Decitabine enhances stem cell antigen-1 expression in cigarette smoke extract-induced emphysema in animal model

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Abstract

Stem cell antigen-1 (*Sca-1*) is a mouse glycosyl phosphatidylinositol-anchored protein and a cell surface marker found on hematopoietic stem cells (HSCs). Despite decades of study, its biological functions remain little known. *Sca-1* is a typical marker of bone marrow-derived HSCs, it is also expressed by a mixture of tissue-resident stem, progenitor cells in nonhematopoietic organs. Endothelial progenitor cell (EPC) is a subtype of HSC and contributes to endothelial repair by homing in on locations of injury. Abnormal genetic methylation has been detected in smoking-related diseases. The present study aimed to investigate the lung function and histomorphology, the expression of *Sca-1* gene in lung tissues, and bone marrow-derived EPCs in cigarette smoke extract (CSE)-induced emphysema mice, and to further determine whether Decitabine (Dec), the most widely used inhibitor of DNA methylation, could protect against the damages caused by CSE. The results of the present study demonstrated that Dec could partly protect against CSE-induced emphysema in mice, enhance *Sca-1* expression in lung tissue, and bone marrow-derived EPCs. The results suggested that the depletion of the progenitor cell pool and DNA methylation of *Sca-1* gene may be involved in the progression of emphysema in mice.

Keywords: Cigarette smoke, Decitabine, emphysema, endothelial progenitor cells, stem cell antigen-1

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Introduction

Stem cell antigen-1 (*Sca-1*) is an 18-kDa mouse glycosyl phosphatidylinositol-anchored cell surface protein (GPI-AP) of the *Ly6* gene family, expressed on the surface of all hematopoietic stem cells (HSCs).¹ Forty years ago, *Sca-1* was first defined as an antigen with increased expression in activated lymphocyte, and being encoded by two species-specific allelic genes: *Ly-6A.2* and *Ly-6E.1*.² Over 20 years ago, *Sca-1*⁺ cells were considered as primitive HSCs and used for colony forming unit of HSCs.^{3,4} Over 10 years ago, *Sca-1*, combined with other markers, was used for purification and identification of HSCs.^{5,6} Despite decades of study, its biological functions remain little known. Although *Sca-1* is a typical marker of bone marrow-derived HSCs, and it also expressed in nonhematopoietic organs, outside of the hematopoietic system, *Sca-1* is similarly expressed by a mixture of tissue-resident stem, progenitor, and differentiated cell types in a wide variety of tissues and organs.¹

Bone marrow-derived endothelial progenitor cell (EPC), a subtype of HSC, plays a fundamental role in the maintenance of endothelial integrity and function, postnatal

vasculogenesis, vascular repairment and tissue regeneration through pivotal bioactivities, differentiating into endothelial cell (EC) and secretion of vasoactive substances that promote angiogenesis, and maintain vascular homeostasis.^{7,8} Our previous study showed decreased and dysfunctional circulating EPCs in patients with chronic obstructive pulmonary disease (COPD).⁹

The advent of genome-wide epigenetic studies allowed for a more comprehensive study of the epigenome in many diseases including pulmonary disease.¹⁰ Methylation is the most abundant epigenetic modification that directly affects the DNA molecule in eukaryotes. Bioinformatics analysis (<http://www.urogene.org/methprimer/>) implied that there were CG dinucleotides in promoter region of *Sca-1* gene, which suggested that the expression of *Sca-1* gene may be regulated by methylation. Decitabine (Dec) is the most widely used inhibitor of DNA methylation which trigger demethylation leading to a consecutive reactivation of epigenetically silenced genes *in vitro* and *in vivo*.¹¹ In the present study, we investigated the lung function and histomorphology, the expression of the *Sca-1* gene in lung tissues and bone marrow-derived EPCs in cigarette smoke extract

(CSE)-induced emphysema mice, and further determine whether Dec could protect against the damages caused by CSE.

