

## Original Article

# URI promotes the migration and invasion of human cervical cancer cells potentially via upregulation of vimentin expression

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**Abstract:** URI is known to act as an oncoprotein in several tumors. Our previous studies have shown that URI is associated with the migration process in cervical and gastric cancer cells, but the mechanisms remain to be determined. Given the fact that URI positively regulates vimentin expression, we therefore investigated how URI regulated vimentin expression affects the migration and invasion of cells from two human cervical cancer cell lines HeLa and C33A, which differentially express URI. We have shown that knock-down of URI in HeLa cells using URI siRNA caused decreased vimentin mRNA and protein levels along with attenuated cell motility. Meanwhile, overexpression of URI by transfection of PCMV6-URI in C33A cells resulted in increased vimentin expression and enhanced cell migration and invasion. We have also used TGF- $\beta$  to induce vimentin expression, which enhanced the cell migration and invasion abilities affected by URI, while inhibition of vimentin by siRNA attenuated URI's effect on cell migration and invasion. In addition, we have performed luciferase reporter and ChIP assays, and the results support that URI indirectly enhances the activity of vimentin promoter. Taken together, our results suggest that URI plays essential roles in the migration and invasion of human cervical cancer cells, possibly via targeting vimentin expression.

**Keywords:** URI, HeLa and C33A cell lines, TGF- $\beta$ , vimentin, migration and invasion

## Introduction

Cervical cancer is one of the most common malignant tumors in female reproductive system. Many risk factors, including early and poly-pregnancy, unhealthy sex activity, and virus (especially HPV, human papillomavirus) infection etc, are known to contribute to the occurrence of cervical cancer [1]. Depends on the stages, treatment of cervical cancer can be combined radiotherapy, chemotherapy with or without surgery [2]. However, the survival rate and curative efficacy will be severely affected once the distal metastasis occurs. Therefore, understanding the metastatic mechanism has been continuously the focused area of cervical cancer research.

URI, a co-chaperone unconventional prefoldin RPB5 interactor, has been shown to possess characteristics of oncoprotein. Accumulating evidences have demonstrated that URI plays

an important role in multiple tumorigenesis and tumor progression including ovarian cancer, hepatocellular carcinoma (HCC), and multiple myeloma [3-7]. A recent study confirmed that URI-regulates OGT, an enzyme catalyzing O-GlcNAcylation, and confers c-MYC-dependent survival functions in response to glucose fluctuations so as to accelerate liver tumorigenesis [8]. As the second most commonly diagnosed female cancer, recurrence and metastasis of cervical cancer are the main reasons causing cancer death, which is also the third leading cause of cancer death for females in developing countries [9, 10]. Epithelial-mesenchymal transition (EMT) program has been implicated as an important molecular mechanism for cervical cancer metastasis [11]. Our previous study has shown that URI promoted cell migration and mRNA expression of vimentin, a mesenchymal marker, in cervical cancer cells [12]. In this study, we further explored the effect and mechanism of URI on

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**Table 1.** Primers for qPCR and ChIP

Gene	Forward (5'-3')	Reverse (5'-3')	Size (bp)
URI	TTTGCAGAAAATGAGCGATG	GCAATTCGGTGTTCCTTT	93
Vimentin	GGACCAGCTAACCAACGACA	AAGGTCAAGACGTGCCAGAG	178
GAPDH1	TCTCTGCTCCTCCTGTTTCA	GCGCCCAATACGACCAAATC	122
Vimentin Promoter:			
Region 1	ACCTGTGCCTTTTCAATTGCT	AAATCACTCAATTCAAGGGCTG	135
Region 2	GCTGCGTAGACAGTTTATCCC	ATTTAGGACAGCAATGCACAG	127
Region 3	AATTGGGGTTTTTAAGCAACAC	GAGGAAACTCGACGTCCGTA	130
Region 4	AAAACCTTCCCGGTGCAAT	TTTGCTCGAATGTGCGGACTT	103
Region 5	AACTTAGGGGCGCTCTGTCC	TTTAGGGGAAACCGTTAGACCAG	146
Region 6	GCAACTCCCGCCGTCGAA	GCGCCTCTGTCCATCGACT	105
Region 7	CCTGAGCCCTGCGTTCCTG	AGGAAAGAGTAAGGAATCCCTC	112
Region 8	GACCCCTCTGTTTCAGTCCC	GGCATGGTCCCGTTACTTCA	145
Region 9	CGCTAGGTCCCTATTGGCTG	GAGGACTGGACCCCGCCGAG	109
Region 10	GTCCAGTCTCTGCCACTC	TCCCGGAGAAGAGGCGAAC	103
GAPDH2	TACTAGCGGTTTTACGGGCG	TCAACAGGAGGAGCAGAGAGCGA	166

the migration and invasion capabilities by altering expression of URI and vimentin in HeLa and C33A cervical cancer cells. We also investigated the relationship between URI and vimentin during cervical cancer metastasis. Our results supported that URI promotes the migration and invasion of cervical cancer cells at least in part by a mechanism relating to URI upregulation of vimentin expression.

### Materials and methods

#### Antibodies and reagents

The western blot polyvinylidene fluoride membranes (0.22  $\mu$ m) were probed with the following antibodies: primary antibody to RMP/URI (5844S) was purchased from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Catalog: D120074) and vimentin (Catalog: RLM-3145) were purchased from Ruiyingbio (China). The secondary antibodies containing anti-rabbit IgG (Catalog: AB-10058) and anti-mouse IgG (Catalog: D111050) were obtained from Sangon Biotech (China). For chromatin immunoprecipitation (ChIP) assay, the anti-RMP/URI antibody (Catalog: 5844S, CST) and Anti-C19orf2 antibody (Catalog: ab72134, Abcam) were both used in this study. Dual-Luciferase Reporter Assay System (Catalog: E1960, Promega). EZ-Magna Chromatin Immunoprecipitation Kit (Catalog:

17-10086) was purchased from Millipore. TGF- $\beta$  (Catalog: 240-B) was purchased from R&D Systems company and dissolved at a concentration of 500 ng/ml for storage. Matrigel Basement Membrane Matrix (Catalog: 3562-34) was purchased from Becton Dickinson company.

