

## Original Article

# Role of AP-1 in the tobacco smoke-induced urocytic abnormal cell differentiation and epithelial-mesenchymal transition *in vivo*

Yi Wang<sup>1\*</sup>, Hao Geng<sup>1\*</sup>, Li Zhao<sup>1</sup>, Zhiqiang Zhang<sup>1</sup>, Dongdong Xie<sup>1</sup>, Tao Zhang<sup>1</sup>, Jie Min<sup>1</sup>, Dexin Yu<sup>1</sup>, Caiyun Zhong<sup>2</sup>

<sup>1</sup>Department of Urology, The Second Affiliated Hospital of Anhui Medical University, Hefei 230032, China;

<sup>2</sup>Department of Nutrition and Food Safety, School of Public Health, Nanjing Medical University, Nanjing 211166, China. \*Equal contributors.

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**Abstract:** Bladder cancer is believed to arise after a series of progressive pathological changes. Cell differentiation exists in almost all cells, when become aberrant, can initiate or promote diseases processes and tumorigenesis. Epithelial-mesenchymal transition (EMT) is a crucial pathophysiological process in cancer initiation and development. Tobacco smoke is an important risk factor of bladder cancer. However, the molecular mechanisms of tobacco smoke-triggered abnormal cell differentiation and EMT in bladder tissues have not been well defined. The current study was designed to investigate the regulatory role of AP-1 in tobacco smoke-triggered urocytic abnormal cell differentiation and EMT *in vivo*. Exposure of male BALB/c mice to tobacco smoke for 12 weeks altered the expression of cell differentiation and EMT markers in bladder tissues. Importantly, we demonstrated that AP-1 modulated tobacco smoke-induced abnormal cell differentiation and EMT, as evidenced by the findings that tobacco smoke elevated AP-1 activation, and tobacco smoke-mediated cell differentiation and EMT were reversed by AP-1 suppression. These data indicated that AP-1 play an important role in tobacco smoke-induced urocytic abnormal cell differentiation and EMT. These findings provide new insights into the mechanism of tobacco smoke associated urocytic tumorigenesis and may help to discover potential targets for novel therapies and chemoprevention.

**Keywords:** Tobacco smoke, bladder cancer, AP-1, abnormal cell differentiation, EMT

## Introduction

In Western countries, bladder cancer is the fifth most common cancer and the most common cancer in urinary malignancies [1]. There were about 382,700 new diagnosed cases and 143,000 deaths in 2013 worldwide [2]. Many factors are strongly associated with bladder cancer; tobacco smoke is one of the major causes of bladder cancer. It has been reported that current smokers have a ~4-fold higher risk of bladder cancer than nonsmokers [3]. It is estimated that approximately half of male bladder cancer patients and a quarter of female patients are associated with smoke habit [4]. Many compounds of tobacco smoke are attributed to its carcinogenic potential, including abnormal cell differentiation, cell hyperproliferation and progression of cancer. Although enor-

mous progress has been made in understanding its molecular mechanisms leading to bladder cancer, the tumorigenic process of tobacco smoke remains largely unknown.

Bladder cancer is a common malignant tumor with high mortality due to its high recurrence rate [5]. Thus, molecular mechanisms of urothelial carcinoma progression are needed to be clarified for early diagnosis and treatment. As with other epithelial malignancies, bladder cancer is believed to arise after a series of progressive pathological changes. Molecular abnormalities in normal appearing and preneoplastic epithelium from patients with cancer and chronic smokers suggest that genetic changes may serve as biomarkers for early diagnosis, risk assessment and monitoring response to chemoprevention [6]. Normal cell differentia-

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tion is essential for various body normal developmental processes, it can be deregulated and become aberrant, which in turn can initiate or promote diseases processes, consisting in cancer [7]. Abnormal cell differentiation exists in all tumors, is the basic feature of cell oncogenic transformation. The aberrant differentiation of bladder epithelial cells should be recognized because of important clinical implications [8]. It is becoming more and more evident that there is a tight contact between abnormal cell differentiation processes and cancer during recent years [7].

Epithelial-mesenchymal transition (EMT) is an important process of cellular transdifferentiation including the changes from an epithelial to a mesenchymal phenotype [9]. These processes have been shown to be of capital relevance in the initiation and development of tumorigenesis [10]. However, the role of tobacco smoke-triggered abnormal cell differentiation and EMT in bladder cancer has not been well defined.

The activator protein-1 (AP-1) is a hetero- or homo-dimeric complex transcription factor composed of the proto-oncogene proteins belonging to the Fos (c-Fos, FosB, Fra-1 and Fra-2), Jun (c-Jun, JunB and JunD) families [11, 12]. In response to various extracellular stimulus, AP-1 bind to the TPA-response element (TRE) to transcriptionally activate target genes that regulate a broad range of normal and pathological cellular events including differentiation, proliferation, survival and apoptosis [13, 14]. It has reported that AP-1 play an important role in the process of tumorigenesis [15, 16]. Previous reports have shown tobacco smoke exposure induces AP-1 activation [1, 17, 18]. However, almost no relevant studies have been done to investigate the regulation of AP-1 on abnormal cell differentiation genes and EMT in tobacco smoke-induced bladder cancer.

In the present study, we assessed the regulation ability of AP-1 in the tobacco smoke-mediated abnormal cell differentiation and EMT alterations in the bladder of BALB/c mice. By using *in vivo* tobacco smoke exposure models, we demonstrate that AP-1 regulated tobacco smoke-induced abnormal cell differentiation and EMT in the bladder tissues of mice. These novel findings suggested the important role of AP-1 in tobacco smoke-associated bladder carcinogenesis and improving our understanding

of these processes may help to discover potential targets for novel therapies and chemoprevention.

