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Original Article

Curcumin reverse cigarette smoke extract-induced human bladder epithelial-mesenchymal transition via suppresses NF-κB signaling in vitro

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Abstract: Epithelial-mesenchymal transition (EMT) is a crucial pathophysiological process in cancer development. Curcumin is a promising chemopreventive agent for several types of cancers. Cigarette smoke (CS) is a crucial factor for bladder cancer, but the role of NF-κB signal pathway in the development of CS-associated BC was not fully elicited. In the present study, SV-HUC-1 cell line is chosen as the suitable model for drug targeting. Meanwhile, the cells were treated with different dose of CSE for 7 days. Protein level and gene expression were analyzed by western blotting and RT-PCR. The cells migration and invasive capacity were analyzed by wound healing and transwell assays. Results showed that exposure of SV-HUC-1 cells to CSE resulted in activation of NF-κB pathways. CSE reduced mRNA and protein expression levels of epithelial markers E-cadherin and ZO-1, while mRNA and protein expression levels of the mesenchymal markers Vimentin and N-cadherin were increased. Pre-treatment of NF-κB inhibitor (PDTC) reversed CSE triggered cells EMT. Curcumin treatment effectively attenuated CSE-triggered activation of NF-κB pathways and EMT alterations in human bladder epithelial cells. Our findings provide new insight into the molecular mechanisms of CS-associated bladder tumorigenesis and may open up new avenues in the search for potential target of bladder cancer intervention.

Keywords: Bladder cancer, cigarette smoke extract, NF-κB pathway, epithelial-mesenchymal transition (EMT) curcumin

Introduction

Bladder cancer (BC) is becoming the leading cause of cancer death in the urinary malignancies, with 42,9793 new cases diagnosed and 16,5084 deaths each year in worldwide [1-5]. Cigarette smoking (CS) is one of the most important risk factors of bladder cancer in humans. Epidemiological and experimental studies have revealed the positive link between CS and risk of bladder cancer development as well as other cancers [6-8]. Cigarette smokers have an approximately three-fold higher risk of bladder cancer than non-smokers [9]. Although enormous progress in understanding its molecular mechanisms leading to bladder cancer development has been made, the molecular pathogenesis remains largely unknown [10].

The epithelial-mesenchymal transition (EMT) is a process that the epithelial cells lose their polarization and junctions between cells, meanwhile with the changed cell shape. The cells become more migratory, invasive, resistance to apoptosis and have the stem cell-like characteristics [11, 12]. EMT involves the loss expression of epithelial markers, such as E-cadherin, ZO-1 and cytokeratins, while an increased expression of mesenchymal markers, such as N-cadherin, Vimentin and fibronectin [13-15]. Previous studies showed that exposure of cells to carcinogens could induce EMT during malignant transformation and also CSE has been documented to promote the EMT which regulates early events in bladder carcinogenesis [16-18]. However, the underlying mechanisms by which CSE induces EMT are poorly understood.

The NF-κB pathway is implicated in important cellular processes, including gene expression, proliferation, apoptosis, angiogenesis, cell motility, and differentiation [19, 20]. While some reports have suggested the function of NF-κB in cancer oncogenesis, its role in EMT regulation has not been well explored. NF-κB pathway triggers a motility and invasive phenotype of gastric cancer cells [21], mammary epithelial cells [22] and breast cells [23]. However, there have been few studies reporting the role of NF-κB pathway in mediating EMT in normal human epithelia bladder cells.

Curcumin as the principle active component of the plant curcuma longa, is well known to posses anti-inflammatory, antioxidant, and antimicrobial activities [24], and has been studied extensively as a chemopreventive agent in a variety of cancers, including those of the breast, liver, prostate, gastrointestinal, and colorectal cancers and as an inhibitor metastasis [25, 26]. But, no reports were regarded the contribution of curcumin in normal human bladder epithelia cells migration.

To data, no studies have been done to examine the action of NF-κB pathway in CSE-induced the EMT of human bladder epithelial cells. Therefore, our study was designed to investigate it. Meanwhile, the preventive effects of curcumin against CSE-induced EMT were also investigated. Finding from this study, we try to demonstrate how CSE induce carcinogenesis in bladder.

Meterials and methods

Materials

An SV-40 immortalized human urothelial cell line (SV-HUC-1) was purchased from the Chinese Academy of Typical Culture Collection Cell Bank. F12K medium was purchased from Gibco (New York, NY, USA). Fetal bovine serum (FBS) was obtained from PAA Laboratories (Pasching, Austria). Curcumin was purchased from Sigma (St Louis, MO, USA, purity: 99.0%). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. P-IKKα/β, IκB, p65, E-cadherin, N-cadherin and vimentin antibodies were all purchased from Cell Signaling Technology (Beverly, MA, USA). P50, ZO-1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primers were synthesized according to published sequences from Invitrogen (Carlsbad, CA, USA). Ammonium pyrrolidinedithiocarbamate (PDTC) was purchased from Beyotime (Shanghai, China). Nuclear and cytoplasmic protein extraction kit was purchased from (Nanjing, China). Sources of other materials are noted accordingly in the text.

Cell culture and treatment

SV-HUC-1 cells were cultured under an atmosphere of 5% CO_2 at 37°C in f12K medium containing antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). The medium was changed every other day, then were reached 80%-90% confluence, then treated with various concentrations of cigarette smoke extract or with PDTC (2 μM) and curcumin (10 μM) for 7 days.

Preparation of CSE

CSE was freshly prepared for each experiment by combusting one commercial cigarette according to the reported method. Commercial cigarettes (Hongtashan filter-tipped cigarettes made in Yunnan, China; each contain 12 mg tar and 1.1 mg nicotine) were smoked. By application of a vacuum, mainstream smoke was drawn through 10 ml of prewarmed (37°C) FBS-free F12K medium supplemented with penicillin and streptomycin at a rate of 5 min/cigarette. The obtained solution was referred to as having 100% strength. Then the CSE stock solution was filtered through a 0.22-μm pore size filter and diluted to the desired concentration with treatment medium. The resulting CSE was applied to epithelial cell cultures within 30 min of preparation. The control solution was prepared using the same protocol, except that the cigarettes were unlit.

