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Clusterin expression level correlates with increased oxidative stress in asthmatics

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ABSTRACT

Background: Oxidative stress is thought to play a role in the pathogenesis of asthma. Clusterin is a sensitive cellular biosensor of oxidative stress and has antioxidant properties. The function and expression of clusterin in patients with asthma have not been fully investigated.
Objective: To investigate whether the expression of clusterin in patients with asthma is regulated by increased oxidative burden and whether clusterin expression could be used to assess the response to inhaled corticosteroids.
Methods: Clusterin levels in serum, induced sputum, and peripheral blood mononuclear cells of patients with asthma were measured by enzyme-linked immunosorbent assay and western blotting and compared with pulmonary function and levels of expression of hyperoxidized peroxiredoxins. Serum concentrations of clusterin in treatment-naive patients were compared before and after inhaled corticosteroid use.
Results: Serum clusterin concentration was significantly elevated in patients with severe asthma and was inversely correlated with pulmonary function. The expression of hyperoxidized peroxiredoxins was greatly increased in peripheral blood mononuclear cells of patients with asthma and was strongly correlated with clusterin expression. Serum clusterin concentrations in treatment-naive patients with asthma were decreased significantly after initial treatment with inhaled corticosteroids.
Conclusion: Clusterin may be a biomarker of asthma severity and the burden of oxidative stress in patients with asthma. Moreover, clusterin may be useful for the prompt assessment of airway inflammation.

Introduction

Asthma is characterized by chronic airway inflammation, and oxidative stress may play a role in its pathogenesis. Oxidative stress not only results from inflammation but also may initiate or enhance inflammation by influencing various signaling pathways.[1–3] Clinical studies have shown increased oxidative stress in patients with asthma, and various oxidants and antioxidants have been associated with asthma.[4–8] The clinical significance of these findings remains unclear because the mechanisms regulating oxidative stress are highly complex, involving many molecules and signaling pathways.

Clusterin (CLU), a widely distributed secretory glycoprotein, is a very sensitive cellular biosensor of oxidative stress.[9–11] Although the function of CLU in oxidative stress has not been determined, it may protect cells from oxidative injury.[12–17] The expression of CLU is increased in several diseases, including diabetes, atherosclerosis, Alzheimer disease, and some malignancies, in which oxidative injury is involved in disease pathogenesis.[11] CLU is strongly expressed in the airway submucosa of smokers and has been found to protect airway fibroblasts from oxidative stress induced by cigarette smoke extract in vitro.[15]

To date, the expression of CLU in patients with asthma has not been studied. The authors hypothesized that CLU expression in these patients might be altered by the increased oxidative burden of the disease and that CLU expression could be used to measure the response to inhaled corticosteroids (ICSs). Therefore, the authors measured CLU expression levels in the serum, induced sputum, and peripheral blood mononuclear cells (PBMCs) of patients with asthma and compared these with serum

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Table 1
Demographic characteristics of study population

	Healthy control (n = 33)	Mild asthma (n = 46)	Moderate persistent asthma (n = 68)	Severe persistent asthma (n = 28)	*P* value
Age, mean ± SD	52.1 ± 8.6	49.5 ± 13.0	51.3 ± 14.4	53.5 ± 12.4	NS[a]
Male/female	17/16	20/26	32/36	13/15	NS[a]
Current/former/nonsmokers	NA	4/15/27	10/20/38	3/10/15	NS[a]
%FEV_1, mean ± SD	NA	89.1 ± 14.3	82.3 ± 16.8	66.6 ± 23.4	<.01[a,b,c]
ICS beclomethasone equivalent dose (μg), mean ± SD	NA	181.8 ± 58.4	386.8 ± 138.1	746.3 ± 313.1	<.01[a,b,c,d]
ACT score, mean ± SD	NA	23.0 ± 1.9	22.55 ± 3.2	20.17 ± 3.6	<.01[a,b,c]

Abbreviations: ACT, Asthma Control Test; %FEV_1, percentage of predicted forced expiration volume in 1 second; ICS, inhaled corticosteroid; NA, not applicable; NS, not significant.
[a]One-way analysis of variance or χ^2 test.
[b]$P < .01$ by 1-way analysis of variance.
[c]$P < .01$ for severe vs mild asthma and severe vs moderate asthma by Student *t* test.
[d]$P < .01$ for moderate vs mild asthma by Student *t* test.

concentrations from treatment-naïve patients before and after treatment with ICSs.