#### Cell culture

Three established cell lines were used in this study. The HeLa cells were gifts from Professor Wei Zhu at Jiangsu University. The C33A

cells (ATCC HTB-31) were purchased from Shanghai Institute of Biochemistry and Cell Biology in China. The 293T cells were supplied by Genepharma company (Shanghai, China). All cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Corning, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, New Zealand) and 1% penicillin/streptomycin (Invitrogen) and cultured at 37°C in a humidified incubator containing 5% CO<sub>2</sub>.

#### Plasmids and cell transfection/treatment

Small interference RNA (siRNA) sequences for human URI and vimentin are listed as the following, URI siRNA: AGAAGGUAGAUAAUGACUAAUUGC. Vimentin siRNA: GCAGGAUGAGAUUCAGAAUUAU. Scrambled control sequence: CGUAAUUCGCGUAUAAUACGCGUAT. All sequences were synthesized by Origene Technologies. For URI overexpression, PCMV6-URI and its control plasmid, PCMV6 (Origene) were transfected into cells. For the analysis of vimentin promoter activity, a 2000 bp upstream fragment from the transcription start site of human vimentin (ID: 7431) promoter was cloned into pGL3/Basic firefly luciferase vector and verified by enzyme digestion and sequencing. Cells were plated on 6-well plates and grown in DMEM supplemented with 10% FBS for 24 hours prior to transfection. Gene knockdown experiment was performed by incubation of 4  $\mu$ M siRNA in 250  $\mu$ l of Opti-MEM media (Invitrogen, Carlsbad, CA) with 10  $\mu$ l of Highperfect transfection

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reagent (Qiagen, USA). For plasmids transfection, cells were transfected with lipofectamine2000 transfection reagent (Catalog: 3778-150, Thermo Fisher Scientific, USA) according to the manufacturer provided protocol. After 20 mins incubation at room temperature for complex formation between the vectors and lipofectamine2000 transfection reagent, the complex was dropped into media and shaken gently. Cells were cultured at 37°C in a humidified incubator containing 5% CO<sub>2</sub> for 24 to 48 hours according to different experimental requirements. To induce vimentin expression, cells were treated with TGF-β which was diluted in serum-free DMEM at a final concentration as indicated.

### *RNA extraction, complementary DNA synthesis and real-time PCR analysis*

Total RNAs were isolated from cells using Trizol reagent (Invitrogen, Carlsbad, CA), and then reverse transcribed with the iScriptcDNA synthesis kit (Bio-Rad, Hercules, CA) following manufacturer's protocol. The complementary DNA product was amplified using sequence specific primers (**Table 1**) and analyzed by real-time PCR using SYBR green detection and performed under standard protocol. Relative RNA quantities were standardized to levels of GAPDH RNA and then calculated using the 2<sup>-ΔΔCt</sup> method.

### *Western blotting analysis*

Whole-cell extracts were prepared in RIPA buffer with 10% phosphatase inhibitor and 10% protease inhibitor cocktail (KangChen, Shanghai, China) and centrifuged at 12,000×g for 15 mins. Protein concentrations were measured using the BCA-assay approach (Eppendorf, Hamburg, Germany). Immunoblotting was performed using specific primary antibodies, and immune-complexes were incubated with the HRP-conjugated secondary antibody and then detected using enhanced chemiluminescence system (Minichemi, China). To ensure equal loading, blots were stripped and re-probed with a specific antibody recognizing GAPDH. Three independent experiments were performed.

### *Migration and invasion assays*

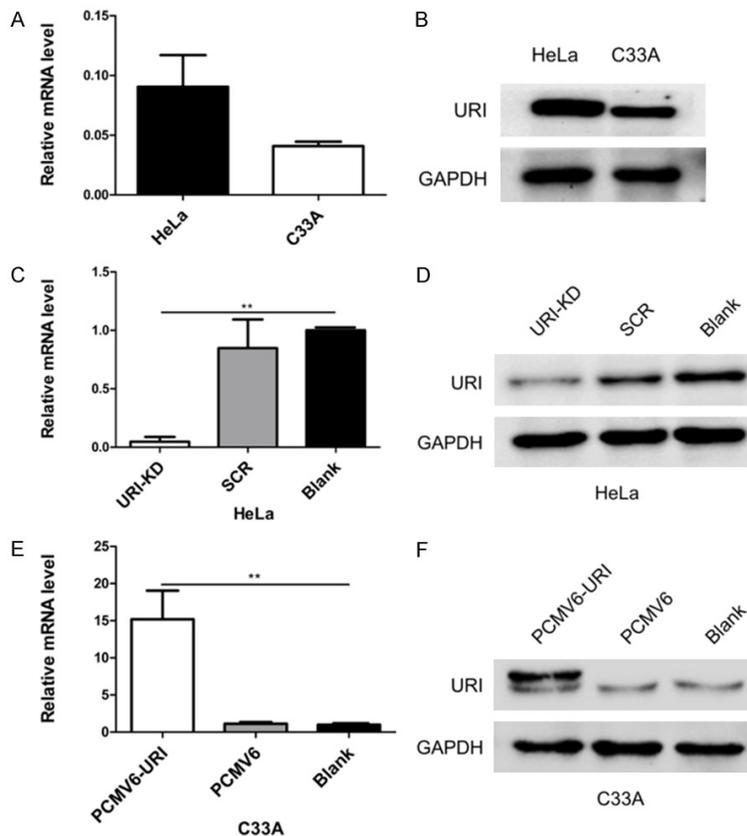
For the migration assay, transwell permeable inserts with 8 mm sizedpores (Corning Inc.,