Cell toxicity assay

SV-HUC-1 cells were seeded in 96-well plates at a plating density of 2×10^3 cells/well in 200 μl of medium. Then the cells were exposed to various concentrations of CSE prepared as previously outlined for 5 days, and the cell viability was determined by MTT assay. For the present study, media containing various concentrations of CSE were changed every day. Five days later, MTT stock solution was added to each well to solubilize the formazan crystals, and plates were incubated for an additional 4 h at 37°C. Afterwards, MTT solution in the medium was removed and the crystals were solubilized in

dimethylsulfoxide (DMSO). Absorbance was measured at 490 nm using a microplate reader. All measurements were performed in triplicate.

Wound healing assay

SV-HUC-1 cells were pretreated with control medium or medium containing CSE (0.1, 0.25 or 0.5%) for 7 days, and then cells were seeded in 6-well plates and incubated for 24 h in serum-free medium before wounding. The scratch wound was generated on the surface of the plates using a pipette tip. Photographic images were taken from SV-HUC-1 cells at 0, and 24 h.

Transwell assay

The invasion assays were performed in a 24-well Boyden chamber with an 8-μm pore size polycarbonate membrane (Millipore, Billerica, MA, USA) coated without or with Matrigel to form a matrix barrier. A total of 100 μl of serum-free medium (containing 1×10^4 cells) was added to the upper compartment of the chamber, whereas the lower compartment was filed with 800 μL of F12K supplemented with 10% FBS. After incubation at 37°C for 48 h, the SV-HUC-1 cells remaining inside the upper chamber were removed with cotton swabs. The cells on the lower surface of the membrane were stained with 0.1% crystal violet after fixation with methanol and then imaged under a light microscope. Subsequently, the cells were bleached with glacial acetic acid and the absorbance of the eluant was measured at 570 nm.

Western blot analysis

SV-HUC-1 cells were harvested, washed with ice-cold phosphate-buffered saline (PBS) and lysed in RIPA buffer (Thermo Scientific, USA). Concentrations of the precipitated proteins in the cell lysates were measured with BCA protein assay (Pierce, Rockford, IL, USA). Afterwards, the proteins were diluted to equal concentrations, boiled for 5 min and separated by 7.5-10% SDS-PAGE, transferred onto polyvinylidene difloride membranes (Millipore). After blocking with 5% milk, the membranes were blocked and incubated with the indicated primary antibodies and secondary antibodies. The blots were subsequently developed using an enhanced chemiluminescence detection kit (Amersham Biosciences, USA) and exposed to film. GAPDH served as the loading control.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated by RNAiso Plus according to the manufacturer's instructions. (Takara, Japan). Then total RNA was transcribed into cDNA using AMV reverse transcriptase (Takara) following the manufacturer's protocol. QRT-PCR was performed using the Power SYBR-Green Master Mix (Takara) and an ABI 7300 Real-Time PCR Detection System (Applied Biosystems). All of the primers were synthesized by Invitrogen. Respectively, normalization was achieved by dividing the expression level of mRNA by its respective GAPDH expression level. Fold-change in the expression of each gene was calculated by a comparative threshold cycle (Ct) method using the formula $2\text{-}\Delta\Delta Ct$. The primers used were: E-cadherin forward, 5'-TCGACACCCGA TTCAAAGTGG-3' and reverse, 5'-TTCCAGAAACGGAGGCCTGAT-3'; vimentin forward, 5'-CCTTGACATTGAGATTGCCA-3' and reverse, 5'-GTATCAACCAGAGGGAGTGA-3'; Zo-1 forward, 5'-GCAGCCACAACCAATTCATAG-3' and reverse, 5'-GCAGACGATGTTCATAGTTTC-3'; N-cadherin forward, 5'-ATCAAGTGCCATTAGCCAAG-3' and reverse, 5'-CTGAGCAGTGAATGTTGTCA-3'; GAPDH forward, 5'-GCTGCCCAACGCACCGAATA-3' and reverse, 5'-GAGTCAACGGATTTGGTCGT-3'.

Immunofluorescence

After CSE treatment for 4 days, SV-HUC-1 cells were fixed in 4% paraformaldehyde for 15 min at room temperature, and were washed three times with TBSTx. Then, membranes were ruptured with 0.1% TritonX-100 for 30 min. Slides were blocked in 5% BSA containing TBSTx for 1 h at room temperature, and then were incubated with a monoclonal E-cadherin (1:150 dilution) and Vimentin (1:100 dilution) antibodies in 5% BSA overnight at 4°C. After washing with TBSTx, cells were incubated with secondary antibodies for 1 h at room temperature. Finally, cells were counterstained with 4,60-diamidino-2-phenylindole (DAPI) before mounting, then cells were washed three times with TBSTx. Stained cells were mounted with fluorescent mounting medium (Dako, Carpinteria, CA, USA). The fluorescent images were obtained using a confocal laser scanning microscope (LSM700; Carl Zeiss Meditec, Gottingen, Germany).