### Materials and methods

#### *Chemicals and reagents*

The primary antibodies for phosphorylated c-Fos, phosphorylated c-Jun, FosB, JunB, E-cadherin, ZO-1, Snail-1, CK13, CK14, N-cadherin and Vimentin were obtained from Cell Signaling Technology (Beverly, MA). CK10, CK20, UpII, GAPDH antibody was from Biogot Technology (Nanjing, China). Primers for E-cadherin, N-cadherin, Vimentin, Snail-1, ZO-1, CK10, CK13, CK14, CK20, UpII and GAPDH were synthesized by Invitrogen (Carlsbad, CA). SR11302 was purchased from Tocris Bioscience (Bristol, UK). Sources of other materials are noted accordingly in the text.

#### *Mice and tobacco smoke exposure*

Thirty-six male BALB/c mice (6-8 weeks old weighting 18-22 g) were purchased from the Animal Research Center of Nanjing Medical University (Nanjing, China). Animals were allowed to acclimate for one week to the onset of experimental exposure. Mice were housed in polypropylene cages, maintained on controlled temperature and humidity, with a 12-h light/dark cycle, and free access to food and water. The animal studies were carried out in strict accordance with the recommendations in the guidelines of the Animal Care and Welfare Committee of Nanjing Medical University.

Six mice were randomly assigned into each group. The control group was exposed to filtered, conditioned air. Mice were exposed to tobacco smoke generated from one of the most consumed Chinese commercial cigarettes (Hongtashan, contains 12 mg tar and 1.1 mg nicotine per cigarette) in a smoking apparatus. One filterless cigarette was combusted to generate tobacco smoke by a smoke machine design by ourselves, which smoked cigarettes and then pumped the mainstream smoke from the burning cigarette at a constant rate (5 min per cigarette). The mainstream smoke was delivered to whole-body exposure chambers with a target concentration of total particulate matter (TPM) of 85 mg/m<sup>3</sup>. BALB/c mice were exposed for 6 hours per day last for 12 weeks.

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The exposure conditions were monitored and characterized as the followings: TSP (0 mg/m<sup>3</sup>), carbon monoxide (13.15±1.96 mg/m<sup>3</sup>) for the control group; TSP (84.29±6.38 mg/m<sup>3</sup>), carbon monoxide (116.62±21.88 mg/m<sup>3</sup>) for tobacco smoke group. After the completion of tobacco smoke exposure, mice were sacrificed and the bladder tissues were collected for analysis.

### *Delivery of specific AP-1 inhibitor in vivo*

Animals were randomly divided into four groups as the followings (n = 6 per group): the control group, mice were exposed to filtered air; tobacco smoke group, mice were exposed to tobacco smoke; tobacco smoke + DMSO group, mice were injected with DMSO and exposed to tobacco smoke; tobacco smoke + SR11302 group, mice were injected with SR11302 and exposed to tobacco smoke. SR11302, a specific AP-1 inhibitor was diluted with DMSO and injected intraperitoneally (1 mg/kg body weight) every day. Following the completion of exposure, mice were sacrificed and bladder tissues were isolated and stored at -80°C until analysis.

### *Western blot analysis*

Bladder tissues were homogenized in a lysate buffer and centrifuged at 4°C for 25 min. Protein concentrations were measured and fifty micrograms of proteins were fractionated by electrophoresis through 7.5-10% SDS-PAGE and then transferred to PVDF membrane (Millipore, Billerica, MA). The membranes were blocked with 5% (w/v) skim milk powder and subsequently probed with primary antibodies overnight at 4°C, and then incubated with horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit secondary antibody. GAPDH served as the loading control.

### *Quantitative real-time PCR*

Total RNA was extracted from bladder samples by using RNAiso Plus according to the manufacturer's instructions (TaKaRa, Japan). The quality and quantity of the RNA samples were analyzed by UV spectrophotometry (260/280 nm ratio). The cDNA was synthesized from two micrograms of total RNA by using AMV Reverse Transcriptase (Promega, Madison, WI). qRT-PCR was performed using the Power SYBR