Materials and methods

Animals

Total of 40 male C57BL/6J mice aged 4–6 weeks old were randomly enrolled in this study. All animals were purchased from Shanghai laboratory animal center of Chinese Academy of Sciences (SLACCAS, Shanghai, China) and fed in a cleaning unit with 23–25°C, 50–60% humidity, and 12 h rhythm of night and day. They were provided free access to water and food. The study was approved by the Institutional Review Board of Central-South University and conformed to the guiding principles for research involving animals and human beings.¹²

Preparation of CSE

The CSE was prepared according to a previously published method¹³ with some modifications. Briefly, one non-filtered Fu-Rong cigarette (Tar: 13 mg, nicotine: 1.0 mg, carbon monoxide: 14 mg/cigarette, China Tobacco Hunan Industrial Co., Ltd., Changsha, China) was burned and the smoke passed through 4 mL of phosphate-buffered saline (PBS) by connecting to a vacuum pump with the constant pressure of -0.1 kPa. This product was further filtered through a filter with $0.22\text{-}\mu\text{mol/L}$ pores (Fisher, Hampton, NH) to remove particles and bacteria and used for intraperitoneal injection. The solution was prepared fresh for each injection.

Preparation of Dec

Five grams of Dec powder (Sigma, USA) were dissolved in 2 mL PBS and further diluted to 25 mg/mL, subpackaged, and stored under -80°C until experiments.

Animal modeling

We did pre-experiment as previously described to establish the doses of CSE¹⁵ and Dec¹⁴ for injection. The mouse emphysema model was established as previously described.¹⁴ Briefly, the C57BL/6J mice were divided into four groups: control group, Dec group, Dec + CSE group, and CSE group ($n=10$ per group). The total experimental period was four weeks. The mice were injected intraperitoneally with PBS, CSE, or Dec on day 0, 11, 15, 17, 19, 22 (Table 3). The dose of intraperitoneal injection of PBS, CSE, Dec were 0.3 mL/20 g, 0.3 mL/20 g, and 2.5 mg/kg (0.3 mL/20 g constant volume) respectively. On day 28, the mice were disposed for the measurement of lung function.

Lung function measurement

Measurement of lung function was performed using small animal spirometer (PLY3211 system, Buxco Electronics, USA) as previously described with a minor modification.¹⁵ Briefly, the mouse was weighed, anesthetized by intraperitoneal injection of 10% chloral hydrate (3 mL/kg body weight), and tracheostomized. The trachea was cannulated, and the cannula was connected to the computer-controlled

small animal spirometer. Airway resistance (Raw), lung dynamic compliance (Cdyn), peak expiratory flow (PEF), and inspiratory time/respiratory time (Ti/Te) were measured according to the manufacturer's instruction.

Histomorphological detection

After lung function measurement, animals were sacrificed by overdose of anesthetization. Lung slices were stained with hematoxylin and eosin (H&E) (Sigma). Emphysema was quantified based on the measurement of the mean linear intercept (MLI) and destructive index (DI) in micrometers. The MLI was measured by dividing the length of a line drawn across the lung section by a total number of intercepts counted within this line at $\times 100$ magnification. The DI was calculated by dividing the defined destructive alveoli by the total number of alveoli counted. Destructive alveolus was defined if at least one of the following alveoli was observed: alveolar wall defects; intraluminal parenchymal rags in alveolar ducts; obviously abnormal morphology; and typically emphysematous changes. The analysis was performed using a microscopic point-count technique at $\times 200$ magnification.¹³ The histomorphology assessment was performed in blinding fashion, in another words, the slide readers were blinded to the specimen processing.

