

# Original article

## Cigarette smoke extract promotes proliferation of airway smooth muscle cells in asthmatic rats via regulating cyclin D1 expression

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**Keywords:** cigarette smoke extract; asthma; airway smooth muscle cells; proliferation; cyclin D1

**Background** Increased proliferation of airway smooth muscle cells (ASMCs) are observed in asthmatic patients and smoking can accelerate proliferation of ASMCs in asthma. To elucidate the molecular mechanisms leading to these changes, we studied *in vitro* the effect of cigarette smoke extract (CSE) on the proliferation of ASMCs and the expression of cyclin D1, an important regulatory protein implicated in cell cycle.

**Methods** ASMCs cultured from 8 asthmatic Brown Norway rats were studied. Cells between passage 3 and 6 were used in the study and were divided into control group, pcDNA3.1 group, pcDNA3.1-antisense cyclin D1 (ascyclin D1) group, CSE group, CSE+pcDNA3.1 group and CSE+pcDNA3.1-ascyclin D1 group based on the conditions for intervention. The proliferation of ASMCs was examined with cell cycle analysis, MTT colorimetric assay and proliferating cell nuclear antigen (PCNA) immunocytochemical staining. The expression of cyclin D1 was detected by reverse transcriptase-PCR (RT-PCR) and Western blotting.

**Results** (1) The percentage of S+G2M phase, absorbance value at 490 nm wavelength ( $A_{490}$ ) and the expression rate of PCNA protein in CSE group were  $(31.22\pm1.17)\%$ ,  $0.782\pm0.221$ ,  $(90.2\pm7.0)\%$  respectively, which were significantly increased compared with those of control group  $((18.36\pm1.02)\%$ ,  $0.521\pm0.109$ , and  $(54.1\pm3.5)\%$ , respectively) ( $P<0.01$ ). After the transfection with antisense cyclin D1 plasmid for 30 hours, the percentage of S+G2M phase,  $A_{490}$  and the expression rate of PCNA protein in ASMCs were much lower than in untreated cells ( $P<0.01$ ). (2) The ratios of  $A_{490}$  of cyclin D1 mRNA in CSE group was  $0.288\pm0.034$ , which was significantly increased compared with that of control group  $(0.158\pm0.006)$  ( $P<0.01$ ). After the transfection with antisense cyclin D1 plasmid for 30 hours, the ratios of  $A_{490}$  of cyclin D1 mRNA in ASMCs was much lower than in untreated cells ( $P<0.01$ ). (3) The ratios of  $A_{490}$  of cyclin D1 protein expression in CSE group was  $0.375\pm0.008$ , which was significantly increased compared with that of control group  $(0.268\pm0.004)$  ( $P<0.01$ ). After the transfection with antisense cyclin D1 plasmid for 30 hours, the ratios of  $A_{490}$  of cyclin D1 protein expression in ASMCs was much lower than in untreated cells ( $P<0.01$ ).

**Conclusion** CSE may increase the proliferation of ASMCs in asthmatic rats via regulating cyclin D1 expression.

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Smoking is an important risk factor involved in the occurrence and development of asthma. It can increase the incidence and severity of symptom of asthma.<sup>1</sup> At present, studies have showed that smoking can reduce the therapeutic effect of corticosteroids on asthma patients.<sup>2</sup> The cigarette smoke can increase airway acute allergic inflammation of guinea pig sensitized by ovalbumin (OVA).<sup>3</sup> In previous investigation, we found that cigarette smoke extract (CSE) could promote proliferation of passively sensitized human airway smooth muscle cells (ASMCs).<sup>4</sup> However, the mechanism has not been elucidated. Airway smooth muscle proliferation plays an important role in the airway remodeling in asthma;<sup>5-7</sup> and the repeatedly rhythmic expression of the cyclin D1 protein is crucial for the maintenance of normal cell proliferation. Cyclin D1 is a key factor in regulating transition from the G1 phase to the S phase in cell cycle,<sup>8</sup> and it also participates in cell cycle regulation of numerous cells. At present, little has been reported about whether CSE takes part in the regulation of the ASMCs proliferation via regulating cyclin D1 expression. This study investigated the effect of CSE on ASMCs proliferation and the probable

mechanism of CSE regulating ASMCs proliferation by expression of cyclin D1 based on the object of ASMCs in asthmatic rat.