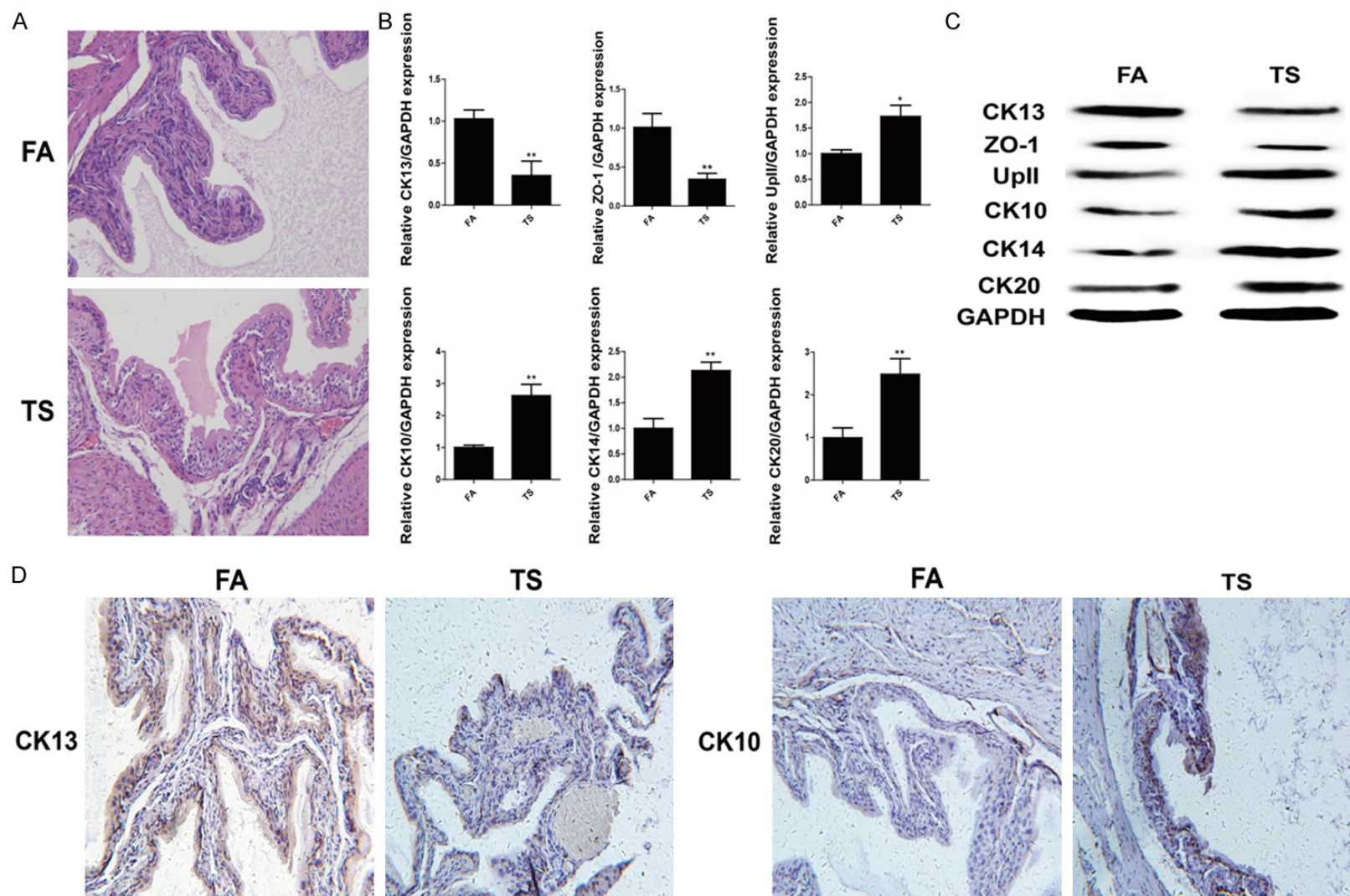
Green Master Mix (TaKaRa, Japan) and an ABI 7300 real-time PCR detection system (Applied Biosystems, CA). The primers used were as follows: ZO-1, forward 5'-GCAGCCACAACCAATTCATAG-3' and reverse 5'-GCAGACGATGTTTCATAGTTTC-3'; CK10, forward 5'-CCATCGATGACCTTAAAAATCAG-3' and reverse 5'-GCAGAGCTACCTCATTCTCATACTT-3'; CK13, forward 5'-AATCCTGCTCTCACCATGAG-3' and reverse 5'-GGTTCTGCATGGTATCTTC-3'; CK14, forward 5'-AAGACCATTGAGGACCTGAGGA-3' and reverse 5'-AGACGGGCATTGTCAATCTG-3'; CK20, forward 5'-ACGCCAGAACAACGAATACC-3' and reverse 5'-TTCAGATGACACGACCTTGC-3'; UpII, forward 5'-AACAACCCACACTTACCACTG-3' and reverse 5'-GGAGCTTGCTGGAATACACCTC-3'; E-cadherin, forward 5'-TCGACACCCGATTCAAAGTGG-3' and reverse 5'-TTCCAGAAACGGAGGCCTGAT-3'; Snail-1, forward 5'-GACCACTATGCCGCGCTCTT-3' and reverse 5'-TCGCTGTAGTTAGGCTTCCGATT-3'; Vimentin, forward 5'-CCTTGACATTGAGATTGCCA-3' and reverse 5'-GTATCAACCAGAGGGAGTGA-3'; N-cadherin forward 5'-ATCAAGTGCCATTAGCCAAG-3' and reverse 5'-CTGAGCAGTGAATGTTGTCA-3'; GAPDH, forward 5'-GCTGCCCAACGCACCGAATA-3' and reverse 5'-GAGTCAACGGATTTGGTCGT-3'. All of the primers were synthesized by Invitrogen (Carlsbad, CA). The 2<sup>-( $\Delta\Delta$ CT)</sup> method was used for the quantification, with GAPDH as the relative abundance normalized to the control.