Corning, NY, USA) were used in a 24-well transwell chamber (Corning Costar, New York, USA). In total, 600 μl complete medium was added to the lower chamber, HeLa cells (1×10<sup>5</sup>) or C33A cells (2×10<sup>5</sup>) were suspended in 200 μl serum-free DMEM and cultured in the upper transwell chamber for 24 hours at 37°C. The non-migrated cells attached to the upper surface of the membrane were removed with a sterile cotton swab, followed by washing with PBS. The migrated cells on the bottom surface of membrane were fixed with 4% paraformaldehyde solution for 30 mins and stained with 0.5% crystal violet for 30 mins at room temperature. For invasion assay, the upper compartment of an 8-mm transwell (Corning Costar, New York, USA) was coated with Matrigel (1 mg/mL) and cultured in a 37°C incubator for 4 hours before starting the invasion assay. Next steps are similar to the migration assay. The number of migrated and invaded cells was calculated under microscope at 5 random fields (200×).

### *Luciferase reporter assay*

293T cells (2.5×10<sup>5</sup>) were seeded in 24-well plates, cultured at 37°C with 5% CO<sub>2</sub> the day before transfection assay. Transient transfection was accomplished with 0.1 μg pGL3-Luc reporter plasmid containing a -2000/+1 bp region of the human vimentin proximal promoter, 0.1 μg of pRL-TK plasmid encoding Renillaformis luciferase, 0.8 μg URI expression (PCMV6-URI) or empty (PCMV6) plasmids. Plasmids and lipofectamine2000 transfection reagents were diluted in serum free medium respectively. 5 mins later, plasmids were mixed with the transfection reagents softly and placed at room temperature for another 20 mins, then the medium was aspirated as much as possible and 400 μl DMEM was added with 10% FBS and the transfection mixture was dropped into every well gently. 6 hours later, the solution was removed and washed using D-Hank's solution before adding 1.5 ml new complete culture medium. After 24 hours' transfection, luciferase activity was detected using Dual-Luciferase Reporter Assay System according to the manufacturer's instructions. The relative luciferase activity is expressed as fold induction of the test plasmid activity compared with the corresponding empty vector (pGL3/Basic, Promega). Each plasmid was assayed in triplicate in three separate experiments.

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**Figure 1.** URI expression and transfection studies in two cervical cancer cell lines. (A, B) Expression of URI in human cervical cancer cell lines was examined by quantitative real-time RT-PCR (A) and western blot (B). URI showed higher expression in HeLa than in C33A cervical cancer cells. (C, D) URI gene knock-down in HeLa cells Transient transfection was performed in HeLa cells using URI siRNA and controls Compared with the blank and scrambled controls, cells transfected with URI siRNA showed significantly decreased URI mRNA (C) and protein (D) as measured by qRT-PCR and western blot and normalized to GAPDH. (E, F) URI overexpression in C33A cells Transient transfection was performed in C33A cells using URI expression and control plasmids Significantly increased URI mRNA (E) and protein (F) levels were detected in cells transfected with pCMV6-URI compared with the blank and pCMV6 controls as demonstrated by qRT-PCR and western blot analysis and normalized to GAPDH. GAPDH was used as an internal control. Error bars represent SEM from at least three independent experiments, \*\* $P < 0.01$ .

### Chromatin immunoprecipitation (ChIP) assay

All ChIP experiments were performed using Millipore EZ-Magna ChIP kit according to the manufacture's protocol. Briefly, HeLa and C33A cell cultures (150-mm diameter plates) were cross-linked with 1% formaldehyde and gentle agitation for 10 mins at room temperature. The cross-linking was then quenched with 0.125 M glycine for 5 mins. Then cells were washed with cold PBS for twice. The following experimental steps were performed on ice or at 4°C. Cells were scraped off into PBS containing protease inhibitor and spinned at 800×g for 5 mins. Cell pellets were resuspended in the lysis buffer for

15 mins on ice. Finally, the chromatin was sonicated in the lysis buffer to obtain DNA fragments at around 300-500 bp. The sonicated samples were centrifuged at 12,000×g for 10 mins and ChIP DNA was extracted as per the kit protocol. Chromatin was pulled down using the following antibodies: anti-RNA Polymerase (For the positive control), Normal Mouse IgG (for the negative control), anti-RMP/URI antibody and Anti-C19orf2 antibody. 4 μg of each antibody was used for ChIP assay. After chromatin precipitation, we performed quantitative PCR using ten sets of primers from vimentin promoter as described in **Table 1**. The ratios for the fold-enrichment of URI were calculated with anti-IgG mouse antibody.