### METHODS

#### Materials

Pathogen-free male Brown Norway rats weighing 200–250 g were provided by Experimental Animal Center, Huazhong University of Science and Technology, China.

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Marlboro cigarettes with filter (Philip Morris Cigarette Factory, USA), which contain 12 mg tar and 10 mg nicotine/cigarette, were used. Mouse monoclonal antibody against  $\alpha$ -smooth muscle actin ( $\alpha$ -SM-actin) and rabbit polyclonal antibody against  $\beta$ -actin were purchased from Santa Cruz Biotechnology Inc., USA. Rabbit polyclonal antibody against cyclin D1 was purchased from Abcam Biotechnology Inc., UK. Mouse monoclonal antibody against proliferating cell nuclear antigen (PCNA) was from Boster Biotechnology Co., China. Streptavidin-peroxidase (SP) kits were obtained from Zhongshan Biotechnology Co. Ltd., Beijing, China; TRIzol reagent and Lipofectamine<sup>TM</sup> 2000 were purchased from Invitrogen, USA. pcDNA3.1+ eukaryotic expression vector was preserved in our laboratory. The pcDNA3.1 plasmid carrying the antisense cyclin D1 (ascyclin D1) gene was constructed by ourselves previously.

### Establishment of the rats asthmatic model

Eight male Brown Norway rats were chosen randomly to duplicate the rat asthmatic model. Rats were actively sensitized by an intraperitoneal injection of 1 mg of OVA together with 200  $\mu$ g Al(OH)<sub>3</sub> in 0.5 ml 0.9% NaCl. One milliliter Bordetella pertussis vaccine containing  $6 \times 10^9$  heat-killed organisms was given intraperitoneally as an adjuvant on the first day of the study (day 0). An intraperitoneal injection of 1 mg OVA and 200  $\mu$ g Al(OH)<sub>3</sub> in 0.5 ml of 0.9% NaCl were given on day 7. On day 14–28, the rats were exposed to aerosolized OVA 3 times a week.<sup>9</sup> Challenges took place for 30 minutes in a 0.8-m<sup>3</sup> chamber, with free-breathing animals being exposed to 2% (w/v) OVA, which was generated by a 402T ultrasonic nebulizer (Siling Instrument, Shanghai, China) with 3 ml/min output. The rats showed symptoms of asthmatic attack such as fidget, bucking, accelerated breathing and mild cyanosis after the rats were challenged.

### Preparation of CSE

Following the method in reference,<sup>10</sup> cigarette smoke derived from one cigarette was drawn slowly into a 50-ml syringe and bubbled through 10 ml of sterile DMEM at room temperature. A cigarette yielded 6 draws of the syringe, with each individual draw taking approximately 6 seconds to complete. The resulting solution was adjusted to pH 7.4 with 0.5 mol/L concentrated NaOH and filtered through a 0.22- $\mu$ m-pore filter before dilution into DMEM as a percentage of the total volume. CSE was applied to ASMCs cultures within 30 minutes of preparation.

### Cell identification, culture and grouping

Primary rat ASMCs were obtained through enzyme digestion.<sup>11</sup> Cells between passage 3 and 6 were used in the experiment. Smooth muscle cells were identified through morphological observation and immuno-cytochemistry staining of  $\alpha$ -SM-actin.