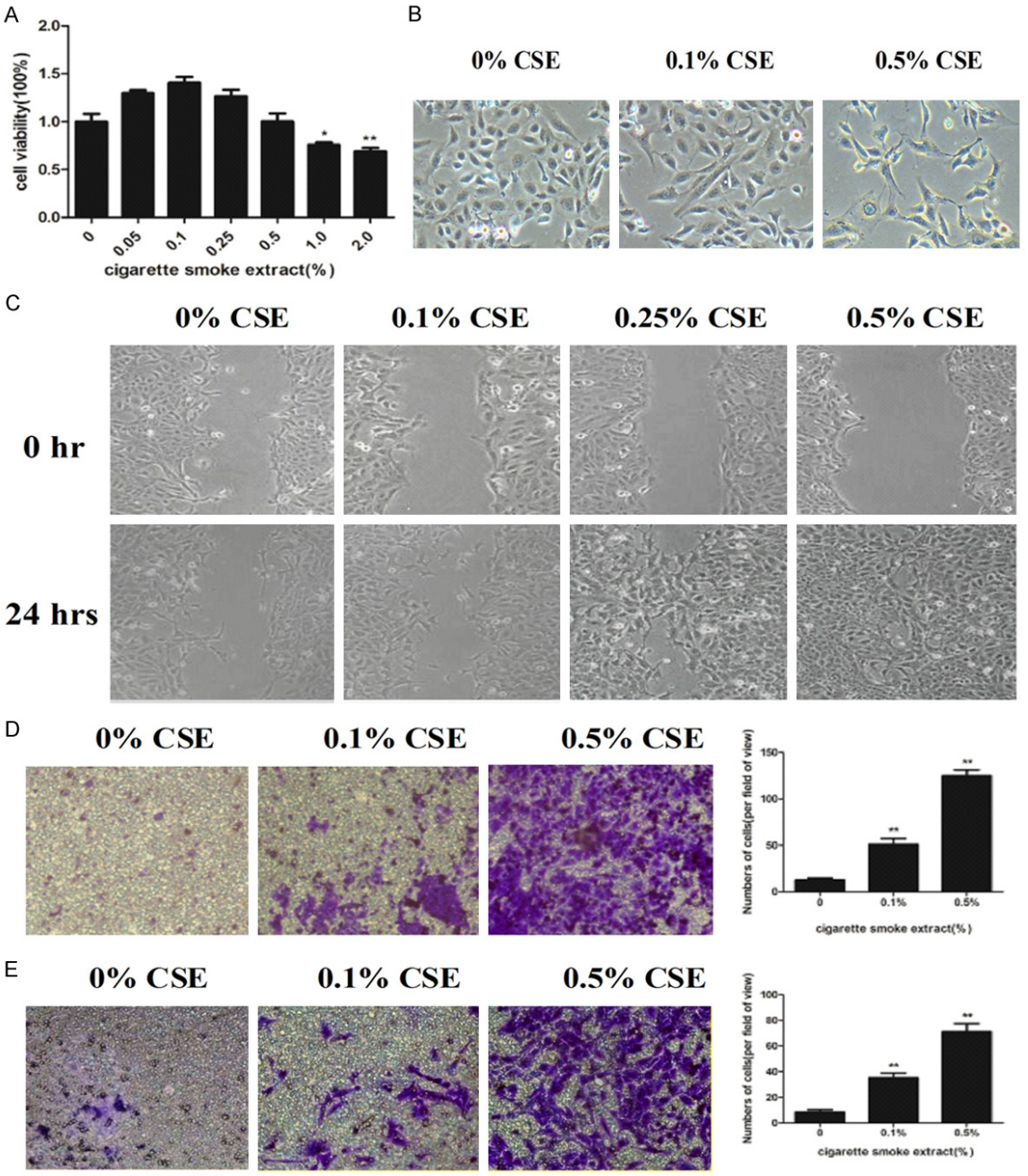

Figure 1. CSE induces EMT morphological change and enhances the invasive capacity of normal human urothelial (SV-HUC-1) cells. A. Effect of cigarette smoke extract (CSE) on the cell viability of SV-HUC-1 cells. The results showed that lower concentrations of CSE (0.05-0.5%) induced mildly enhanced cell viability in the SV-HUC-1 cells. Additionally, cell viability decreased below 80% when cells were exposed to 1% or higher CSE concentrations, which proved to be toxic to the SV-HUC-1 cells. B. CSE induced morphological change from an epithelial to a spindle-like mesenchymal shape, as shown by morphological examination of the SV-HUC-1 cells following CSE treatment for 7 days. SV-HUC-1 cells became longer and thinner, some of which generated a slender tail. C. CSE enhanced migratory capacity of SVHUC-1 cells, as determined by wound healing assay. D, E. CSE enhanced the invasive capacity of the SV-HUC-1 cells, as determined by Transwell invasion assay. The 0.25% CSE and 0.5% CSE groups revealed that CSE had a strong stimulative effect on the invasion capacity of the SV-HUC-1 cells. Similarly, invasion of cells through reconstituted matrigel matrices was enhanced by CSE. The subsequent absorbance assay confirmed this change. Data are expressed as mean ± SD. *P<0.05, **P<0.01, compared with the control group. CSE cigarette smoke extract; EMT epithelial-mesenchymal transition.