### Statistical analysis

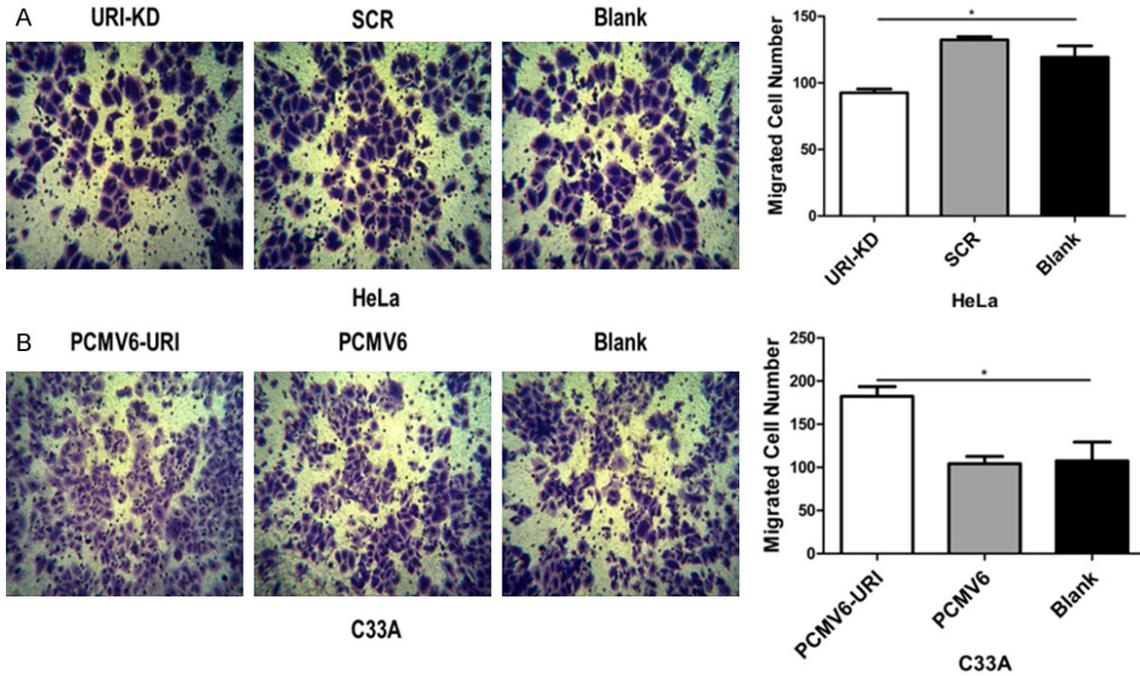
All statistical analyses were performed using GraphPad Prism software version 5.0 for Windows (La Jolla, CA, USA). Statistical significance in different groups was assessed using a one-way analysis of variance (one-way ANOVA). All data were presented as the mean ± SEM of triplicate independent experiments.  $P$ -values <0.05 were considered statistically significant.

## Results

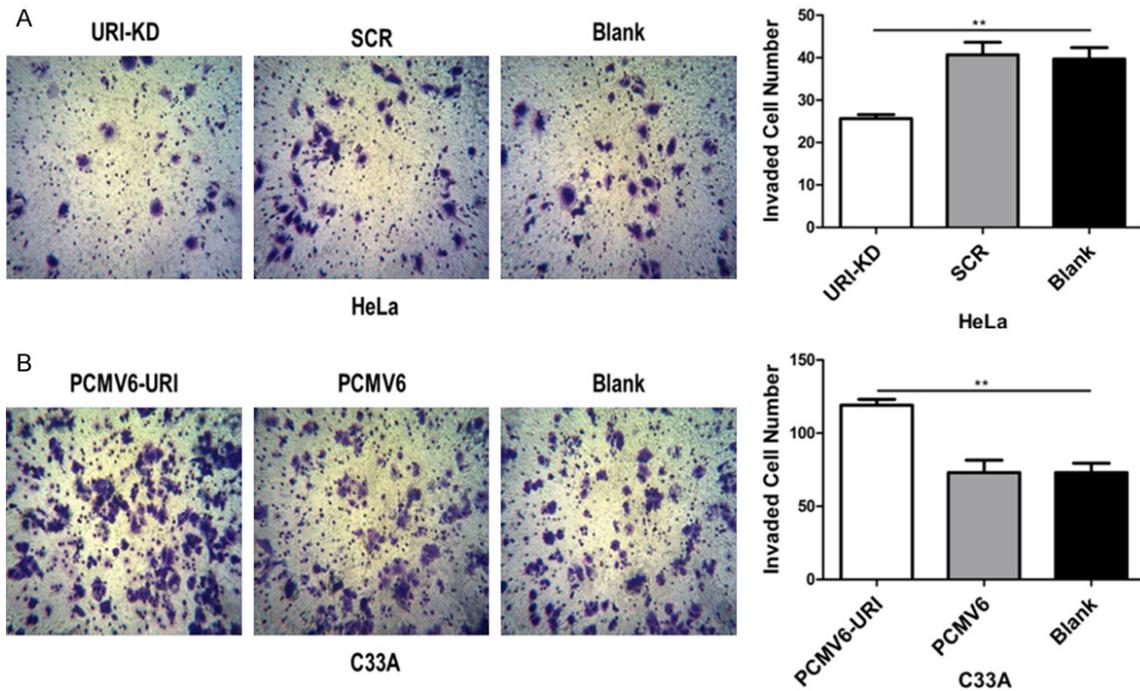
### URI promotion of cervical cancer cell migration and invasion in vitro

URI mRNA and protein levels were detected in two cervical cancer cell lines using qRT-PCR and western blot, and HeLa cells showed a higher basic level expression of URI than C33A cells (**Figure 1A** and **1B**). URI siRNA transfection was hereby applied for silencing of URI in HeLa cells (**Figure 1C** and **1D**) and pCMV6-URI plasmid transfection was applied for overexpression of exogenous URI in C33A cells (**Figure 1E** and **1F**). Transwell (without Matrigel) migra-