Isolation, culture, and identification of EPCs

Mononuclear cells (MNCs) from bone marrow of C57BL/6J mice was isolated by Ficoll density gradient centrifugation (Histopaque-1083, Sigma), and then inoculated into culture flask with density of $(3\text{--}5) \times 10^6/\text{mL}$, cultured with endothelial growth medium-2 (EGM-2) in presence of 5% fetal bovine serum (SingleQuots®, Lonza, Switzerland) under an atmosphere of 95% humidity, 5% CO₂ at 37°C.¹⁶ The culture fluid was replaced totally by fresh culture medium in day 4 of the culture to remove the unattached cells. Then half replacement by the fresh medium was performed every three days. The cell harvest was performed on day 7 of the culture. EPCs were characterized by double-positive staining with ulex europaeus agglutinin-1 (UEA-1) lectin and acetylated low-density lipoprotein (acLDL).¹⁷ Briefly, after being washed with PBS, the slides of cells were incubated with $7.5\text{ }\mu\text{g/mL}$ 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled acLDL (DiI-acLDL, Molecular Probes, USA) at 37°C for 4 h and fixed with 4% paraformaldehyde for 10 min. After being washed again, the cells were treated with $10.0\text{ }\mu\text{g/mL}$ fluorescein isothiocyanate (FITC)-labeled UEA-1 (FITC-UEA-1, Sigma) for 30 min. Finally, the cells were treated with $1\text{ }\mu\text{g/mL}$ 4',6-diamidino-2-phenylindole (DAPI) for 5 min before identification was performed using a laser scanning confocal microscope (LSCM, Olympus, Japan). Fifteen random view-fields were involved to calculate the positive rate of amphiphilic cells.

Western blotting

Briefly, EPCs were washed three times with ice cold PBS, then lysed in RIPA lysate (Applygen Technologies Inc,

Beijing, China) for 30 min on ice. Lung tissues were homogenized manually in a glass homogenizer and lysed in RIPA lysate for 30 min on ice. Solution of EPCs or lung tissue was centrifuged at 4°C, 12,000 g for 5 min. Bicinchoninic acid assay (BCA) protein quantitation kit (Wellbio Inc, Changsha, China) was used for protein measurement. Protein (30–60 µg) was mixed 1:1 with 2× sodium dodecyl sulfate (SDS) loading buffer (20% glycerol, 4% SDS, 3.12% dithiothreitol, 0.2% bromophenol blue, and 0.1 mol/L Tris HCl, PH 6.8, all from Sigma), and incubated at 100°C for 4 min. Equal amount of proteins for each sample were separated by 10–12% SDS-polyacrylamide gel run at 120 V for 90 min and blotted onto a polyvinylidene difluoride micro-porous membrane (Millipore, Billerica, MA). Membranes were incubated with a 1:200 dilution of primary antibody (mouse monoclonal antibody, Biolegend, USA) overnight, and then washed for three times with Tris-buffered-saline with Tween (TBS-T) and revealed using secondary anti-rat antibody with horseradish peroxidase conjugate (1:3000, 1 h) followed by washing with TBS-T again. Immunoreactive bands were developed using ECL chemiluminescent substrate (Thermo, USA).

RNA extraction and quantitative real-time PCR

Total RNA was extracted from the tissues or cells using Trizol reagent (Invitrogen, USA). First-strand cDNA was synthesized using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, USA) according to the manufacturer's instructions, and used as the template for quantitative RT-PCR analysis. DNase-treated samples were subjected to RT-PCR using SYBR Green qPCR Master Mix (Applied Biosystems, USA) on Bio-rad CFX96 Real Time system (Bio-Rad, USA) with β-actin used as an internal control. The PCR amplification conditions consisted of 10 min at 95°C followed by 40 cycles of denaturation step at 95°C for 15 s, and annealing and extension for 1 min at 60°C. Data were analyzed using the comparative Ct ($\Delta\Delta Ct$) method.¹⁸ The primer sequences were as follow: β-actin, 5'-CAT CCTGCGTCTGGACCTGG-3' (forward), and 5'-TAATGT CACGCACGATTTCC-3' (reverse); Sca-1, 5'-ACACCGAG CCCAGGTAACCC-3' (forward), and 5'-CTGGTCCGC TCAGGACAGCA-3' (reverse).

Statistical analysis

Analyses were performed using SPSS for Windows 16.0 (SPSS Inc., Chicago, IL). All data were expressed as means ± standard deviation (SD). Analysis of differences among groups were performed using analysis of variance (one-way ANOVA), followed by *post hoc* analysis as appropriate. Values of $P < 0.05$ were considered statistically significant.