Methods

Study Design

The patient cohort consisted of 142 randomly recruited patients with asthma who visited the asthma clinic at Asan Medical Center (Seoul, Korea) and participated in the Cohort for Reality and Evolution of Adult Asthma in Korea study, a prospective, longitudinal, multicenter study of Korean patients with asthma.[18,19] Asthma severity was assessed using the 2002 Global Initiative for Asthma guidelines. As healthy controls, 33 patients who visited the health promotion center in the same hospital were recruited.

In addition, 37 treatment-naive patients with asthma were enrolled to assess the effects of ICS on serum CLU concentration. To analyze CLU expression patterns in patients with asthma without considering the effects of controller medications, CLU concentrations were measured in PBMC lysates and the supernatants of induced sputum obtained from 20 of the treatment-naive patients. As controls, serum, induced sputum, and PBMCs were obtained from 14 healthy subjects. This study was approved by Asan Medical Center institutional review board.

Measurements of CLU and Hyperoxidized Peroxiredoxins in Biologic Samples

Serum concentrations of CLU were measured using a quantitative sandwich enzyme-linked immunosorbent assay kit for human CLU (BioVendor LCC, Candler, North Carolina), according to the manufacturer's instructions. PBMC lysates[20] and induced sputum supernatant[21] were prepared as previously described, and CLU expression was measured by western blotting using a commercial rabbit antihuman CLU-α/β (H-330) polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, California). The expression of hyperoxidized peroxiredoxins (PRDX-SO_3) in PBMCs also was measured by western blotting using a commercial rabbit anti–PRDX-SO_3 polyclonal antibody (ab16830, Abcam, Cambridge, United Kingdom).

Statistical Analyses

All statistical analyses were performed using SPSS 12.0 (SPSS, Inc, Chicago, Illinois). Normality of distributions was assessed by the Kolmogorov-Smirnov test. Normally distributed data were analyzed by 1-way analysis of variance and the Student *t* test; non-normally distributed data were analyzed by the Kruskal-Wallis and Mann-Whitney *U* tests. Dichotomous variables were analyzed by χ^2 tests. Correlations of normally and non-normally distributed data were evaluated by the Pearson and Spearman tests, respectively.

Results

The clinical characteristics of patients with asthma and healthy control subjects are listed in Table 1. There were no between-group differences in age and no differences in sex between asthma groups. Lung function (percentage of forced expiration volume [FEV]) and Asthma Control Test score were significantly lower in patients with severe asthma than in those with mild or moderate asthma ($P < .01$). Mean ICS dose (beclomethasone equivalent) was significantly higher with increasing asthma severity.

Serum CLU concentration was significantly higher in patients with severe asthma than in those with mild to moderate asthma and in healthy subjects (Fig 1). Serum CLU level was inversely correlated with the percentage of predicted FEV in 1 second (FEV_1; $r = -0.193$, $P = 0.024$; Fig 2A) and positively correlated with age ($r = 0.217$, $P < .01$; Fig 2B) in all patients with asthma. However, CLU concentrations did not differ by sex or smoking history (Table 2).

To investigate the relation between CLU expression and airway inflammation, CLU expression was measured in the supernatants of induced sputum and in PBMC lysates. CLU expression was significantly higher in samples from patients with asthma than in those from controls (Fig 3). The relation between CLU and oxidative stress was assessed by measuring the expression of PRDX-SO_3, a biomarker of cellular oxidative stress, in PMBC lysates from patients with asthma and from healthy subjects. PRDX-SO_3 expression was significantly greater in PBMCs from patients with asthma than in samples from healthy subjects ($P < .001$), with the expression levels of PRDX-SO_3 strongly correlated with those of CLU in PBMCs (Fig 4).