### *Histological analysis and Immunohistochemistry*

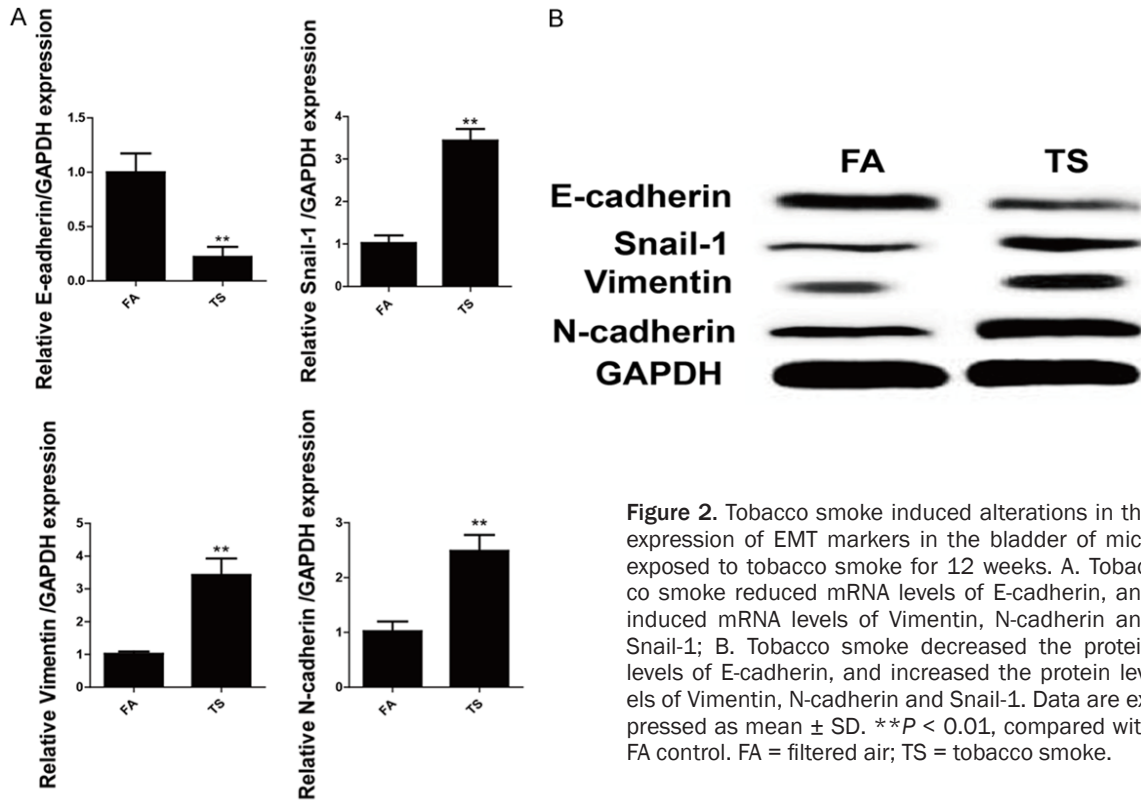
The bladder tissues were isolated and fixed in paraformaldehyde. After tissues fixation, these were subjected to dehydration and the embedded in paraffin. Thin sections (5  $\mu$ m) of the paraffin embedded tissues were cut using a microtome. Subsequently, the sections were de-waxed in xylene, rehydrated in a series of different grades of alcohol and washed with distilled water. The sections were stained with hematoxylin or incubated with the primary antibody. After counterstained with eosin or incubated with biotinylated goat anti-rabbit for 1 h, the sections were dehydrated in graded ethanol series and dehydration in xylene.

### *Statistical analysis*

The SPSS 16.0 software system was used for statistical analysis. All data were expressed as mean  $\pm$  standard deviation. One-way ANOVA



**Figure 1.** Tobacco smoke induced alterations in the expression of cell differentiation markers in the bladder of mice exposed to tobacco smoke for 12 weeks. A. Tobacco smoke-induced the morphological changes of bladder cells. B. Tobacco smoke reduced mRNA levels of CK13 and ZO-1, and induced mRNA levels of CK10, CK14, CK20 and UpII, detected by q-PCR after normalization to GAPDH. C. Tobacco smoke decreased the protein levels of CK13 and ZO-1, and increased the protein levels of CK10, CK14, CK20 and UpII. GAPDH was used as loading control for Western blotting. D. Tobacco smoke decreased CK13 protein expression and increased UpII protein expression shown by immunohistochemical staining. Data are expressed as mean  $\pm$  SD. \* $P < 0.05$ , \*\* $P < 0.01$ , compared with FA. FA = filtered air; TS = tobacco smoke.



**Figure 2.** Tobacco smoke induced alterations in the expression of EMT markers in the bladder of mice exposed to tobacco smoke for 12 weeks. A. Tobacco smoke reduced mRNA levels of E-cadherin, and induced mRNA levels of Vimentin, N-cadherin and Snail-1; B. Tobacco smoke decreased the protein levels of E-cadherin, and increased the protein levels of Vimentin, N-cadherin and Snail-1. Data are expressed as mean  $\pm$  SD.  $**P < 0.01$ , compared with FA control. FA = filtered air; TS = tobacco smoke.

was used for comparison of statistical differences among multiple groups, followed by the LSD significant difference test. Unpaired Student's t-test was also used for two-group comparisons.  $P < 0.05$  indicated statistical significance.

### Results

#### *Tobacco smoke induced urocytic abnormal cell differentiation and EMT in vivo*

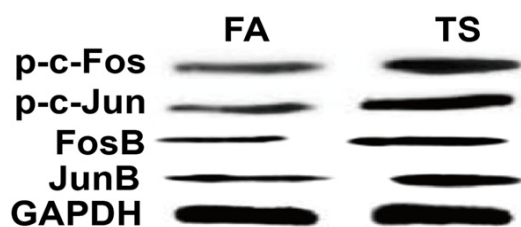
Tobacco smoke-triggered abnormal cell differentiation and EMT are critically involved in neoplastic transformation. In the current study, we investigated whether long term tobacco smoke induces abnormal cell differentiation and EMT in the bladder tissues of mice. Male BALB/c mice were exposed to tobacco smoke for 12 consecutive weeks, and then the morphological changes, the expression levels of the cell differentiation markers and EMT markers in bladders were examined. Tobacco smoke-induced the morphological changes of bladder cells were shown as **Figure 1A**. Our results demonstrated that tobacco smoke exposure decreased the mRNA expression of CK13, ZO-1, UplI, and E-cadherin, and increased the

mRNA expression levels of CK10, CK14, CK20, Snail-1, Vimentin, and N-cadherin (**Figures 1B and 2A**). As while, we found that tobacco smoke exposure reduce CK13, ZO-1, and E-cadherin protein expression, and elevate CK10, CK14, CK20, UplI, Snail-1, Vimentin, and N-cadherin protein levels (**Figures 1C, 1D and 2B**).