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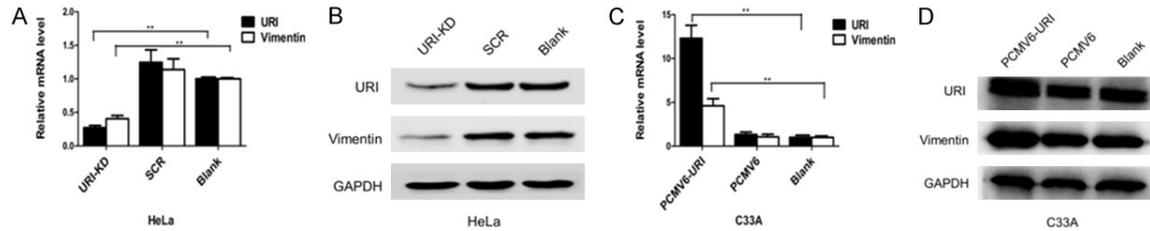
**Figure 2.** The effect of URI on migration of cervical cancer cells. Cells were reseeded into transwell chamber (without Matrigelcoated) after 48 hours for transfection and cultured for another 24 hours, and then stained with crystal violet. Compared with blank and scrambled controls, more number of migrated HeLa cells on the filter surface was seen in the group with URI gene knockdown (A). The number of migrated C33A cells on the filter surface significantly increased in the group transfected with URI expression plasmid (pCMV-URI) compared with the blank and empty vector (PCMV6) control groups (B). Bar graph represented the number of migrated cells (right panel). The results represent mean of three independent experiments. Data was expressed as mean  $\pm$  SEM of three separated experiments, \* $P < 0.05$ .



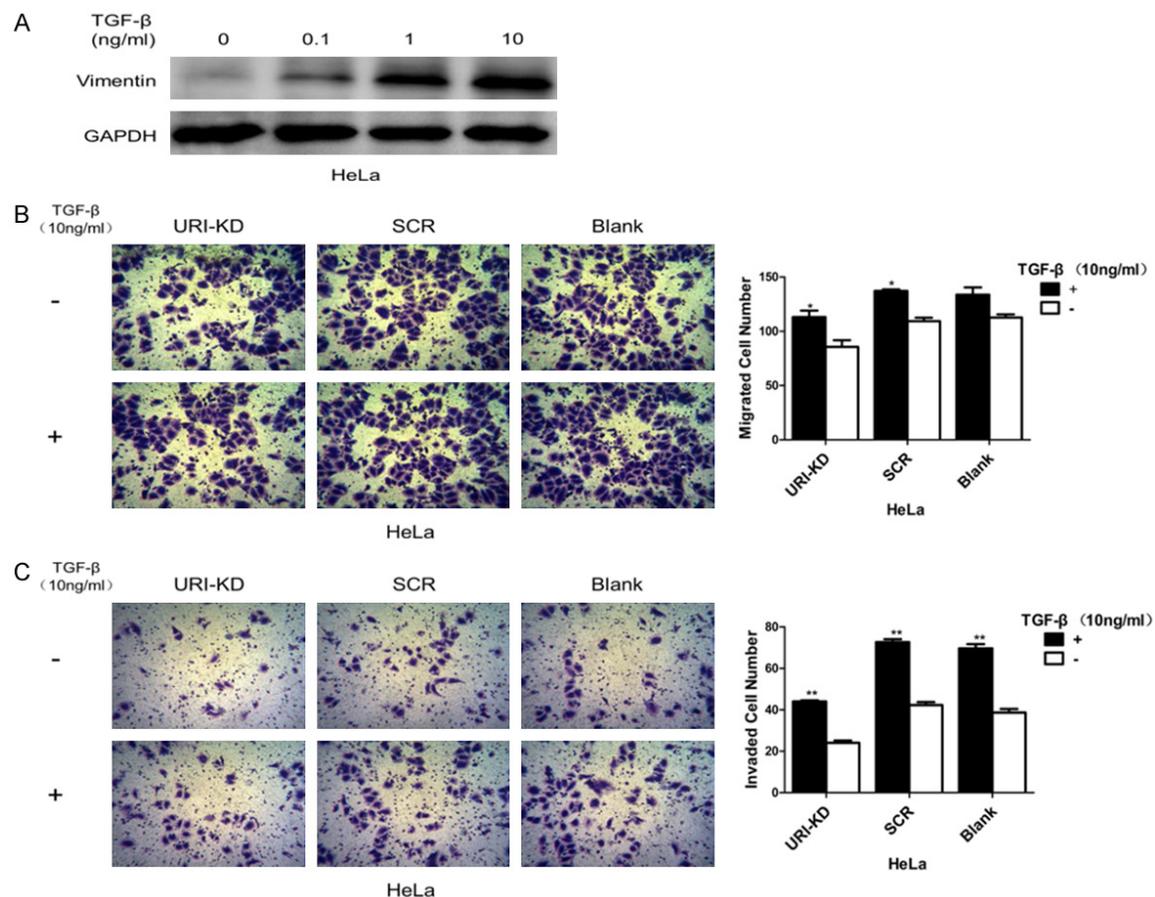
**Figure 3.** The effect of URI on invasion of cervical cancer cells. At 48 hours after transfection, cells were reseeded into transwell chamber (with Matrigelcoated) for 24 hours and then stained with crystal violet. Down-regulation of URI significantly decreased the invaded ability of HeLa cells. The cells transfected with URI siRNA (URI-KD) were

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much less than the ones transfected with scrambled sequence (SCR) and blank groups (A). Cells in URI overexpression (PCMV6-URI) group were more than that of control (PCMV6 and Blank) groups (B), indicating that Up-regulation of URI promoted invasion of C33A cells. Bar graph represented the number of invaded cells (right panel). All the experiments were performed in triplicate. The mean value of invaded cells was shown in right panel. Data was mean  $\pm$  SEM, \*\* $P < 0.01$ .