ASMCs were seeded at a density of  $1 \times 10^5$  cells/ml in a

culture flask or  $1 \times 10^4$  cells/well in 96-well plates in DMEM with 10% fetal bovine serum (FBS). Cultivated cells, after reaching 70%–80% confluence, were serum-deprived for 24 hours to synchronize at the G0 phase. Subsequently, 10% FBS and DMEM were added to the cultures with or without various treatment for 24 hours. The ASMCs were divided into 6 groups according to different treatment: control group, pcDNA3.1 group, pcDNA3.1-antisense cyclin D1 (ascyclin D1) group, CSE group, CSE+pcDNA3.1 group and CSE+pcDNA3.1-ascyclin D1 group. pcDNA3.1 group, CSE+pcDNA3.1 group, pcDNA3.1-ascyclin D1 group and CSE+pcDNA3.1-ascyclin D1 group were transfected with Lipofectamine 2000 empty plasmid and antisense plasmid 6 hours before CSE treatment. Each group had 4 replicated wells. CSE of 5% concentration was used within 30 minutes of preparation. The related indicators were detected after treated by 5% CSE for 24 hours.

### Construction and transfection of antisense cyclin D1 recombinant plasmid

The constructed expression plasmids of the antisense cyclin D1 gene with the pcDNA3.1 plasmid were preserved in our laboratory. The antisense cyclin D1 full length fragment and the pcDNA3.1 vector were digested by *Bam*HI, *Xho*I, as Yan et al described.<sup>12</sup> Then, T4 DNA ligase ligated cyclin D1 fragment to pcDNA3.1 vector. The constructed expression plasmids were obtained after selected by ampicillin. The antisense cyclin D1 sequence was in the downstream of T7 promoter in constructed expression plasmids. Plasmid DNA was extracted using the Endo-free plasmid mini kit (Omega Bio-Tek, Lutterworth, Leicestershire, UK). Cells were seeded onto a 24-well plate followed by the transient transfection of the cyclin D1 recombinant plasmid. The transfection was performed with Lipofectamine 2000 according to the manufacturer's instructions.

### Analysis of ASMC cell cycle by flow cytometer

Cells in each group were collected and re-suspended in phosphate buffer solution (PBS) at  $1 \times 10^6$  cells/ml, then the suspension was fixed with precooled 75% ethanol for 12 hour at  $-20^\circ\text{C}$ . Fixed cells were stained with Propidium Iodide (PI) containing 5  $\mu$ g/ml ribonuclease (RNase) A for 30 minutes at room temperature in the dark, then the percentage of the cells at different phases in the cell cycle was analyzed by a Flow Cytometer (FACsort, BD, USA).

### Measurement of ASMCs proliferation by MTT colorimetric assay

MTT (5 mg/ml) was added to the wells (20  $\mu$ l/well) at the end of the experimental period. After a 4-hour incubation at room temperature, medium was removed from the well and DMSO was added (150  $\mu$ l/well). The plates were agitated at room temperature for 10 minutes. The value of absorbance of each well was read at 490 nm wavelength ( $A_{490}$ ) on an enzyme-linked immunosorbent assay reader.

### Detection of expression of PCNA protein in ASMCs by immunocytochemistry staining

The ASMCs were seeded onto 6-well plates at a density of  $1 \times 10^5$  cells/ml with covering slides, cultivated, and divided into groups as mentioned before. After fixed with cool absolute alcohol, the expression of PCNA was detected using a Streptavidin/peroxidase kit. The primary antibody was replaced with PBS in negative control. The criteria for judgment were that the cell positive for PCNA staining was brown in nuclei. On each slide, 200 cells were randomly counted under light microscope and then the positive percentage was calculated.