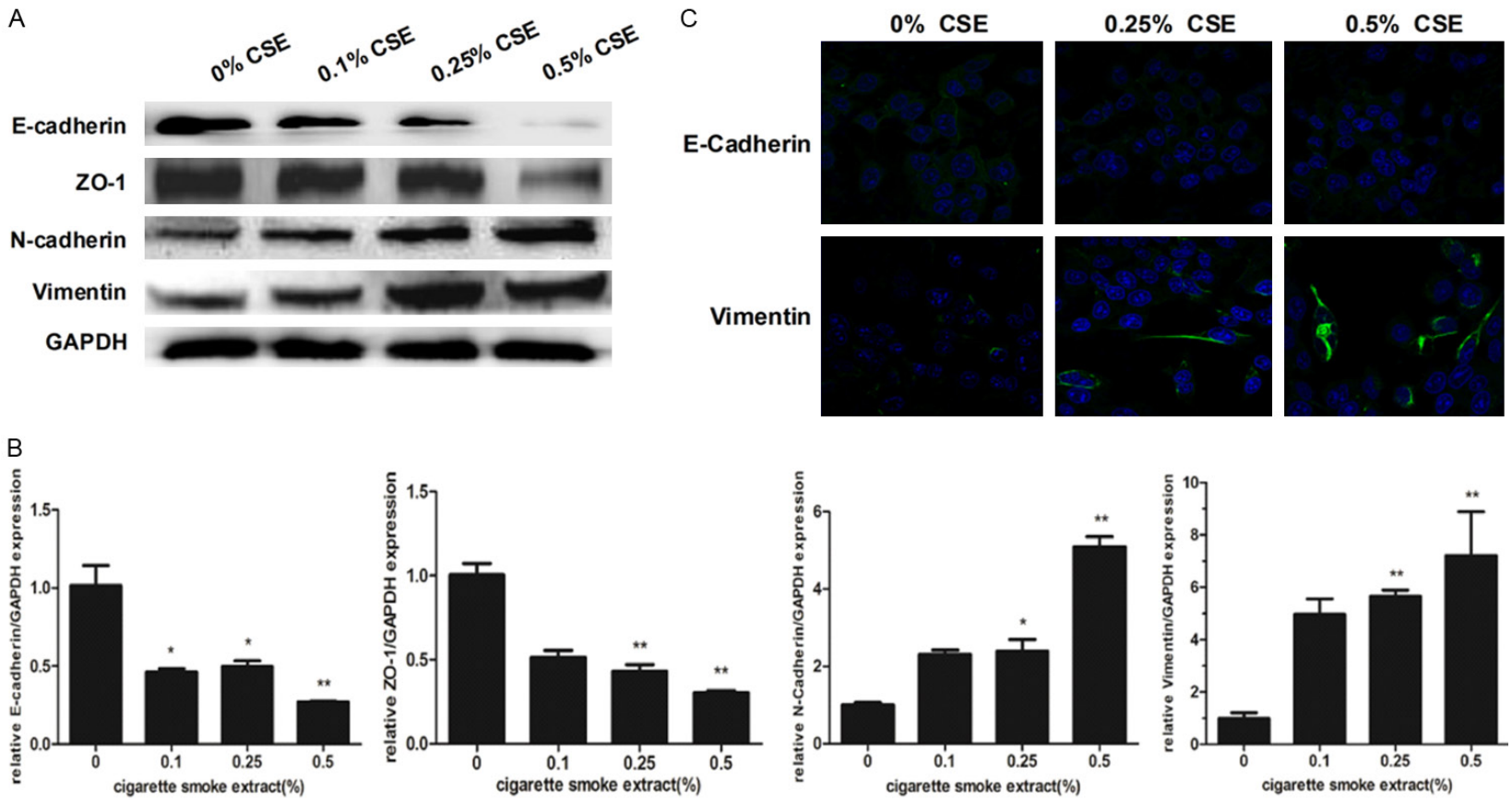

Figure 2. CSE alters the expression of EMT markers in SV-HUC-1 cells following CSE treatment for 7 days. A. Cigarette smoke extract (CSE) increased the protein expression of epithelial markers E-cadherin and ZO-1, and decreased protein expression of mesenchymal markers vimentin and N-cadherin in the SV-HUC-1 cells, as shown by western blotting. GAPDH was used as a loading control. B. CSE enhanced the expression of E-cadherin and Zo-1 mRNAs, and decreased the expression of Vimentin and N-cadherin mRNAs, as detected by quantitative reverse transcriptase-polymerase chain reaction after normalization to GAPDH. C. CSE decreased E-cadherin protein expression and increased Vimentin protein expression in SV-HUC-1 cells shown by immunoflorescent staining. Data are expressed as mean ± SD. *P<0.05, **P<0.01, compared with the control group.

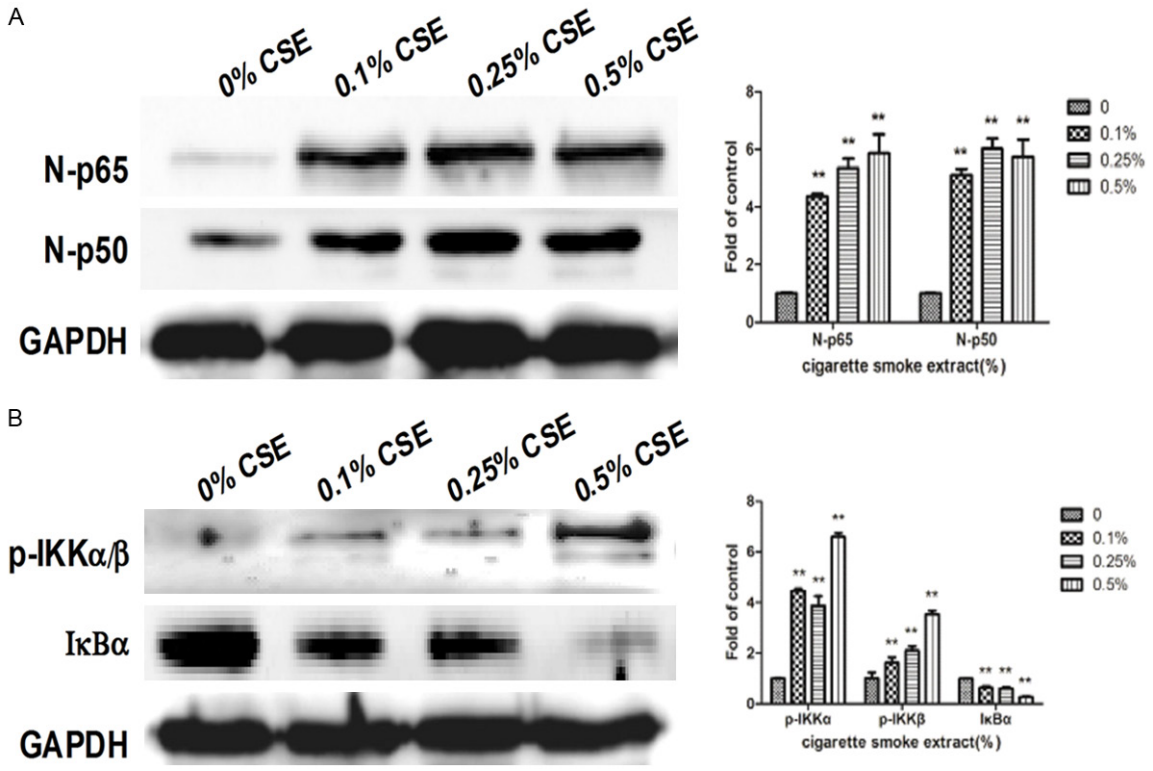

Figure 3. CSE–induced EMT is associated with activate NF-κB activation in SV-HUC-1 cells. A. CSE activated NF-κB activation in SV-HUC-1 cells. The levels of p50 and p65 in nuclear were increased, the active form NF-κB was measured by Western blotting following CSE treatment. B. CSE increased IKK protein activation, but IκB was subsequently degradation. Glyceraldehyde 3-phosphate dehydrogenase was used as loading control. One representative image of more than three independent experiments is shown. Data are expressed as mean ± SD. *P<0.05, **P<0.01, compared with control group.