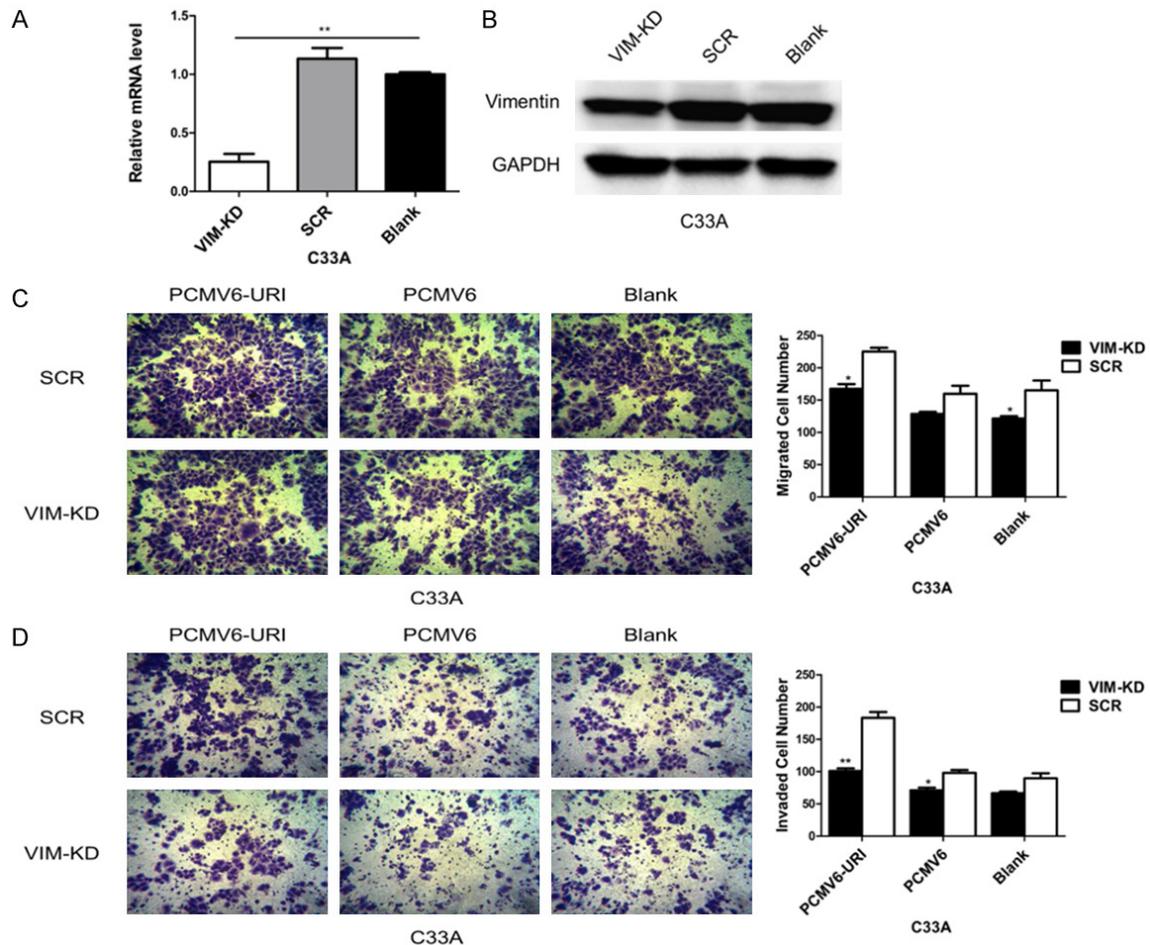
**Figure 4.** URI regulates vimentin expression in vitro. (A, B) URI knock-down in HeLa cells decreased vimentin expression. vimentin showed decreased expression in HeLa cells transfected with URI siRNA compared with the scrambled and blank control groups as detected by qRT-PCR (A) and western blot (B). (C, D) Overexpression of URI increased vimentin expression in C33A cells. vimentin mRNA (C) and protein (D) levels increased in cells transfected with pCMV-URI expression plasmid compared with the empty vector pCMV6 and blank control groups. The mRNA and protein levels were quantified relative to the loading control (GAPDH). Error bars represent SEM from at least three independent experiments, \*\* $P < 0.01$ .



**Figure 5.** URI-knockdown attenuated cell motility of HeLa cells mediated by vimentin. (A) vimentin expression induced by TGF- $\beta$ . Immunoblotting for vimentin in HeLa cells showed that TGF- $\beta$  dose-dependently increase vimentin expression. Transwell assay (with or without Matrigel coated) was conducted in HeLa cells at 48 hours after transfection of URI siRNA or scrambled sequence and treated with or without 10 ng/ml TGF- $\beta$ . Cells were reseeded into chamber and cultured in 24-well plates for 24 hours and then stained with crystal violet. The number of migrated (B) and invaded (C) cells in URI knockdown (URI-KD) group with 10 ng/ml TGF- $\beta$  treatment is higher compared with

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that without TGF- $\beta$  treatment. Bar graph represented the number of migrated or invaded cells (right panel). All the experiments were performed in triplicate. The mean value of migrated cells was shown in right panel. Data was mean  $\pm$  SEM, \* $P$ <0.05, \*\* $P$ <0.01.



**Figure 6.** vimentin-knockdown decreased cell motility of C33A cells mediated by URI. Transfection of vimentin specific siRNA in C33A cells leads to significantly decreased vimentin mRNA (A) and protein (B) expression compared with the scrambled and control groups as detected by qRT-PCR and western blot. For transwell assay (with or without Matrigel coated), cells were transfected PCMV6-URI or PCMV6 vectors and treated with vimentin siRNA or scrambled sequence for 48 hours. Cells were reseeded into chamber and cultured in 24-well plates for 24 hours and then stained with crystal violet. Both the migrated (C) and the invaded (D) cells with or without URI overexpression were decreased with vimentin knocked down (VIM-KD). Bar graph represented the number of migrated or invaded cells (right panel). Analysis was performed as mean of cells numbers  $\pm$  SEM for three independent experiments. \* $P$ <0.05, \*\* $P$ <0.01.