### Reverse transcriptase-PCR (RT-PCR)

Total RNA was isolated from the cells using TRIzol reagent according to the manufacturer's instruction. cDNA was generated by reverse transcription using oligo(dT) primer and moloney murine leukemia virus (MMLV) reverse transcriptase and then used as a template in the subsequent polymerase chain reaction analysis. The cyclin D1 primers were forward 5'-CTG ACT GCC GAG AAG TTG TG-3' and reverse 5'-GAG GGT GGG TTG GAA ATG A-3', and the size of amplified product is 248 bp. Forward 5'-ACC CAG ATC ATG TTT GAG ACC T-3' and reverse 5'-TCA GGA GGA GCA ATG ATC TTG-3' primers were used to amplify  $\beta$ -actin which has the size 644 bp. PCR reaction conditions were: a single predenaturation step at 94°C for 5 minutes, followed by 32 cycles of denaturation at 94°C for 40 seconds, annealing at 57°C for 40 seconds, and elongation at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were inspected by electrophoresis on 2.0% agarose gel. The  $A_{490}$  of each strap was measured and analyzed by Quantity one 4.6 (Bio-Rad Hercules, CA, USA). The results were expressed as the ratio of  $A_{490}$  of cyclin D1 mRNA to that of  $\beta$ -actin. The experiments were repeated 3 times.

### Western blotting

At the end of culture, cells were collected and lysed. After determining the protein concentrations, 20  $\mu$ l protein was loaded in each lane and subjected to SDS-PAGE gel (12%) electrophoresis, and then followed by transfer to cellulose nitrate membranes for immunoblotting. The membranes were blocked for 2 hours with Tris-Buffered saline with Tween-20 (TBST) containing 5% (w/v) non-fat dry milk, and then incubated with antibodies against cyclin D1 or against  $\beta$ -actin both at a dilution of 1:1000 overnight at 4°C. After washed with TBST, the membrane was incubated with horseradish peroxidase-conjugated secondary anti-rabbit antibody at a dilution of 1:10 000 for 1 hour. The immunoreactive bands were visualized with enhanced chemiluminescence and captured on X-ray film. Optical density of the bands was measured with a gel imaging analysis system. The results were expressed as the ratio of  $A_{490}$  of cyclin D1 protein expression to that of  $\beta$ -actin. The experiments were repeated 3 times.

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD). All

statistical analyses were performed with statistical software (SPSS for windows v13.0, Statistical Product and Service Solutions Inc., USA). *F*-test was used for the comparison of variance among several groups. The significance of difference between two groups was tested by *q* test. Differences were considered to be statistically significant at  $P < 0.05$ .

## RESULTS

### Cell identification

The cells were observed by an inverted phase-contrast microscope. They appeared spindle-shaped, as well as the characteristic "hill and valley" appearance (Figure 1). The cells were identified with anti-smooth muscle-actin antibody using an immunocytochemistry method to show that the cells were of airway smooth muscle origin (Figure 2).

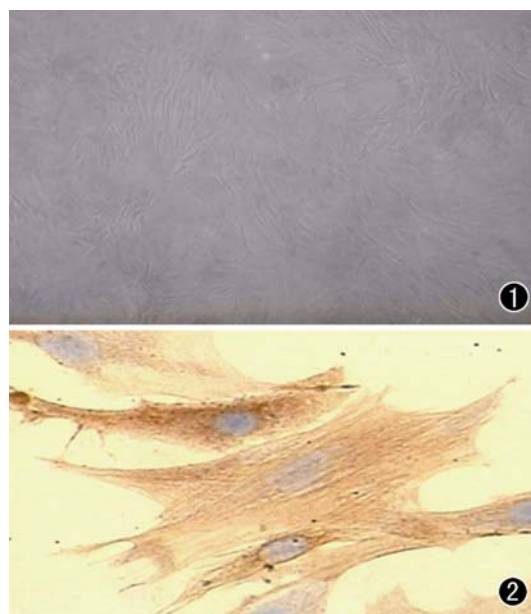


**Figure 1.** ASMCs displayed the characteristic "peak and valley" appearance (Original magnification  $\times 100$ , by phase contrast microscope).

**Figure 2.** Positive expression of ASMCs  $\alpha$ -SM-actin (Immunocytochemistry staining, original magnification  $\times 600$ ).