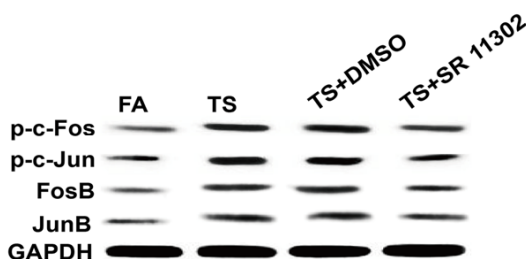
#### *Tobacco smoke-mediated urocytic abnormal cell differentiation and EMT was associated with AP-1 activation*

AP-1 is implicated in several cellular events including differentiation, proliferation, and carcinogenesis. The role of AP-1 in tobacco smoke-induced urocytic abnormal cell differentiation and EMT regulation has not been well revealed. To determine whether tobacco smoke-induced abnormal cell differentiation and EMT in the bladder of mice are associated with AP-1 activation, the expression levels of phosphorylated c-Fos, phosphorylated c-Jun, FosB, and JunB were measured. It was found that tobacco smoke exposure increased the activation of AP-1 proteins as indicated by elevated levels of phosphorylated c-Fos, phosphorylated c-Jun, FosB, and JunB (**Figure 3**).

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**Figure 3.** Tobacco smoke increased AP-1 activation in the bladder of mice. Western blotting analyses of phosphorylated c-Fos, phosphorylated c-Jun, FosB and JunB after exposed to tobacco smoke for 12 weeks. FA = filtered air; TS = tobacco smoke.



**Figure 4.** SR11302 attenuated tobacco smoke induced AP-1 activation in the mice bladder. Western blotting analyses of phosphorylated c-Fos, phosphorylated c-Jun after exposed to tobacco smoke for 12 weeks. FA = filtered air; TS = tobacco smoke.

### *SR11302 reversed tobacco smoke-induced AP-1 activation in mice bladder*

Since above results revealed that AP-1 may play an important role in the onset and progression of abnormal cell differentiation and EMT. Herein, our study was designed to determine the role of AP-1 in urocytic abnormal cell differentiation and EMT regulation. We first treated the male BALB/c mice with the specific AP-1 inhibitor SR11302 (1 mg/kg body weight) every day. Western blot analyses results revealed that SR11302 markedly decreased tobacco smoke increased the expression levels of phosphorylated c-Fos, phosphorylated-c-Jun, FosB, and JunB (**Figure 4**).

### *AP-1 suppression attenuated tobacco smoke-elicited abnormal cell differentiation and EMT*

To further determine the role of AP-1 in tobacco smoke-triggered abnormal cell differentiation and EMT in the mice bladder tissues, the expression of the cell differentiation and EMT markers were examined after treatment with

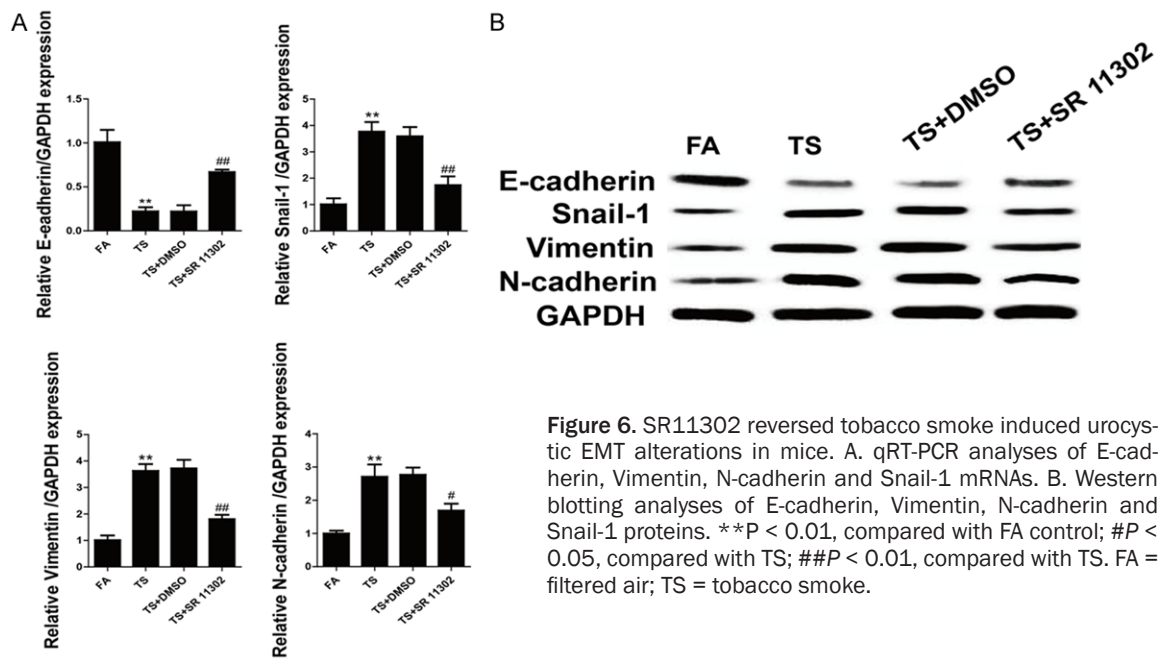
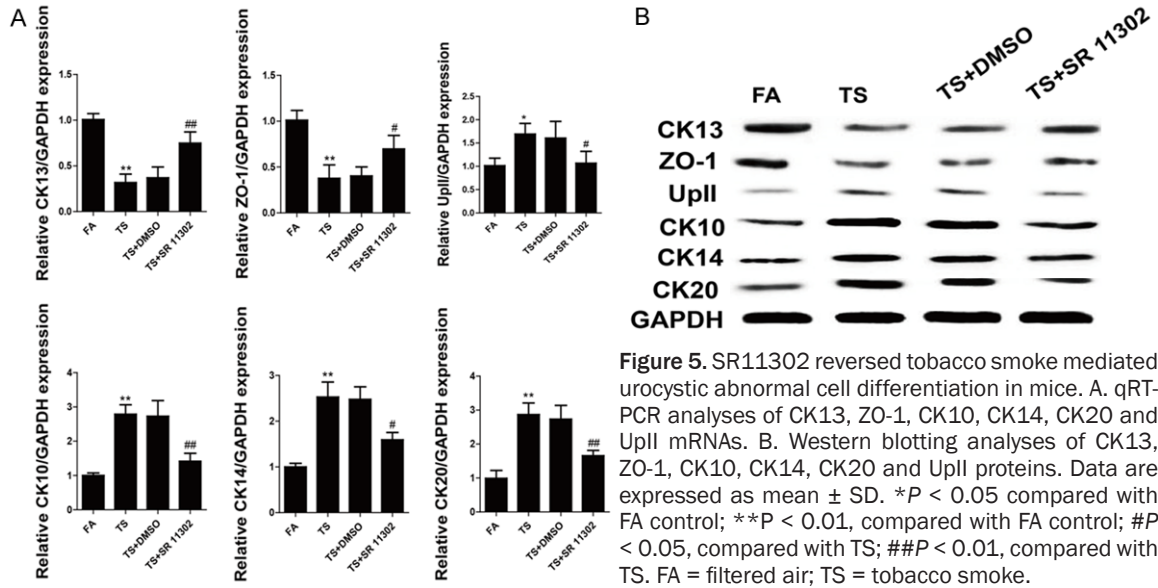
SR11302 and tobacco smoke. We found that tobacco smoke-induced alterations in the mRNAs of CK13, ZO-1, E-cadherin, CK10, CK14, CK20, UpII, Snial-1, Vimentin, and N-cadherin were effectively attenuated by AP-1 inhibitor (SR11302) (**Figures 5A** and **6A**). Western blot analyses demonstrated that treated with SR11302 reversed both tobacco smoke-mediated decrease of CK13, ZO-1, and E-cadherin levels, and the increase of CK10, CK14, CK20, UpII, Snial-1, Vimentin, and N-cadherin in the bladders of BALB/c mice (**Figures 5B** and **6B**). These data demonstrated the regulatory role of AP-1 in tobacco smoke-induced abnormal cell differentiation and EMT in the bladder tissues.