Results

Lung function

As shown in Figure 1, abrupt ascents were noted in the maximal expiratory flow volume curves of the Dec + CSE group (Figure 1c) and CSE group (Figure 1d), the descending limbs of which demonstrated prolonged expiratory

phases compared with those of the controls (Figure 1a) and Dec group (Figure 1b).

Quantitatively, Raw was significantly higher in the Dec + CSE group ($P < 0.01$) and CSE group ($P < 0.01$) compared with the controls. However, Raw was significantly lower in the Dec + CSE group compared with the CSE group ($P < 0.05$). Cdyn, PEF, and Ti/Te were significantly lower in Dec + CSE group ($P < 0.01$ or $P < 0.05$) and CSE group ($P < 0.01$) compared with the controls. Cdyn was significantly higher in the Dec + CSE group compared with the CSE group ($P < 0.05$). There were no significant differences between the Dec + CSE group and the CSE group regarding PEF and Ti/Te values ($P > 0.05$). There were no significant differences between the controls and the Dec group regarding any of the parameters described above ($P > 0.05$) (Table 1).

Lung histomorphology

As shown in Figure 2, the lung tissues of the Dec + CSE group (Figure 2c) and CSE group (Figure 2d) exhibited enlarged alveolar spaces, thinner alveolar septa, and damaged alveolar walls. Alveolar spaces appeared to be smaller in the Dec + CSE group compared with the CSE group. Quantitative analysis showed that MLI and DI were significantly higher in the Dec + CSE group and CSE group compared with the controls ($P < 0.01$). However, MLI and DI were significantly lower in the Dec + CSE group compared with the CSE group ($P < 0.01$ or $P < 0.05$). There were no significant differences between the controls and the Dec group in any of the parameters described above ($P > 0.05$) (Table 2).

Culture and identification of EPCs

As shown in Figure 3, on day 1 of the culture, the MNCs isolated from bone marrow were round and were similar in size, the cells were suspended in the culture media (Figure 3a). On day 4 of the culture, the cells appeared to be clustered together; they were larger in size and were oval, spindle or polygonal in shape (Figure 3b). On day 7 of the culture, the cells followed fusiform or polygonal patterns and contacted each other in an attempt to form a capillary structure (Figure 3c). LSCM demonstrated that the cells on day 7 of the culture displayed red cytoplasm when taking up Dil-acLDL (Figure 4a), green cytomembrane when binding FITC-UEA-1 (Figure 4b), and orange cells when taking up both Dil-acLDL and binding FITC-UEA-1 (Figure 4c). The positive rate of amphophilic cells was 95.39 ± 3.03 on day 7 of the culture.

Expression levels of Sca-1 protein in lung tissues and bone marrow-derived EPCs

As shown in Figure 5, the expression levels of the Sca-1 protein in lung tissues and EPCs were significantly lower in the Dec + CSE group ($P < 0.01$) and CSE group ($P < 0.01$) compared with the controls. However, the expression levels of the Sca-1 protein in lung tissue and EPCs were significantly higher in the Dec + CSE group compared with the CSE group ($P < 0.01$). Notably, the expression levels of the Sca-1 protein in EPCs were significantly lower in the Dec group compared with the controls ($P < 0.05$).

Figure 1 Maximal expiratory flow volume curve. Of each picture, upper part is expiratory phase and lower part is inspiratory phase. Compared with controls (a) and Dec group (b), Dec + CSE group (c), and CSE group (d) have a abrupt ascending and the descending limb concave to the axis showing prolonged expiratory phase. Dec, Decitabine; CSE, cigarette smoke extract

Table 1 Lung function

	Raw (cm H ₂ O·mL ⁻¹ ·min ⁻¹)	Cdyn (mL/cm H ₂ O)	PEF (mL/s)	Ti/Te
N (n = 10)	0.59 ± 0.10	1.01 ± 0.17	4.47 ± 0.72	0.88 ± 0.14
Dec (n = 10)	0.56 ± 0.10	1.00 ± 0.17	4.33 ± 0.75	0.85 ± 0.16
Dec + CSE (n = 10)	1.85 ± 0.34†‡	0.67 ± 0.10†‡	3.36 ± 0.61†‡	0.73 ± 0.12*
CSE (n = 10)	2.46 ± 0.44†‡§	0.52 ± 0.10†‡§	2.81 ± 0.53†‡	0.62 ± 0.11†‡