### Effect of CSE on ASMCs proliferation

#### Cell cycle analysis by flow cytometry

As illustrated in Table, as compared with control group, the proportion of cells at G0/G1 phase was decreased significantly ( $(68.78 \pm 1.53)\%$ ,  $P < 0.01$ ) in CSE group, while the proportion of cells at S+G2M phase was increased significantly ( $(31.22 \pm 1.17)\%$ ,  $P < 0.01$ ). Compared with control group, the proportion of cells at G0/G1 phase was increased obviously to  $(93.05 \pm 1.76)\%$  in antisense plasmid group, while the proportion of cells at S+G2M phase was decreased distinctly to  $(6.95 \pm 1.03)\%$  correspondingly ( $P < 0.01$ ). Compared with CSE group, the proportion of cells at G0/G1 phase was increased markedly and the proportion of cells at S+G2M phase was decreased significantly ( $P < 0.01$ ) in CSE+pcDNA3.1-ascyclin D1 group.

**Table.** Effect of 5% CSE on proliferation of ASMCs in asthmatic rat

| Groups              | n | Cell cycle (%)          |                         | MTT ( $A_{490}$ )        | PCNA (%)              |
|---------------------|---|-------------------------|-------------------------|--------------------------|-----------------------|
|                     |   | G0/G1                   | S+G2M                   |                          |                       |
| Control group       | 8 | 81.64±1.17 <sup>†</sup> | 18.36±1.02 <sup>†</sup> | 0.521±0.109 <sup>†</sup> | 54.1±3.5 <sup>†</sup> |
| PcDNA3.1 group      | 8 | 81.80±1.13              | 18.20±1.04              | 0.501±0.112              | 51.2±3.3              |
| Antisense group     | 8 | 93.05±1.76              | 6.95±1.03               | 0.301±0.082              | 28.8±3.0              |
| CSE group           | 8 | 68.78±1.53 <sup>*</sup> | 31.22±1.17 <sup>*</sup> | 0.782±0.221 <sup>*</sup> | 90.2±7.0 <sup>*</sup> |
| PcDNA3.1+CSE group  | 8 | 69.95±1.25              | 30.05±1.09              | 0.780±0.209              | 87.6±5.7              |
| Antisense+CSE group | 8 | 84.80±1.72 <sup>‡</sup> | 15.20±1.02 <sup>‡</sup> | 0.422±0.102 <sup>‡</sup> | 40.2±3.2 <sup>‡</sup> |

Cells from 8 rats were used for the experiments. Data are expressed as mean±SD. <sup>\*</sup> $P < 0.01$ , compared with control group; <sup>†</sup> $P < 0.01$ , compared with antisense group; <sup>‡</sup> $P < 0.01$ , compared with CSE group.