## Discussion

Bladder cancer is still one of the major causes of cancer-related death worldwide and the first leading cause of cancer mortality in the urinary malignancies [19]. The relationship between bladder cancer and tobacco smoke has been established [20, 21]. Tobacco smoke is one of the primary causes of bladder cancer, which promote the initiation and progression of urocytic tumorigenesis. However, the underlying molecular mechanisms by which tobacco smoke causes the initiation and development of bladder cancer remain to be well established. In the current study, we revealed that tobacco smoke triggered abnormal cell differentiation and EMT in the mice bladder. Furthermore, our data demonstrated that AP-1 regulated tobacco smoke-induced urocytic abnormal cell differentiation and EMT *in vivo* tobacco smoke exposed model.

Bladder cancer is believed to arise after a series of progressive pathological changes. Cell differentiation exists in almost all cells of the body, when become aberrant, we can initiate or promote diseases processes, consisting in tumorigenesis. It has been documented that tobacco smoke induces cell aberrant differentiation in many cell types [13, 22, 23]. Tobacco smoke is one of the key risks of bladder cancer. Tobacco smoke triggered abnormal cell differentiation may play an important role in the onset and progression of bladder cancer. Cytokeratins expression are the most commonly used markers to investigate the differentiation status of the epithelial cells [24].

## AP-1 regulate smoke-induced bladder abnormal cell differentiation and EMT



Cytokeratins are believed to be involved in the precancerous lesions, and their expression profiles are quite specific in the early cancer lesions [25]. Each type of epithelium has its specific pattern of cytokeratin polypeptides expression, are thought to be epithelial differentiation of different types and stages of molecular markers. UPII is a high specificity and moderate sensitivity marker in detection of primary and metastatic bladder cancers. ZO-1 is a commonly

used epithelial marker [3, 26, 27]. In the present study, we also found that tobacco smoke altered the expression of cell differentiation markers, including decreased ZO-1, CK13, and increased CK10, CK14, CK20 and UplI. These results revealed that long-term tobacco smoke exposure induced abnormal cell differentiation in the bladder tissues of male BALB/c mice. Tobacco smoke-induced changes of morphological were observed (**Figure 1A**).

## AP-1 regulate smoke-induced bladder abnormal cell differentiation and EMT

EMT is an areversible morphogenetic process capable of converting polarized epithelial cells into mesenchymal-like cells, which involved in cancer initiation and development [28, 29]. It has been reported that tobacco smoke promotes the EMT program, resulting in loss of epithelial traits and the acquisition of mesenchymal features [30-32]. Consistent with previous reports, our results revealed that long time (12 weeks) exposure to tobacco smoke induce bladder EMT in an animal model. We showed that tobacco smoke exposure reduced E-cadherin expression, and increased Snail-1, Vimentin and N-cadherin expression levels.

Nonetheless, the underlying molecular mechanisms of abnormal cell differentiation and EMT induction by tobacco smoke are not well understood. The transcription factor AP-1 is a complex of proto-oncogene c-Jun and c-Fos, plays a critical role in the regulation of cell differentiation, proliferation, migration and carcinogenic process. However, the role of AP-1 on tobacco smoke-induced bladder abnormal cell differentiation and EMT has not been well characterized. Many studies have demonstrated that tobacco smoke activated AP-1 via MAPK [3, 26, 27], our previous studies also shown that tobacco smoke activated AP-1 through MAPK pathway [1, 17, 18]. Few studies shown that tobacco smoke activated AP-1 may be associated with nuclear factor kappa B [33, 34]. In the present study we focused on whether AP-1 play an important role in tobacco smoke-induced abnormal cell differentiation and EMT in the bladder tissues. Our results showed that tobacco smoke-induced gastric elicited abnormal cell differentiation and EMT was associated with upregulation of AP-1 activation in the mice bladder. To determine the role of AP-1 in urocytic abnormal cell differentiation and EMT regulation, the male BALB/c mice were treated with a specific AP-1 inhibitor SR11302 (1 mg/kg body weight). As expected, SR11302 down-regulated the activation of AP-1 in the stomach of mice, as indicated by attenuated the levels of phosphorylated c-Fos, phosphorylated c-Jun, FosB, and JunB. Furthermore, suppression of AP-1 reversed tobacco smoke-triggered alterations in cell differentiation and EMT markers, including decreased CK13, ZO-1, and E-cadherin levels, and the increase of CK10, CK14, CK20, UplI, Snial-1, Vimentin, and N-cadherin. Collectively, these data obtained clearly indi-

cated that AP-1 positively regulates tobacco smoke-mediated bladder abnormal cell differentiation and EMT in the animal model.