Lung function of C57BL/6J mouse was measured 28 days after the start of the experiment. N, normal control; Dec, Decitabine; CSE, cigarette smoke extract; Raw, airway resistance; Cdyn, lung dynamic compliance; PEF, peak expiratory flow; Ti, inspiratory time; Te, expiratory time. Values are means ± standard deviation.

* $P < 0.05$ vs. N.

† $P < 0.01$ vs. N.

‡ $P < 0.01$ vs. Dec.

§ $P < 0.05$ vs. Dec + CSE.

Expression levels of *Sca-1* mRNA in lung tissues and bone marrow-derived EPCs

As shown in Figure 6, the expression levels of *Sca-1* mRNA in lung tissues and EPCs were significantly lower

in the Dec + CSE group ($P < 0.01$) and the CSE group ($P < 0.01$) compared with the controls. The expression levels of *Sca-1* mRNA in lung tissue and EPCs were significantly higher in the Dec + CSE group compared with the

Figure 2 Histomorphological changes of lung tissues. Lung tissues of Dec + CSE group (c) and CSE group (d) exhibited enlarged alveolar space, thinner alveolar septum, and destroyed alveolar wall when compared with controls (a) and Dec group (b). Alveolar space of Dec + CSE group (c) seems to be smaller than that of CSE group (d). Scale bar represents 50 μ m. Dec, Decitabine; CSE, cigarette smoke extract

Table 2 Lung histomorphology

	MLI (μ m)	DI (%)
N (n = 10)	28.95 $\pm$ 4.66	10.22 $\pm$ 1.28
Dec (n = 10)	28.14 $\pm$ 4.60	10.22 $\pm$ 1.34
Dec + CSE (n = 10)	53.06 $\pm$ 7.64*†	34.55 $\pm$ 5.85*†
CSE (n = 10)	67.16 $\pm$ 9.15*†§	43.58 $\pm$ 6.60*†‡

N, normal control; Dec, Decitabine; CSE, cigarette smoke extract; MLI, mean linear intercept; DI, destructive index. Values are means $\pm$ standard deviation.

* $P < 0.01$ vs. N.

† $P < 0.01$ vs. Dec.

‡ $P < 0.05$ vs. Dec + CSE.

§ $P < 0.01$ vs. Dec + CSE.

CSE group ($P < 0.05$). However, the expression levels of *Sca-1* mRNA in lung tissue were significantly lower in the Dec group compared with the controls ($P < 0.01$).

Discussion

The present study demonstrated that the expression levels of *Sca-1* protein and *Sca-1* mRNA were significantly decreased in the lung tissue in emphysema mice, which was consistent with what was seen in bone marrow-derived EPCs. Dec could partly protect against CSE-induced emphysema in mice, enhance *Sca-1* expression in lung tissue and bone marrow-derived EPCs. These findings suggested that the depletion of the progenitor cell pool and DNA methylation of *Sca-1* gene may be involved in the progression of emphysema in mice.

Sca-1 was used as a candidate marker in the search for tissue-resident stem cells.¹ *Sca-1* immunohistochemistry revealed a linear staining pattern on the endothelial surface of large and small pulmonary arteries and veins and alveolar capillaries. Even more, the change of *Sca-1* was consistent with the number of cells which derived from bone marrow, thereby marking cells of bone marrow origin.¹⁹ *Sca-1*+ cells improve endothelial dysfunction compared to *Sca-1* depleted cells.¹⁷ So, *Sca-1* may be responsible for the repairment of lung tissue in emphysema mice and play a role in wound healing of lung tissue. In the present study, decreased expressions of *Sca-1* were observed both in bone marrow-derived EPCs and lung tissues in emphysema mice. EPCs are the precursors of ECs. Accumulating evidence indicated that EPCs derived from bone marrow

contribute to re-endothelialization of injured vessels as well as neo-vascularization of ischemic lesions in either a direct or an indirect way under physiological and pathological conditions.^{20,21} EPCs were demonstrated to be required for tissue repairing and airway remodeling in lungs.^{22,23} Peripheral infusion of bone marrow-derived EPCs was helpful for vascular repair or damage healing processes by homing in impaired location.²⁴ So, the results of the present study suggested that the decreased *Sca-1* expression in EPCs may contribute to the emphysema together with the direct *Sca-1* suppression in lung tissue.