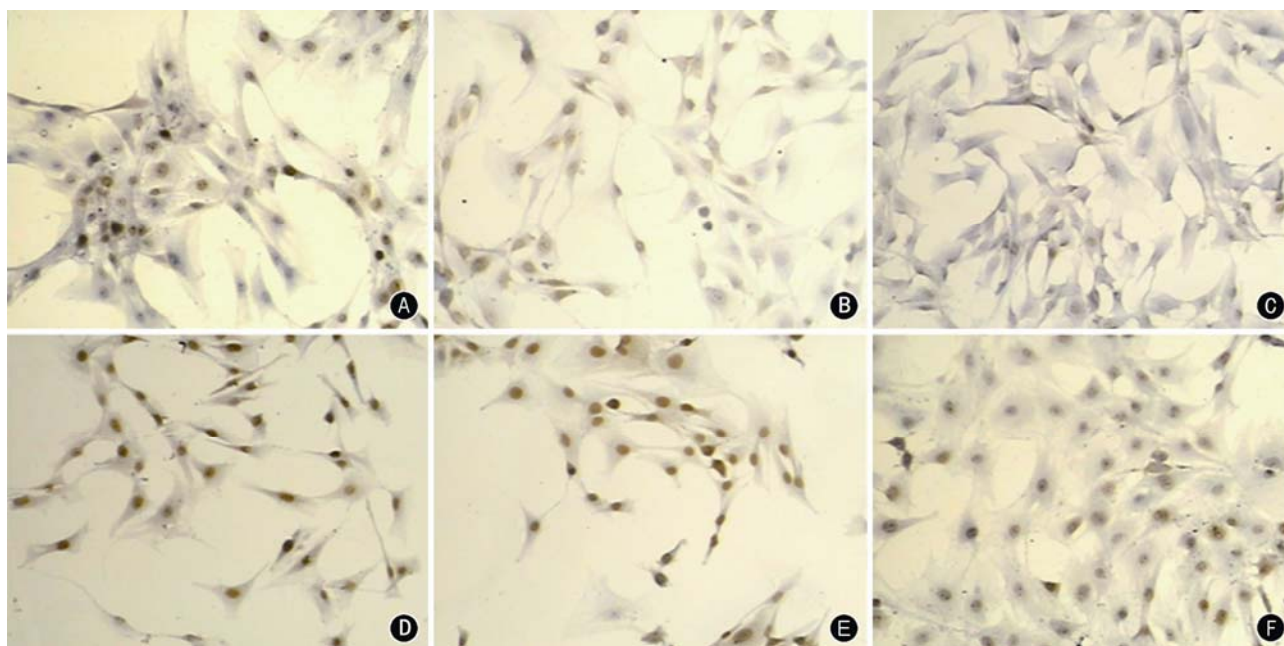


**Figure 3.** Effect of 5% CSE on expression of PCNA protein in ASMCs (Immunocytochemical staining, original magnification×100). **A:** Brown-yellow particles were observed in ASMCs nuclei in control group. **B:** The number and staining intensity of ASMCs positive for staining were moderate in pcDNA3.1 group. **C:** Amount of ASMCs positive for staining was reduced in pcDNA3.1-ascyclin D1 group. **D:** ASMCs positive for staining were very high in amount and staining intensity in CSE group. **E:** The number and staining intensity of ASMCs positive for staining were comparatively high in CSE+pcDNA3.1 group. **F:** Brown-yellow particles can be observed in few ASMCs nuclei in CSE+pcDNA3.1-ascyclin D1 group.

#### Measurement of ASMCs proliferation by MTT colorimetric assay

As show in Table,  $A_{490}$  in CSE group was increased significantly (0.782±0.221) as compared with that in control group (0.521±0.109). There was a significant difference between two groups ( $P < 0.01$ ). As compared with control group,  $A_{490}$  in antisense plasmid group was decreased significantly (0.301±0.082) ( $P < 0.01$ ). The  $A_{490}$  in CSE+pcDNA3.1-ascyclin D1 group was decreased distinctly as compared with CSE group. There was a significant difference between two groups ( $P < 0.01$ ).

#### Expression of PCNA in ASMCs

As shown in Table and Figure 3, compared with expression rate of PCNA in control group ((54.1±3.5)%), the expression rate of PCNA in CSE group was increased significantly ((90.2±7.0)%) ( $P < 0.01$ ). As compared with control group, the expression rate of PCNA in antisense plasmid group was decreased significantly ((28.8±3.0)%) ( $P < 0.01$ ). The expression rate of PCNA in CSE+pcDNA3.1-ascyclin D1 group was decreased

significantly as compared with CSE group ( $P < 0.01$ ).

#### Effect of CSE on cyclin D1 mRNA expression in ASMCs

As shown in Figure 4, compared with the ratios of  $A_{490}$  of cyclin D1 mRNA in control group (0.158±0.006), the ratios of  $A_{490}$  of cyclin D1 mRNA in CSE group was increased significantly (0.288±0.034) ( $P < 0.01$ ). Compared with control group, the ratio of  $A_{490}$  of cyclin D1 mRNA in antisense plasmid group was decreased distinctly (0.102±0.010) ( $P < 0.01$ ). The ratio of  $A_{490}$  of cyclin D1 mRNA in CSE+pcDNA3.1-ascyclin D1 group was decreased remarkably as compared with that in CSE group ( $P < 0.01$ ).