Our present study demonstrated for the first time that AP-1 play an important role in tobacco smoke-induced abnormal cell differentiation and EMT in the bladder tissues of male BALB/c mice. These findings provide new insight into the mechanisms of tobacco smoke-associated bladder carcinogenesis and improving our understanding of these precancerous lesions processes may help to discover potential targets for novel therapies and chemoprevention.

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Dexin Yu, Department of Urology, The 2nd Affiliated Hospital of Anhui Medical University, Hefei 230032, China. Tel: +86 13705690883; Fax: +86 0551 63869522; E-mail: ay2fy\_uro@126.com; Caiyun Zhong, Department of Nutrition and Food Safety, School of Public Health, Nanjing Medical University, Nanjing 211166, China. Tel: +86 25 86868450; Fax: +86 25 86868499; E-mail: cyzhong@njmu.edu.cn

### References

- [1] Yu D, Geng H, Liu Z, Zhao L, Liang Z, Zhang Z, Xie D, Wang Y, Zhang T, Min J and Zhong C. Cigarette smoke induced urocytic epithelial mesenchymal transition via MAPK pathways. *Oncotarget* 2017; 8: 8791-8800.
- [2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; 65: 5-29.
- [3] Liang Z, Xie W, Wu R, Geng H, Zhao L, Xie C, Li X, Zhu M, Zhu W, Zhu J, Huang C, Ma X, Wu J, Geng S, Zhong C and Han H. Inhibition of tobacco smoke-induced bladder MAPK activation and epithelial-mesenchymal transition in mice by curcumin. *Int J Clin Exp Pathol* 2015; 8: 4503-4513.
- [4] Letasiova S, Medve'ova A, Sovcikova A, Dusinska M, Volkovova K, Mosoiu C and Bartonova A. Bladder cancer, a review of the environmental risk factors. *Environ Health* 2012; 11 Suppl 1: S11.



