYJ20160428101420063). The funders had no role in study design, data collection and analysis, manuscript preparation or decision to publish.

Disclosure of conflict of interest

None.

Address correspondence to: Tao Zeng, Department of Medical Laboratory, School of Laboratory Medicine and Biotechnology, Southern Medical University, Shatai Road, Baiyun District, Guangzhou 510515, P. R. China. Tel: +86-020-62786961; Fax: +86-020-62786961; E-mail: zengt@smu.edu.cn

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.
[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
[3] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
[4] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
[5] Small W Jr, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, Jhingran A, Kitchener HC, Mileshkin LR, Viswanathan AN and Gaffney DK. Cervical cancer: a global health crisis. Cancer 2017; 123: 2404-2412.
[6] Masson C, Menaa F, Pinon-Lataillade G, Frobert Y, Chevillard S, Radicella JP, Sarasin A and Angulo JF. Global genome repair is required to activate KIN17, a UVC-responsive gene involved in DNA replication. Proc Natl Acad Sci U S A 2003; 100: 616-21.
[7] Cloutier P, Lavallée-Adam M, Faubert D, Blanchette M and Coulombe B. Methylation of the DNA/RNA-binding protein Kin17 by METTL22 affects its association with chromatin. J Proteomics 2014; 100: 115-24.
[8] Miccoli L, Biard DS, Frouin I, Harper F, Maga G and Angulo JF. Selective interactions of human kin17 and RPA proteins with chromatin and the nuclear matrix in a DNA damage- and cell cycle-regulated manner. Nucleic Acids Res 2003; 31: 4162-75.
[9] Zeng T, Gao H, Yu P, He H, Ouyang X, Deng L and Zhang Y. Up-regulation of kin17 is essential for proliferation of breast cancer. PLoS One 2011; 6: e25343.
[10] Ruan LQ, Jiang QG and Zhang HT. Relationships of kin17 protein expression with clinical features and prognosis of colorectal cancer. Transl Cancer Res 2018; 7: 1072-1078.
[11] Zhang Y, Huang S, Gao H, Wu K, Ouyang X, Zhu Z, Yu X and Zeng T. Upregulation of KIN17 is associated with non-small cell lung cancer invasiveness. Oncol Lett 2017; 13: 2274-2280.
[12] Zhang Y, Gao H, Gao X, Huang S, Wu K, Yu X, Yuan K and Zeng T. Elevated expression of kin17 in cervical cancer and its association with cancer cell proliferation and invasion. Int J Gynecol Cancer 2017; 27: 628-633.
[13] Fan C, Tang Y, Wang J, Wang Y, Xiong F, Zhang S, Li X, Xiang B, Wu X, Guo C, Ma J, Zhou M, Li X, Xiong W, Li Y, Li G and Zeng Z. Long noncoding RNA LOC284454 promotes migration and invasion of nasopharyngeal carcinoma via modulating the Rho/Rac signaling pathway. Carcinogenesis 2019; 40: 380-391.
[14] Hao L, Rong W, Bai L, Cui H, Zhang S, Li Y, Chen D and Meng X. Upregulated circular RNA circ_0007534 indicates an unfavorable prognosis in pancreatic ductal adenocarcinoma and regulates cell proliferation, apoptosis, and invasion by sponging miR-625 and miR-892b. J Cell Biochem 2019; 120: 3780-3789.
[15] Cildir G, Low KC and TergaonKαr V. Noncanonical NF-Kαppa B signaling in health and disease. Trends Mol Med 2016; 22: 414-429.
[16] Huang H, LangenKαmp E, Georganaki M, Loskog A, Fuchs PF, Dieterich LC, Kreuger J and Dimberg A. VEGF suppresses T-lymphocyte infiltration in the tumor microenvironment

through inhibition of NF-Kαppa B-induced endothelial activation. FASEB J 2015; 29: 227-38.
[17] Kαrin M, Cao Y, Greten FR and Li ZW. NF-KαppaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002; 2: 301-10.
[18] Kαrin M and Greten FR. NF-Kαppa B: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005; 5: 749-59.
[19] Nieto MA, Huang RY, Jackson RA and Thiery JP. EMT: 2016. Cell 2016; 166: 21-45.
[20] Pastushenko I and Blanpain C. EMT transition states during tumor progression and metastasis. Trends Cell Biol 2019; 29: 212-226.
[21] Yeung KT and Yang J. Epithelial-mesenchymal transition in tumor metastasis. Mol Oncol 2017; 11: 28-39.
[22] Yan S, Wang Y, Yang Q, Li X, Kong X, Zhang N, Yuan C, Yang N and Kong B. Low-dose radiation-induced epithelial-mesenchymal transition through NF-Kαppa B in cervical cancer cells. Int J Oncol 2013; 42: 1801-6.
[23] Alidadiani N, Ghaderi S, Dilaver N, Bakhshamin S and Bayat M. Epithelial mesenchymal transition transcription factor (TF): the structure, function and microRNA feedback loop. Gene 2018; 674: 115-120.
[24] Assani G and Zhou Y. Effect of modulation of epithelial-mesenchymal transition regulators Snail1 and Snail2 on cancer cell radiosensitivity by targeting of the cell cycle, cell apoptosis and cell migration/invasion. Oncol Lett 2019; 17: 23-30.
[25] Kim SO and Kim MR. [6]-gingerol prevents disassembly of cell junctions and activities of MMPs in invasive human pancreas cancer cells through ERK/NF-Kαppa B/snail signal transduction pathway. Evid Based Complement Alternat Med 2013; 2013: 761852.
[26] Yang Y, Li Y, Wang K, Wang Y, Yin W and Li L. P38/NF-Kαppa B/snail pathway is involved in caffeic acid-induced inhibition of cancer stem cells-like properties and migratory capacity in malignant human keratinocyte. PLoS One 2013; 8: e58915.