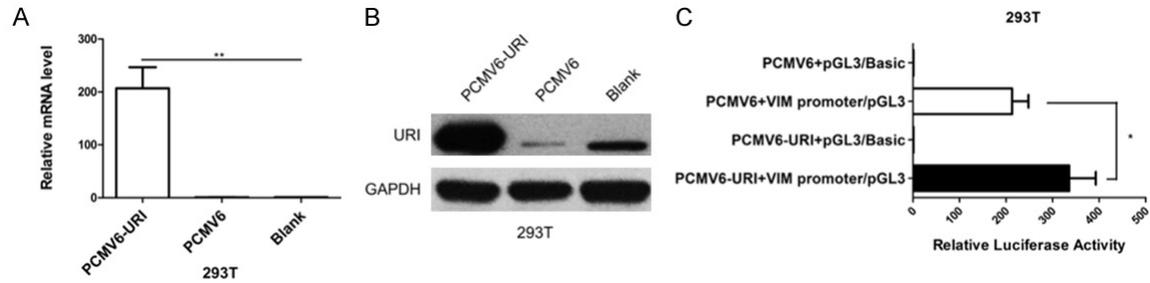
tion assay showed that fewer cells migrated through the chamber in URI knockdown of HeLa cells than in control cells (Figure 2A), and the number of migrated cells was higher in URI overexpressed C33A cells than in control cells (Figure 2B). To explore whether URI affects invasion ability of cervical cancer cells, we performed transwell (with Matrigel) invasion assay and the results showed that the cell number invaded through the matrigel was significantly decreased in the URI knocked-down HeLa cells

(Figure 3A), and significantly increased in URI force-expressed C33A cells (Figure 3B) respectively compared to the control groups.

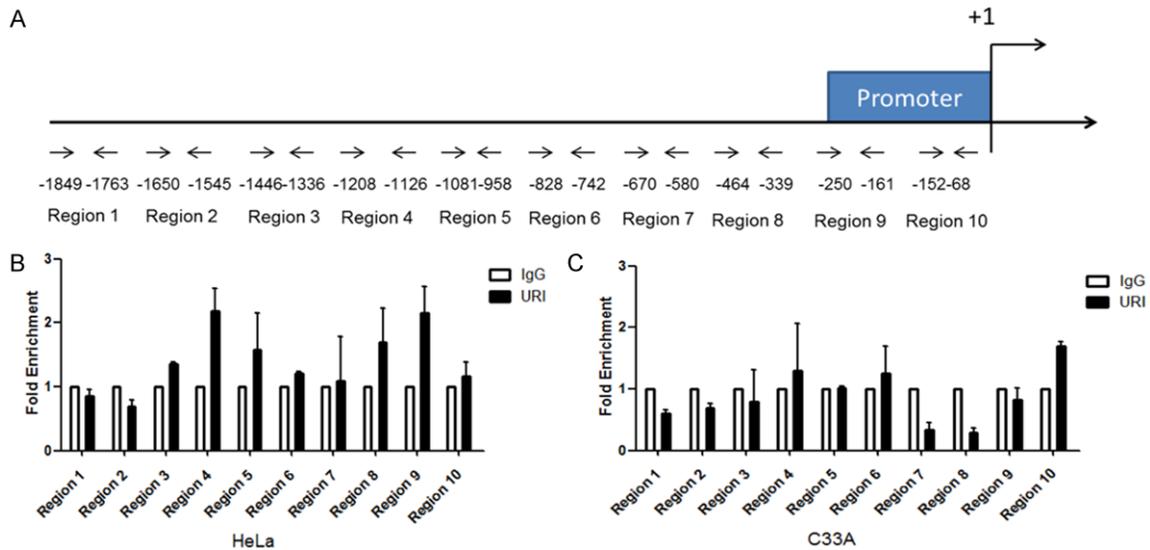
### *Attenuation of TGF- $\beta$ -enhanced HeLa cell migration and invasion after URI knockdown*

As illustrated in Figure 4A and 4B, vimentin mRNA and protein expression decreased after URI was knocked down in HeLa cells, while forced URI expression in C33A cells increased

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**Figure 7.** URI invigorates vimentin promoter activity. 293T cells were transfected with pCMV6-URI or pCMV6 plasmids for 24 hours and URI mRNA (A) and protein (B) levels were detected using qRT-PCR and western blot. URI was weakly expressed in 293T cells as shown in the groups of pCMV6 and blank controls. URI significantly increased after cells were transfected with URI expression plasmid pCMV-URI. GAPDH was used as an internal control. Error bars represent SEM from at least three independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ . (C) Vimentin promoter activity was determined using the luciferase reporter assay. Co-transfection of pCMV6-URI into 293T cells significantly increased the luciferase activity mediated by vimentin promoter/pGL3 or pGL3/Basic plasmids.