## AP-1 regulate smoke-induced bladder abnormal cell differentiation and EMT

- [5] Shang A, Yang M, Shen F, Wang J, Wei J, Wang W, Lu W, Wang C and Wang C. MiR-1-3p suppresses the proliferation, invasion and migration of bladder cancer cells by up-regulating SFRP1 expression. *Cell Physiol Biochem* 2017; 41: 1179-1188.
- [6] Wistuba II, Mao L and Gazdar AF. Smoking molecular damage in bronchial epithelium. *Oncogene* 2002; 21: 7298-7306.
- [7] Rivlin N, Koifman G and Rotter V. p53 orchestrates between normal differentiation and cancer. *Semin Cancer Biol* 2015; 32: 10-17.
- [8] Shim J, Go H, Lim YS, Moon KC, Ro JY and Cho YM. Hepatoid differentiation in renal cell carcinoma: a rare histologic pattern with clinical significance. *Ann Diagn Pathol* 2014; 18: 363-368.
- [9] Martinez-Ramirez AS, Diaz-Munoz M, Butanda-Ochoa A and Vazquez-Cuevas FG. Nucleotides and nucleoside signaling in the regulation of the epithelium to mesenchymal transition (EMT). *Purinergic Signal* 2017; 13: 1-12.
- [10] Fici P, Gallerani G, Morel AP, Mercatali L, Ibrahim T, Scarpì E, Amadori D, Puisieux A, Rigaud M and Fabbri F. Splicing factor ratio as an index of epithelial-mesenchymal transition and tumor aggressiveness in breast cancer. *Oncotarget* 2017; 8: 2423-2436.
- [11] Kappelmann M, Bosserhoff A and Kuphal S. AP-1/c-Jun transcription factors: regulation and function in malignant melanoma. *Eur J Cell Biol* 2014; 93: 76-81.
- [12] Alam M, Kashyap T, Pramanik KK, Singh AK, Nagini S and Mishra R. The elevated activation of NFkappaB and AP-1 is correlated with differential regulation of Bcl-2 and associated with oral squamous cell carcinoma progression and resistance. *Clin Oral Investig* 2017; [Epub ahead of print].
- [13] Zhong CY, Zhou YM, Douglas GC, Witschi H and Pinkerton KE. MAPK/AP-1 signal pathway in tobacco smoke-induced cell proliferation and squamous metaplasia in the lungs of rats. *Carcinogenesis* 2005; 26: 2187-2195.
- [14] Papoudou-Bai A, Hatzimichael E, Barbouti A and Kanavaros P. Expression patterns of the activator protein-1 (AP-1) family members in lymphoid neoplasms. *Clin Exp Med* 2016; [Epub ahead of print].
- [15] Wang F, Lv P, Gu Y, Li L, Ge X and Guo G. Galectin-1 knockdown improves drug sensitivity of breast cancer by reducing P-glycoprotein expression through inhibiting the Raf-1/AP-1 signaling pathway. *Oncotarget* 2017; 8: 25097-25106.
- [16] Fan F, Bashari MH, Morelli E, Tonon G, Malvestiti S, Vallet S, Jarahian M, Seckinger A, Hose D, Bakiri L, Sun C, Hu Y, Ball CR, Glimm H, Sattler M, Goldschmidt H, Wagner EF, Tassone P, Jaeger D and Podar K. The AP-1 transcription factor JunB is essential for multiple myeloma cell proliferation and drug resistance in the bone marrow microenvironment. *Leukemia* 2016; [Epub ahead of print].
- [17] Geng H, Zhao L, Liang Z, Zhang Z, Xie D, Bi L, Wang Y, Zhang T, Cheng L, Yu D and Zhong C. Cigarette smoke extract-induced proliferation of normal human urothelial cells via the MAPK/AP-1 pathway. *Oncol Lett* 2017; 13: 469-475.
- [18] Liu Z, Liu J, Zhao L, Geng H, Ma J, Zhang Z, Yu D and Zhong C. Curcumin reverses benzidine-induced epithelial-mesenchymal transition via suppression of ERK5/AP-1 in SV-40 immortalized human urothelial cells. *Int J Oncol* 2017; 50: 1321-1329.
- [19] Harb-de la Rosa A, Acker M, Kumar RA and Manoharan M. Epigenetics application in the diagnosis and treatment of bladder cancer. *Can J Urol* 2015; 22: 7947-7951.
- [20] Masaoka H, Matsuo K, Ito H, Wakai K, Nagata C, Nakayama T, Sadakane A, Tanaka K, Tamakoshi A, Sugawara Y, Mizoue T, Sawada N, Inoue M, Tsugane S, Sasazuki S; Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan. Cigarette smoking and bladder cancer risk: an evaluation based on a systematic review of epidemiologic evidence in the Japanese population. *Jpn J Clin Oncol* 2016; 46: 273-283.
- [21] Wyszynski A, Tanyos SA, Rees JR, Marsit CJ, Kelsey KT, Schned AR, Pendleton EM, Celaya MO, Zens MS, Karagas MR and Andrew AS. Body mass and smoking are modifiable risk factors for recurrent bladder cancer. *Cancer* 2014; 120: 408-414.
- [22] Martinez-Garcia E, Irigoyen M, Anso E, Martinez-Irujo JJ and Rouzaut A. Recurrent exposure to nicotine differentiates human bronchial epithelial cells via epidermal growth factor receptor activation. *Toxicol Appl Pharmacol* 2008; 228: 334-342.
- [23] Tian D, Zhu M, Chen WS, Li JS, Wu RL and Wang X. Role of glycogen synthase kinase 3 in squamous differentiation induced by cigarette smoke in porcine tracheobronchial epithelial cells. *Food Chem Toxicol* 2006; 44: 1590-1596.
- [24] de Andrade NP, Rodrigues MF, Rodini CO and Nunes FD. Cancer stem cell, cytokeratins and epithelial to mesenchymal transition markers expression in oral squamous cell carcinoma derived from orthotopic xenotransplantation of CD44 high cells. *Pathol Res Pract* 2017; 213: 235-244.
- [25] Saitoh T, Sato K, Tonogi M, Tanaka Y and Yamane GY. Expression of Cytokeratin 13, 14, 17, and 19 in 4-nitroquinoline-1-oxide-induced

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- oral carcinogenesis in rat. *Bull Tokyo Dent Coll* 2016; 57: 241-251.
- [26] Liang Z, Xie W, Wu R, Geng H, Zhao L, Xie C, Li X, Huang C, Zhu J, Zhu M, Zhu W, Wu J, Geng S and Zhong C. ERK5 negatively regulates tobacco smoke-induced pulmonary epithelial-mesenchymal transition. *Oncotarget* 2015; 6: 19605-19618.
- [27] Liang Z, Wu R, Xie W, Geng H, Zhao L, Xie C, Wu J, Geng S, Li X, Zhu M, Zhu W, Zhu J, Huang C, Ma X, Zhong C and Han H. Curcumin suppresses MAPK pathways to reverse tobacco smoke-induced gastric epithelial-mesenchymal transition in mice. *Phytother Res* 2015; 29: 1665-1671.
- [28] Du B and Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. *Molecules* 2016; 21.
- [29] Guo J, Li X, Tan Z, Lu W, Yang G and Guan F. Alteration of N-glycans and expression of their related glyco genes in the epithelial-mesenchymal transition of HCV29 bladder epithelial cells. *Molecules* 2014; 19: 20073-20090.
- [30] Shin VY, Jin HC, Ng EK, Sung JJ, Chu KM and Cho CH. Activation of 5-lipoxygenase is required for nicotine mediated epithelial-mesenchymal transition and tumor cell growth. *Cancer Lett* 2010; 292: 237-245.
- [31] Zhang L, Gallup M, Zlock L, Basbaum C, Finkbeiner WE and McNamara NA. Cigarette smoke disrupts the integrity of airway adherens junctions through the aberrant interaction of p120-catenin with the cytoplasmic tail of MUC1. *J Pathol* 2013; 229: 74-86.
- [32] Zhang H, Liu H, Borok Z, Davies KJ, Ursini F and Forman HJ. Cigarette smoke extract stimulates epithelial-mesenchymal transition through Src activation. *Free Radic Biol Med* 2012; 52: 1437-1442.
- [33] Yang BC, Yang ZH, Pan XJ, Xiao FJ, Liu XY, Zhu MX and Xie JP. Crotonaldehyde-exposed macrophages induce IL-8 release from airway epithelial cells through NF- $\kappa$ B and AP-1 pathways. *Toxicol Lett* 2013; 219: 26-34.
- [34] Li YT, He B, Wang YZ and Wang J. Effects of intratracheal administration of nuclear factor- $\kappa$ B decoy oligodeoxynucleotides on long-term cigarette smoke-induced lung inflammation and pathology in mice. *Respir Res* 2009; 10: 79.