Statistical analysis

Statistical analyses were performed with SPSS 16.0. All data are expressed as mean ± standard deviation. One-way ANOVA was used for comparison of statistical differences among multiple groups, followed by the LSD significant difference test. In case of comparison between two groups, an unpaired Student's t-test was used. A value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

CSE induces EMT in SV-HUC-1 cells

CSE is the most important risk factor for bladder cancer, and CSE-induced EMT is critically involved in CSE-associated malignant transformation. To study the cell viability of the SV-HUC-1 cells after treatment with CSE, the cells were incubated with CSE (0, 0.05, 0.1, 0.25, 0.5, 1 and 2%) for 7 days and examined by MTT assay. The results showed that the cell viability decreased below 80% when the cells were exposed to 1% or higher CSE concentrations, which proved to be toxic to the SV-HUC-1 cells (**Figure 1A**). Therefore, 0.5% CSE was selected as the maximum concentration for the following experiments.

The process of EMT is manifested by alterations in cell morphology and invasive capacity, as well as expression of epithelial and mesenchymal markers. Treatment of the SV-HUC-1 cells with CSE for 7 days resulted in significant morphological change from a urothelial oblate-shape to a spindle-like mesenchymal form (**Figure 1B**). Additionally, the cells became dispersive and some SV-HUC-1 cells even generated a tail-like change after treatment with CSE. To further examine the effect of CSE on EMT, wound healing assays and transwell assays were carried out to analyze SV-HUC-1 cell migratory and invasive capacities in response to CSE. CSE treatment significantly

increased SV-HUC-1 cell migration. Similarly, invasion of cells through reconstituted matrigel matrices was enhanced by CSE (**Figure 1C-E**). To determine whether molecular alterations of EMT occurred in the CSE-treated cells, the expression levels of EMT markers were determined. Exposure of the SV-HUC-1 cells to CSE resulted in decreased protein expression of the epithelial markers E-cadherin and ZO-1. In contrast, the protein levels of mesenchymal markers Vimentin and N-cadherin were increased, as shown by western blot analyses (**Figure 2A**). Moreover, qRT-PCR analyses revealed similar changes in the mRNA levels of epithelial and mesenchymal markers in the SV-HUC-1 cells exposed to CSE (**Figure 2B**). Immunoflorescent staining also showed that CSE decreased E-cadherin protein expression and increased Vimentin expression in SV-HUC-1 cells (**Figure 2C**). Meanwhile, the higher the concentration of CSE, the more obvious was the observed change. Collectively, data from the morphological, invasive and molecular changes demonstrated that CSE exposure induced EMT in human urothelial cells in a dose-dependent manner.

CSE-induced urocystic EMT is associated with NF-κB activation

To determine whether the CSE-induced proliferation correlated with NF-κB pathway, we examined the protein levels of p65 and p50 in nuclear extract of SV-HUC-1 (As shown in **Figure 3A**). The nucleus protein levels of both p65 and p50 were significantly increased. Furthermore, we also examined the upstream of NF-κB pathway. As shown in **Figure 3B**, we found that the expression of IκB kinase (p-IKKα/β) was clearly increased in cytoplasm, then the IκB was subsequently degradation and the expression was reduced obviously.

Inhibition of NF-κB attenuates CSE-induced EMT in SV-HUC-1 cells

Since the above results revealed that CSE-induced EMT was associated with NF-κB activation in SV-HUC-1 cells, we next explored the role of NF-κB in urocystic EMT regulation. SV-HUC-1 cells were treated for 7 days with 2 μM PDTC, a highly specific NF-κB inhibitor. As expected, PDTC downregulated p65 and p50 in the SV-HUC-1 cells (**Figure 4A**). Inhibition of NF-κB decreased CSE-mediated migration and invasion capacities of SV-HUC-1 cells (**Figure 4B-D**). Furthermore, inhibition of NF-κB by PDTC resulted in upregulation of the protein and mRNA levels of the epithelial markers E-cadherin and ZO-1, as well as downregulation of the mesenchymal markers vimentin and N-cadherin (**Figure 5A-C**). Therefore, these results suggest that activity NF-κB plays an important role in CSE-induced EMT in SV-HUC-1 cells.

Curcumin reversed CSE-triggered EMT change in SV-HUC-1 cells

In order to determine the effects of curcumin on CSE-mediated EMT in the human bladder epithelial cells, SV-HUC-1 cells were received curcumin (10 μM) and exposed to CSE for 7 days. We also explore the influence of curcumin on CSE-mediated activation of NF-κB pathway; we further examined the changes in NF-κB activation following curcumin treatment. Western blot analyses showed that curcumin inhibited CSE-induced NF-κB activation in a dose-dependently manner (**Figure 6A**). Meanwhile, CSE-induced alterations in mRNA and protein expressions of the EMT markers, including decreases of the epithelial markers E-cadherin and ZO-1, and increases of the mesenchymal markers vimentin and N-cadherin, were effectively attenuated with curcumin treatment (**Figure 6B** and **6C**). These data indicated that curcumin reversed CSE-induced human bladder epithelial cells EMT changes in vitro.

Discussion

Bladder cancer is one of the leading causes of cancer-related death in the world. The relationship between the occurrence of bladder cancer and cigarette smoke (CS) has been established. The underlying molecular mechanisms by which CS causes bladder cancer development remain to be investigated. In the present study, we revealed that CSE induced EMT in normal human bladder epithelial SV-HUC-1 cells. Most importantly, our study provides the first repot to explore the role of NF-κB pathway in CSE-induced EMT in SV-HUC-1 cells. Meanwhile, our data indicated that curcumin prevented CSE-triggered NF-κB pathway activation and effectively attenuated CSE-induced EMT of bladder epithelial cells. These data suggested the important role of NF-κB activity in CSE-associated EMT and provide critical information about the molecular mechanisms of CSE-