Study on the expression of *Sca-1* gene in the lung of variously aged CD-1 mice showed that the expression of *Sca-1* tends to decrease with age in the postnatal life.²⁵ Interestingly, the number and function of EPCs in young mice were superior to those in old mice.²⁶ Furthermore, there was an inverse relationship between age and levels of circulating EPCs in healthy humans aged 1–81 years.²⁷ Our previous study showed decreased and dysfunctional circulating EPCs in patients with COPD.⁹ Thereby, the results of the present study were consistent with the theory that COPD or at least emphysema represents accelerated lung aging induced in part by oxidative damage from cigarette smoke components.^{28,29} Decreased *Sca-1* may indicate that an individual's youth is slipping away, and the aging process is progressing. The present study was also useful for understanding the association between emphysema, a degenerative disease, and the depletion in the progenitor cell pool.

Sca-1 is the most common marker used to enrich adult HSCs. Embryonic and fetal *Sca-1* expression is consistent with the emergence and expansion of definitive HSCs.¹ More and more researchers considered that *Sca-1* not only was a surface marker of stem/progenitor cells, but played an important role in self-renew and differentiation of stem/progenitor cells. *Sca-1* has been shown to be necessary for HSC activity and play a role in HSC self-renewal.³⁰ *In vivo* and *in vitro* analyses demonstrated that *Sca-1* was required directly for the self-renew of mesenchymal progenitors, and *Sca-1*^{−/−} mice exhibit age-related osteoporosis characterized by deterioration in bone material, microarchitectural, and mechanical properties.³¹ *Sca-1* knockout impairs myocardial progenitor cell function, resulting in early-onset cardiac contractile deficiency.³² *Sca-1* was demonstrated to be close associated with c-Kit signaling in stem and progenitor cells.³⁰ C-Kit/c-Kit ligand interaction of bone

Figure 3 The morphological changes of bone marrow-derived EPCs during culture. On day 1 of the culture, the cells formed round, the sizes of cells were almost same, and the cells were suspended in the culture medium (a). On day 4 of the culture, the cells attempted to attach each other, and their sizes were getting enlarged, and their shapes became oval, spindle or polygonal (b). On day 7 of the culture, the cells shaped fusiform or polygon patterns and contacted each other to attempt to form capillary structure (c). Scale bar represents 50 μ m. EPCs, endothelial progenitor cells

Table 3 Experiment schedule

Day	0	11	15	17	19	22
N (<i>n</i> = 10)	PBS	PBS	PBS	PBS	PBS	PBS
Dec (<i>n</i> = 10)	PBS	PBS	Dec	Dec	Dec	PBS
Dec + CSE (<i>n</i> = 10)	CSE	CSE	Dec	Dec	Dec	CSE
CSE (<i>n</i> = 10)	CSE	CSE	PBS	PBS	PBS	CSE

All treatments were conducted by intraperitoneal injection. All animals were disposed on day 28 after the start of treatment. N, normal control; Dec, Decitabine; CSE, cigarette smoke extract.