**Figure 8.** Chromatin immunoprecipitation (ChIP) assay in two cervical cancer cells. A. Respective regions of human vimentin promoters were as illustrated. B. Chromatin immunoprecipitation (ChIP) analysis in HeLa cervical cancer cells. C. Chromatin immunoprecipitation (ChIP) analysis in C33A cervical cancer cells. The data exhibited for the real-time PCR ratios for the fold-enrichment of URI at different regions of vimentin promoter. GAPDH was used as internal reference, mouse anti-IgG antibody was used as a control. No significant enrichment was detected for either of the vimentin promoter regions analyzed. Error bars represent SEM from at least three independent experiments.

vimentin mRNA and protein expression (**Figure 4C** and **4D**). As vimentin is a widely recognized marker of the EMT-like phenotype, which can be induced by EMT inducer TGF- $\beta$  [13], we then used TGF- $\beta$  to induce vimentin expression in HeLa cells to determine whether URI is related to vimentin in promoting cell migration and invasion. The results showed that TGF- $\beta$  can induce the expression of vimentin in a dose-dependent manner (**Figure 5A**). URI knockdown in HeLa cells supplied with 10 ng/ml TGF- $\beta$  increased the number of cells migrating and

invading through the chamber compared with control groups (**Figure 5B** and **5C**).

### *URI promotion of vimentin knockdown-attenuated C33A cell migration and invasion*

By siRNA assay, vimentin mRNA and protein expression was reduced in C33A cells (**Figure 6A** and **6B**). Migration and invasion assays showed that knockdown of vimentin reduced the cell number of migration and invasion but promoted by overexpressed URI (**Figure 6C** and

**6D**). These data suggested that URI promoted cell migration and invasion mediated to a certain extent via vimentin in cervical cancer cells.

### *URI upregulation of vimentin transcription indirectly through enhancing the promoter activity*

Transcription factors or cofactors affect mRNA transcription through influencing target gene promoter activity [14]. We carried out a dual luciferase reporter gene experiments. The over-expressed URI mRNA and protein were detected in PCMV6-URI transfected 293T cells (**Figure 7A** and **7B**). The vimentin promoter activity increased about 1.57 fold in PCMV6-URI and vimentin promoter/pGL3 cotransfected 293T cells than in PCMV6 transfected group, indicating that URI enhanced vimentin promoter activity (**Figure 7C**). Furthermore, 10 pairs of primers were designed according to 2000bp upstream of vimentin promoter region (**Table 1** and **Figure 8A**) and a ChIP experiment was conducted both in HeLa and in C33A cells. However, there were no statistical differences compared with control group in two cells (**Figure 8B** and **8C**). This indicated that the transcriptional regulation of URI on vimentin was likely to be through an indirect manner.

## Discussion

### *URI promotes migration and invasion of cervical cancer cells possibly via vimentin*

Our studies confirm that knockdown or overexpression of URI inhibited or promoted the migration and invasion of cervical cancer cell lines. Although we have recently shown that URI can induce vimentin expression in cervical and gastric cancer cells [12, 15], it is not clear whether URI regulates cell migration and invasion associated with vimentin. Vimentin is a member of the type III intermediate filament (IF) protein family, which expressed in endothelial and other mesenchymal cells. As one of the key components of the cellular cytoskeleton, vimentin played an important role in maintaining cell shape and integrity and was also involved in cytoskeletal interactions such as adhesion and migration [16]. Aberrant expression of vimentin has been found in several tumors and is considered as a marker of cancer prognosis and a potential molecular target for cancer treatment [17-20]. EMT is a cellular process associated with cancer invasion and

metastasis in cervical cancer and other tumors [21, 22]. Vimentin, as a cytoskeletal protein is closely related to cell adhesion, migration, and invasion, and thus caused tumor metastasis [23-25]. Vimentin is now recognized not only as a canonical marker but also as a driver of epithelial-mesenchymal transition (EMT) [26]. TGF- $\beta$  has been identified as important inducer of EMT during development as well as during carcinogenesis, which prominently induces EMT via a group of specific transcription factors [27, 28]. In the present experiments, TGF- $\beta$  enhanced cell migration and invasion capacities inhibited by URI knockdown in HeLa cells, whereas siRNA-mediated silencing of vimentin attenuated the migration and invasion capacities enhanced due to overexpression of URI in C33A cells. These results confirm that the effect of URI on cell motility and invasion is related to vimentin, as well as EMT.

### *Vimentin may be an indirect target for URI*

It has been found that URI is able to regulate IL-6 transcription in hepatocellular carcinoma and multiple myeloma [6, 7]. In our previous work, we have shown that in cervical and gastric cancer cells, URI upregulated vimentin expression at both mRNA and protein levels, but the mechanism of URI-mediated vimentin expression is not known. To determine whether URI regulates vimentin transcription associated with the region of the vimentin promoter, we performed a luciferase reporter assay in 293T cells, which has a high transfection efficiency. Our results showed that overexpression of URI enhanced luciferase activity. In order to verify whether the URI-dependent regulation of vimentin depends on the ability of URI binding to its promoter, ChIP experiments were performed in HeLa cells and C33A cells. Although URI functioned as a regulator for vimentin transcription by activating its promoter, there is no sufficient evidence to show that URI acts as a transcription factor directly binds to the promoter of vimentin. Vimentin may be an indirect target for URI in two cell lines. Based on above facts, we assumed that, as a molecular chaperone, URI may regulate the expression of vimentin through other transcription factors or signal transduction pathway, although the mechanism remains to be explored. URI has been shown as a phosphorylated target of the mTOR/S6K1 pathway and contributes to rapamycin-

sensitive transcription [29, 30]. TGF- $\beta$ -induced EMT is also regulated by activation of the mTOR pathway [31]. Therefore, URI regulates vimentin expression and EMT through the mTOR pathway may not be excluded.

Taken together, our data suggest that URI promotes cervical cancer cell motility mediated at least in part by upregulation of vimentin expression, and by activating indirectly its promoter activity. URI knockdown accompanied by silencing of vimentin would reduce the potential for migration and invasion in cervical cancer cells, as well as metastasis in cervical cancer.

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### Disclosure of conflict of interest

None.

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