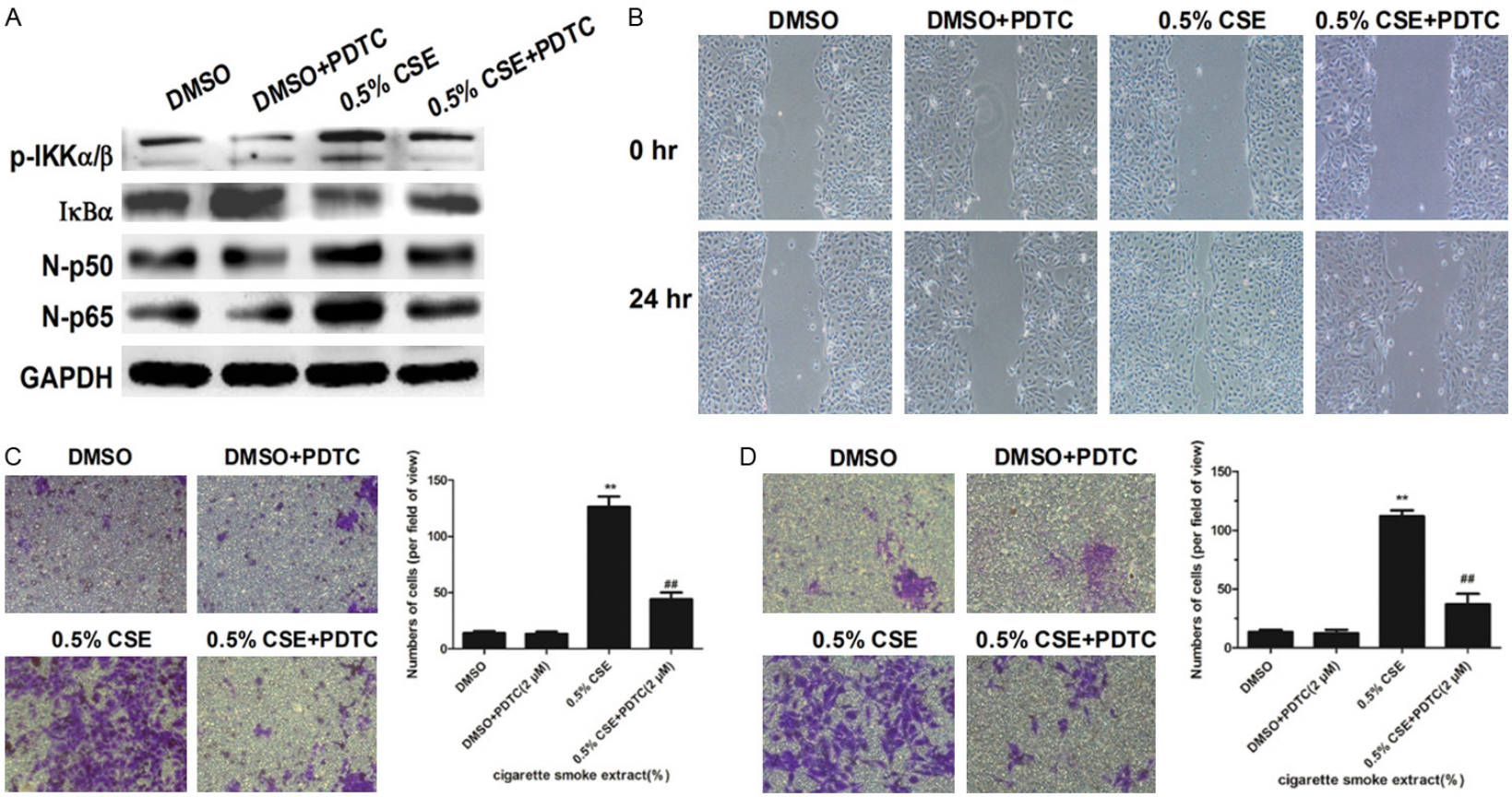

Figure 4. Inhibition of NF-κB reverses CSE-triggered EMT morphological change and migratory and invasive capacities in human bladder epithelial (SV-HUC-1) cells. NF-κB inhibitor PDTC suppressed NF-κB activation in the SV-HUC-1 cells. SV-HUC-1 cells were treated with various concentrations of CSE together with a highly specific NF-κB inhibitor (PDTC) for 7 days. A. PDTC suppressed the activation of p65 and p50 induced by CS exposure, as measured by Western blotting. B. Inhibition of NF-κB reduced CSE-triggered migratory capacity of SV-HUC-1 cells shown by wound healing assay. C, D. PDTC decreased invasive capacity of SV-HUC-1 cells as determined by invasion and migration assay. Data are expressed as mean ± SD. *P<0.05, **P<0.01, compared with the control group. CSE cigarette smoke extract; EMT epithelial-mesenchymal transition.