Figure 4 The identification of EPCs by double positive staining with Dil-acLDL and FITC-UEA-1. The laser scanning confocal microscope (LSCM) illustrated that the cells on day 7 of the culture displayed red cytoplasm when uptaking Dil-acLDL (a), green cytomembrane when binding FITC-UEA-1 (b), orange when double positively staining with Dil-acLDL and FITC-UEA-1 (c), and blue when staining with DAPI in nuclear localization (d). Scale bar represents 10 μ m. Dil, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; acLDL, acetylated low density lipoprotein; FITC, fluorescein isothiocyanate; UEA-1, ulex europaeus agglutinin-1; DAPI, 4',6'-diamidino-2-phenylindole

Figure 5 Expressions of *Sca-1* protein in lung tissues and EPCs. There were pictures of expressions of *Sca-1* protein in lung tissues (a) and EPCs (b), and corresponding quantitative analysis (c). *Sca-1*, stem cell antigen-1; N, normal controls; D, Dec, Decitabine; C, CSE, cigarette smoke extract; EPCs, endothelial progenitor cells. $\star P < 0.05$ vs. group N; $\star P < 0.01$ vs. group N; $\Delta P < 0.05$ vs. group Dec; $\blacktriangle P < 0.01$ vs. group Dec; $\blacklozenge P < 0.01$ vs. group Dec + CSE

Figure 6 Expressions of *Sca-1* mRNA in lung tissues and EPCs. *Sca-1*, stem cell antigen-1; EPCs, endothelial progenitor cells; N, normal control; Dec, Decitabine; CSE, cigarette smoke extract. $\star P < 0.01$ vs. group N; $\blacktriangle P < 0.01$ vs. group Dec; $\blacklozenge P < 0.01$ vs. group Dec + CSE

marrow-derived EPCs was influenced in a CSE-induced emphysema model.³³ EPCs with normal self-renew, proliferatory capacity, differentiative potential are essential for their own trans-endothelia shift, homing to injured location, repairment, and regeneration of various tissues and organs. Oxidative stress could lead to premature EPCs “aging”.³⁴ Our previous study showed that the chronic stimulation of CSE decreased the proliferation capacity of EPCs.¹⁶ The problem whether there is any relevance between *Sca-1* expression and EPCs function needs further study.

The in-depth study of epigenetics benefits the research on mechanisms in many diseases. In COPD, the produced

oxidative stress induced by cigarette smoking further damages the lung leading to acquired genetic changes, including DNA methylation, due to an inefficient DNA repair machinery.³⁵ DNA methylation was catalyzed by DNA methyltransferases (DNMTs) family³⁶ and played an important role in maintaining cell's identity by affecting the gene expression. Dec, an S-phase-specific inhibitor of DNMT, inhibits DNMT and demethylates DNA, leading to changes in gene reactivation. The molecular mechanism of active demethylation in mammalian cells is not well understood but seems to be linked to the DNA repair machinery.³⁷ Typically, methylation in the promoter region of a gene is associated with repression. Genetic abnormal methylation has been detected in smoker without or with cancer.³⁸ In the present study, the decreased expression of *Sca-1* gene in emphysema mice could be partly up-regulating by Dec. Lung morphological and functional changes in emphysema mice also could be ameliorated by Dec. These results suggested that *Sca-1* gene methylation might be involved in the progress of emphysema. At the same time, we noticed that the expression of *Sca-1* protein in bone marrow-derived EPCs was significantly lower in Dec-induced animals than in controls, and the expression of *Sca-1* mRNA in lung tissue was significantly lower in Dec-induced animals than in controls, which may be the result of side-effects incurred by using Dec alone in healthy individuals or pushing EPCs into differentiation.

Conclusion

The results of the present study demonstrated that the expression of *Sca-1* was decreased in lung tissue and bone marrow-derived EPCs in mice with emphysema, Dec

exerted beneficial effects on *Sca-1* expression and partially protected against CSE-induced emphysema. Our results suggested that the depletion of the progenitor cell pool and DNA methylation of *Sca-1* gene may be involved in the progression of emphysema in mice. The issue of whether a relationship exists between *Sca-1* expression and EPC function warrants further study, and the exact mechanism by which *Sca-1* plays a role in emphysema should be investigated via future studies.

Authors' contributions: Z-HH conceived the study, its design, performed the experiment, data analysis, and drafted the manuscript. YC conceived the study and its design. PC coordinated the study and critically revised the manuscript. S-DH, J-RY, and DL helped in operation and data collection. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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