#### Effect of CSE on cyclin D1 protein expression in ASMCs

As shown in Figure 5, the ratio of  $A_{490}$  of cyclin D1 protein expression in CSE group was increased significantly (0.375±0.008) as compared with that in control group (0.268±0.004) ( $P < 0.01$ ). The ratio of  $A_{490}$

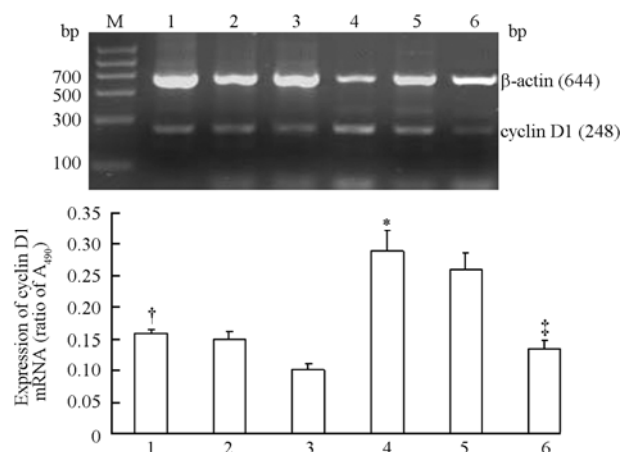


**Figure 4.** Effect of 5% CSE on cyclin D1 mRNA expression in ASMCs. 1: control group; 2: pcDNA3.1 group; 3: pcDNA3.1-ascyclin D1 group; 4: CSE group; 5: CSE+pcDNA3.1 group; 6: CSE+pcDNA3.1-ascyclin D1 group. Cells from 8 different rats were used for the experiments. Data are expressed as mean±SD. \* $P < 0.01$ , compared with control group; <sup>†</sup> $P < 0.01$ , compared with antisense group; <sup>‡</sup> $P < 0.01$ , compared with CSE group. M: marker.

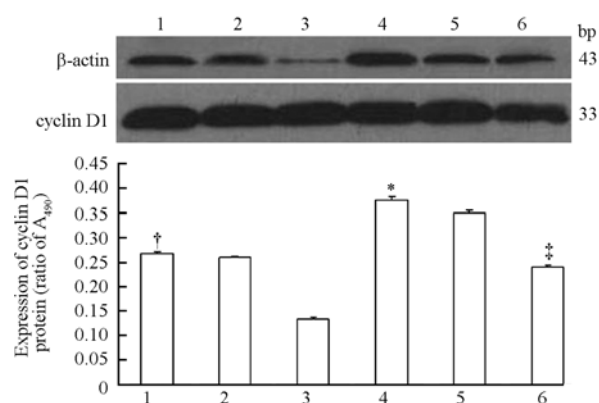


**Figure 5.** Effect of 5% CSE on cyclin D1 protein expression in ASMCs. 1: control group; 2: pcDNA3.1 group; 3: pcDNA3.1-ascyclin D1 group; 4: CSE group; 5: CSE+pcDNA3.1 group; 6: CSE+pcDNA3.1-ascyclin D1 group. Cells from 8 different rats were used for the experiments. Data are expressed as mean±SD. \* $P < 0.01$ , compared with control group; <sup>†</sup> $P < 0.01$ , compared with antisense group; <sup>‡</sup> $P < 0.01$ , compared with CSE group.

of cyclin D1 protein expression in antisense plasmid group was decreased significantly ( $0.135 \pm 0.002$ ) as compared with control group ( $P < 0.01$ ). The ratio of A<sub>490</sub> of cyclin D1 protein expression in CSE+pcDNA3.1-ascyclin D1 group was decreased obviously as compared with that in CSE group ( $P < 0.01$ ).