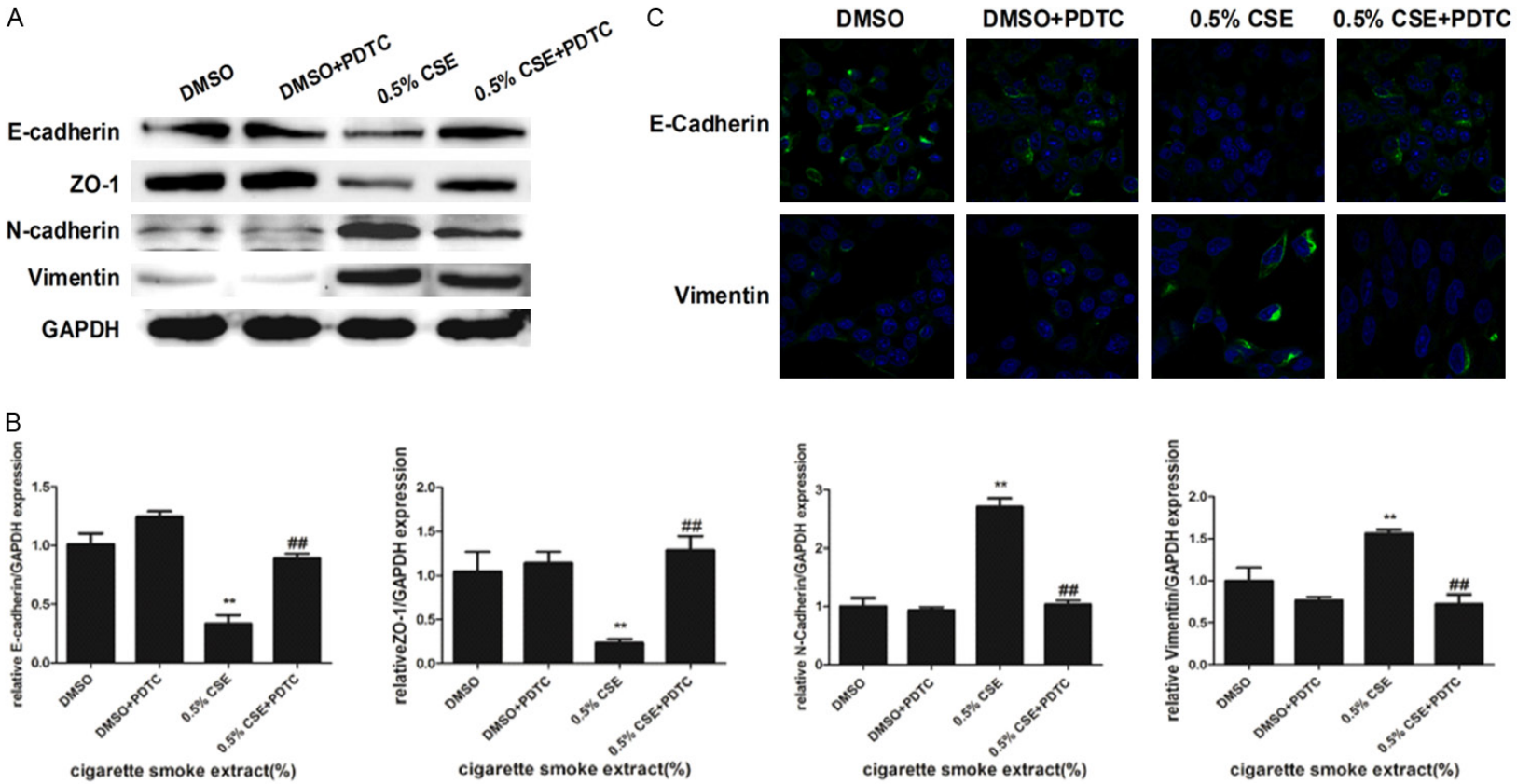

Figure 5. Inhibition of NF-κB attenuates CSE-induced alterations in EMT markers' expression inhuman bladder epithelial (SV-HUC-1) cells. Inhibition of NF-κB ameliorated CSE-induced decreases in the protein expression levels of E-cadherin and ZO-1, as well as increases in protein expression levels of Vimentin and N-cadherin, as measured by Western blotting. A. Inhibition of NF-κB improved CSE-induced alterations in the expression of E-cadherin, ZO-1, Vimentin and N-cadherin mRNAs. B. NF-κB down expression attenuated CSE-reduced E-cadherin protein expression and its increased Vimentin protein expression in SV-HUC-1 cells, C. As shown by immunoflorescent staining. NF-κB down expression attenuated CSE-reduced E-cadherin protein expression and its increased Vimentin protein expression in SV-HUC-1 cells. **P*<0.05, ***P*<0.01, compared with control group; #*P*<0.05, ##*P*<0.01, compared with CSE control group.

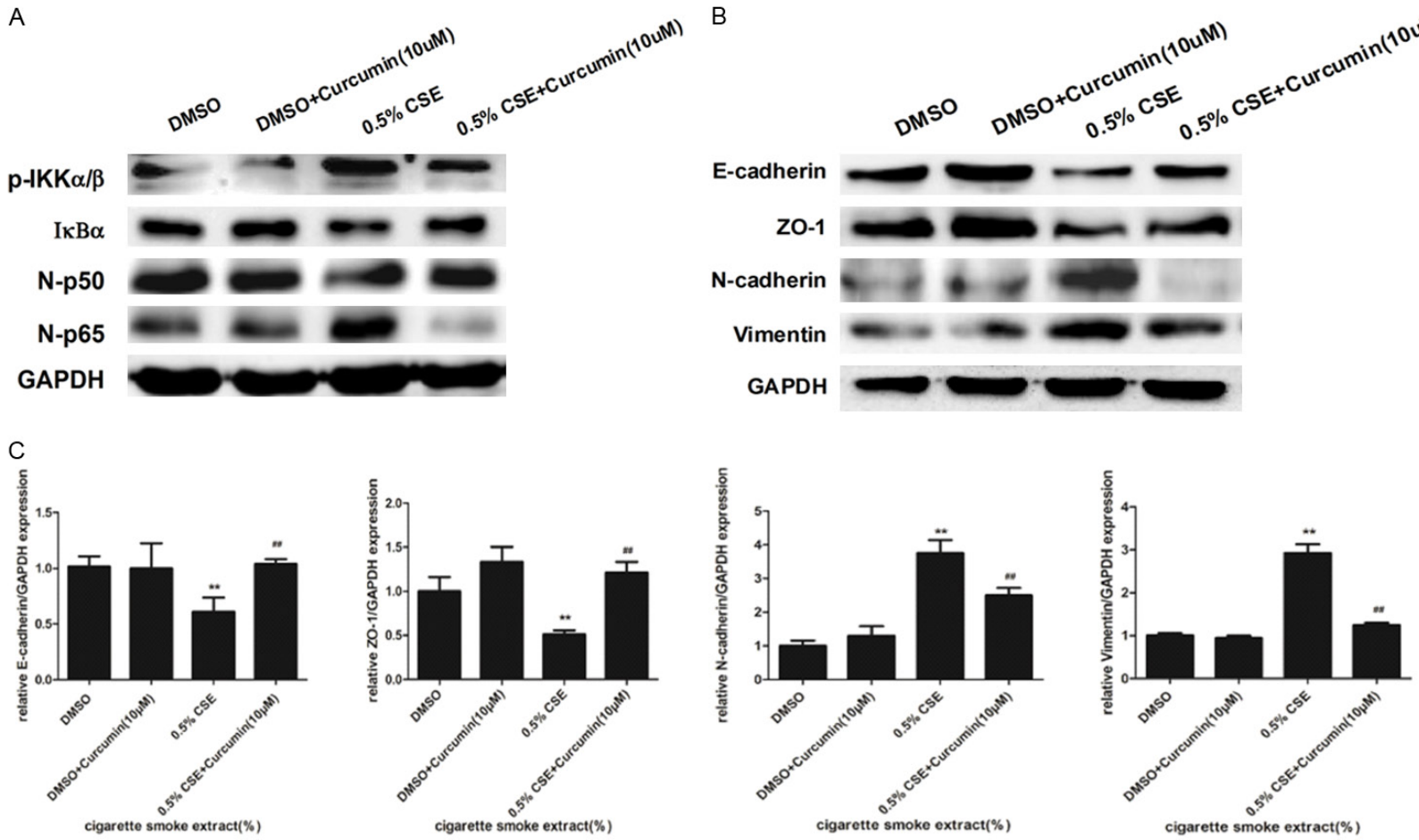

Figure 6. Curcumin reversed CS-induced EMT in SV-HUC-1 cells through inhibited NF-κB pathway. A. The activation of NF-κB was inhibited obviously by curcumin. B. Western blotting analyses of E-cadherin, ZO-1, Vimentin, and N-cadherin proteins. C. Quantitative real-time polymerase chain reaction analyses of E-cadherin, ZO-1, Vimentin, and N-cadherin mRNAs. **P*<0.05, ***P*<0.01, compared with control group; #*P*<0.05, ##*P*<0.01, compared with CSE control group.

related bladder tumorigenesis as well as search for the potential target of bladder cancer intervention.

Metastasis is a multistep process including local invasion, intravasation, transport, extravasation and colonization by which tumor cells disseminate from their primary site and form secondary tumors at a distant site. This process is essentially dependent on the prominent biological event referred to as epithelial-mesenchymal transition (EMT) [27-29]. Evidences have revealed that exposure of cells to carcinogens induces EMT during transformation and tumor formation [29-32], suggesting the important role of EMT in the initiation of tumorigenesis by promoting cell malignant transformation. For example, Goregory et al demonstrated that exposure of epithelial alveolar type II cells to tobacco-specific nitrosamine NNK induces EMT [29]. It has been documented that CS promoted EMT, resulting in loss of cellular polarity, downregulation of epithelial cadherin, loss of cell-cell adhesion and increased mobility [33, 34]. In agreement with previous reports, we showed in the present study that exposure to CSE induced EMT in SV-HUC-1 cells, as manifested by morphological change from epithelial to mesenchymal form, increased migration and invasion capacity, as well as alterations in the expression of EMT markers, including decreased epithelial markers E-cadherin and ZO-1, and increased mesenchymal markers Vimentin and N-cadherin. Taken together, our data revealed that CSE induced EMT in vitro setting.

Several cell signaling factors has been implicated in CS induced EMT. Nevertheless, the underlying mechanisms of EMT induction by CS are poorly understood. Activation of NF-κB pathway is increasingly being recognized as a key mechanism for EMT progress, which remains unexplored in the development of bladder cancer [35]. It has been shown that NF-κB triggers a motility and invasive phenotype of breast, gastric, ovarian cells [36-38]. In human ovarian cancer cells, suppression of metastasis upon blocked of NF-κB activity [38]. Notably, evidences have suggested that NF-κB regulates the expression of multiple genes involved in tumor metastasis, including those encoding MMPs, IL-6, VGFR and Vimentin. Several NF-κB target genes can be induced EMT [39]. To data, no studies have been done to investigate the function of NF-κB pathway in CS-induced EMT in human bladder epithelial cells. Work presented in this indicated that CSE-induced EMT was associated with activation of NF-κB pathway in vitro. Our results demonstrated that the expression of p65 and p50 in the nucleus were increased while its expression in the cytosol was decreased. The IκBα in the cytosol, which is the major inhibitor of NF-κB pathway, was decreased after CSE treatment. To further determined the role of NF-κB in EMT regulation, the inhibitory effect of NF-κB on CS was mimicked with a highly specific NF-κB inhibitor. Inhibition of NF-κB was reversed the mesenchymal-like morphological changes triggered by CSE. Moreover, the enhanced invasive capacities as well as alterations in EMT marker expression were attenuated by inhibition of NF-κB pathway. Collectively, the present study is the first clearly indicate that NF-κB positively regulated CSE-induced urocystic EMT. Base on the important role of the NF-κB signaling pathway in the development of cancer, a new choice has been provide for tumor treatment. Nevertheless, how to block the NF-κB signaling pathway is a reasonable manner requires further exploration.

Curcumin is a dietary polyphenol that has various biological activities and excellent tolerance when administrated systemically. The potent anticancer property of curcumin is attributable to its ability to regulate numerous proteins and cellular signal pathways, altering the expression and activities of genes involved in cell proliferation, apoptosis, and metastasis [40]. In oral squamous cells, curcumin may inhibit EMT via p53-dependent signaling through Alan's study [41]. However, the precise molecular mechanism for the anti-cancinogenic activity of curcumin is still not fully understood. In the present study, our results illustrated the CSE-triggered EMT alteration was reversed by 10 um curcumin in vitro. Moreover, our in vitro results further revealed that administration of curcumin at 10 um for 7 days, prevented CSE-mediated activation of NF-κB pathway. These data suggest that curcumin modulates NF-κB signal pathway and its downstream target genes to exhibit its protective effects against CSE-induced EMT in human bladder epithelial SV-HUC-1 cells.

In summary, exposure to CSE induced EMT in normal human bladder epithelial cells and acti-

vated the NF-κB pathway, while the special inhibitor inhibited NF-κB activity and afterwards restrained the EMT triggered by CSE. Meanwhile, we found that curcumin effectively attenuated CSE exposure triggered NF-κB activation and EMT alterations in SV-HUC-1 cells. All of these data provide important information that inhibition of NF-κB pathway may be a therapeutic target for the treatment of early stage bladder cancer and regarding the chemopreventive function of curcumin against CSE-associated bladder cancer.

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Disclosure of conflict of interest

None.

Abbreviations

SV-HUC-1, SV-40 immortalized human uroepithelial cell; CS, Cigarette smoke; CSE, Cigarette smoke extract; MTT, Thiazolyl blue tetrazolium bromide; RT-PCR, Reverse transcription-polymerase chain reaction.

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