## DISCUSSION

Airway remodeling is one of the important characteristics of asthma as well as the significant pathology base of the irreversible airflow obstruction and airway hyperresponsiveness,<sup>13</sup> among which the proliferation of ASMCs play a key role in airway remodeling.

Cigarette smoke contains many toxic substances,

including nicotine, tar, benzo-pyrene, quinonoid compound and oxidants,<sup>14</sup> among which nicotine can promote DNA synthesis and cell proliferation. In this study, we used CSE to stimulate primary cultured ASMCs in asthmatic rats *in vitro* and evaluated the effect of CSE on ASMCs proliferation through MTT colorimetric assay, PCNA immunocytochemical staining and cell cycle analysis. We found that compared with control group, the proliferation of rat ASMCs accelerated obviously, percentage of cells at S+G2M phase increased distinctly, and percentage of cells at G0/G1 phase decreased significantly in CSE stimulated group. These results indicated that CSE could promote the proliferation of ASMCs in asthmatic rat model. Similar conclusion was obtained in the investigation of the effect of CSE on proliferation of rat pulmonary artery smooth muscle cells by Hu et al.<sup>15</sup>

The process that various of mitogenic factors outside the cell promote the proliferation of ASMCs through different signal transduction pathway is finally implemented through the accomplishment of cell division cycle.<sup>16</sup> The process of cell cycle depends on precise and rigorous regulation caused by levels of regulatory factors to cell cycle.<sup>17</sup> There are two critical restrictive sites in cell cycle: G1/S phase and G2M phase.<sup>18</sup> Cyclin D plays a leading role at G1 phase, at early of which cyclin D is synthesized; and the concentration of cyclin D reaches the summit at the anaphase of G1. Cyclin D is degraded before it synthesizes cyclinE.<sup>19</sup> The compound conjugated by cyclin D and cyclin dependent kinase (CDK<sub>4</sub>/CDK<sub>6</sub>) at G1 phase can cause the phosphorylation of retinoblastoma (Rb) family. The phosphorylation of Rb leads itself separated from nuclear factor E2F and drives the cell into S phase from G1 phase which promote the cell proliferation.<sup>20-22</sup> Cyclin D1 is an isoforms of cyclin D, which is required in proliferation of most tissue and has been lucubrated deeply. When ASMCs in asthmatic rat were transfected by plasmid expressing antisense cyclin D1 and blank plasmid, we found that the ability of proliferation of ASMCs in antisense plasmid transfected group was impaired significantly, and this impairment could not be reversed or recovered even stimulated by CSE. This revealed that the proliferation of ASMCs can be inhibited through the inhibition of the activity of cyclin D1. Thus cyclin D1 plays an important role in the process of ASMCs proliferation. Our research showed that after stimulated by CSE, the expressions of cyclin D1 in asthmatic rat ASMCs were increased significantly both at transcription and translation level, and the cell proliferation accelerated evidently. The above impact caused by CSE could be inhibited obviously by transfecting antisense cyclin D1 plasmid, disclosing CSE may promote the proliferation of ASMCs through promoting the expression of cyclin D1. The mechanism may be following: some airway inflammations caused by chemical substance in CSE which can stimulate inflammatory cells to secrete manifold mediators of inflammation, the latter transmit signals through signal

transduction pathway and finally act on cyclin to influence the proliferation of ASMCs.

In conclusion, the present investigation demonstrates CSE can promote the proliferation of ASMCs in asthmatic rat. Cyclin D1 plays an important role in promoting the proliferation of ASMCs in asthmatic rat. CSE probably affect the proliferation of ASMCs through regulating the expression of cyclin D1. All these findings suggest that cyclin D1 may be a new therapeutic target for the treatment of ASMCs proliferation and airway remodeling in asthma patients.

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