To test the effects of ICSs on serum CLU, its concentrations were measured before and after initial ICS treatment in 37 treatment-

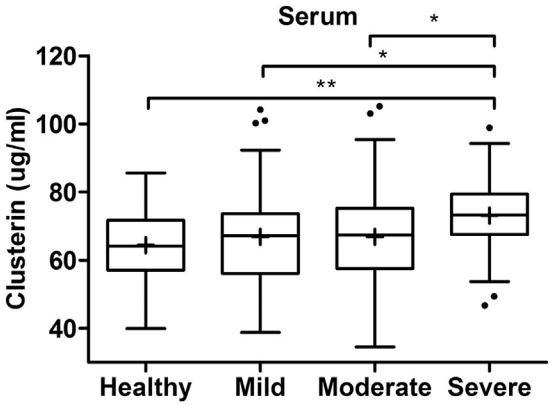

Figure 1. Expression of clusterin in serum from patients with asthma and healthy controls. $^{*}P < .05$; $^{**}P < .01$, by Mann-Whitney *U* tests.

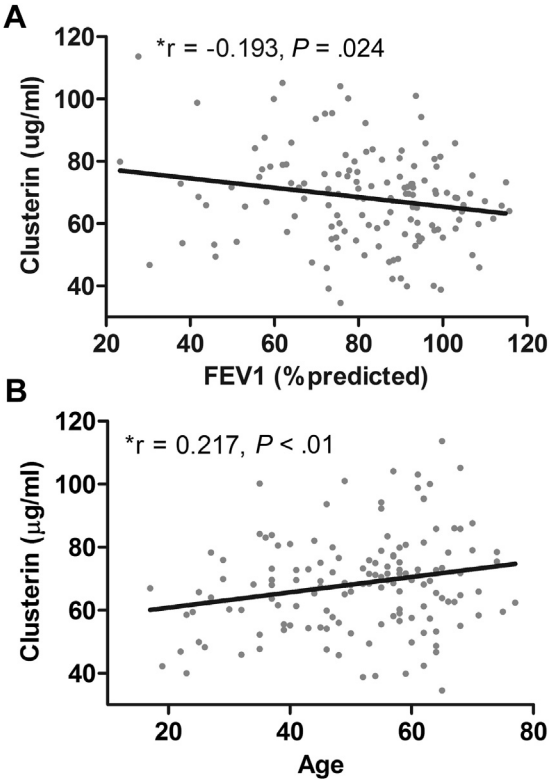

Figure 2. Correlation *(A)* between serum clusterin levels and percentage of predicted forced expiration volume in 1 second (FEV_1) and *(B)* between serum clusterin levels and age in patients with asthma. *Statistical significance of the correlations was determined by nonparametric Spearman rank correlation.

naive patients with asthma (Figure 5). Serum CLU concentration was significantly lower after than before ICS treatment (82.1 ± 16.6 and 76.3 ± 13.8 μg/mL, respectively, mean ± SD; $P = .016$). The mean dose of ICSs prescribed initially in steroid-naive patients was 434.6 ± 217.1 μg (beclomethasone equivalent dose, mean ± SD). There was no significant correlation between the initial prescribed dose of ICSs and changes in CLU levels after treatment (Pearson $r = 0.277$, $P = .097$).

Discussion

This study showed that CLU expression was stronger in biologic samples from patients with asthma than in samples from control subjects. Moreover, CLU expression was associated with cellular oxidative stress, suggesting that CLU may be a useful biomarker of oxidative stress in patients with asthma.

Oxidative stress is important in the pathogenesis of asthma. It has been associated with many pathophysiologic changes in this disease, such as increased production of chemoattractants, increased lipid peroxidation, stimulation of vascular permeability, and increased airway remodeling.[22] Increased oxidative stress also has been related to the development of severe asthma refractory to antiasthma medications.[7]

Despite the apparent importance of oxidative stress in asthma, many antioxidants have limited therapeutic benefits, with at best moderate effects on airway inflammation. These findings indicate the need to investigate antioxidants appropriate to treat each subtype of asthma and the ideal situations for their use based on exact assessments of oxidative stress status in patients with asthma. Furthermore, it is necessary to clarify the precise role of molecules regulating oxidative stress in the pathogenesis of asthma.

Clusterin, also called apolipoprotein J, is an extracellular chaperone glycoprotein composed of 2 35- to 40-kDa subunits linked by disulfide bonds. There is a conserved 14-bp CLU-specific element

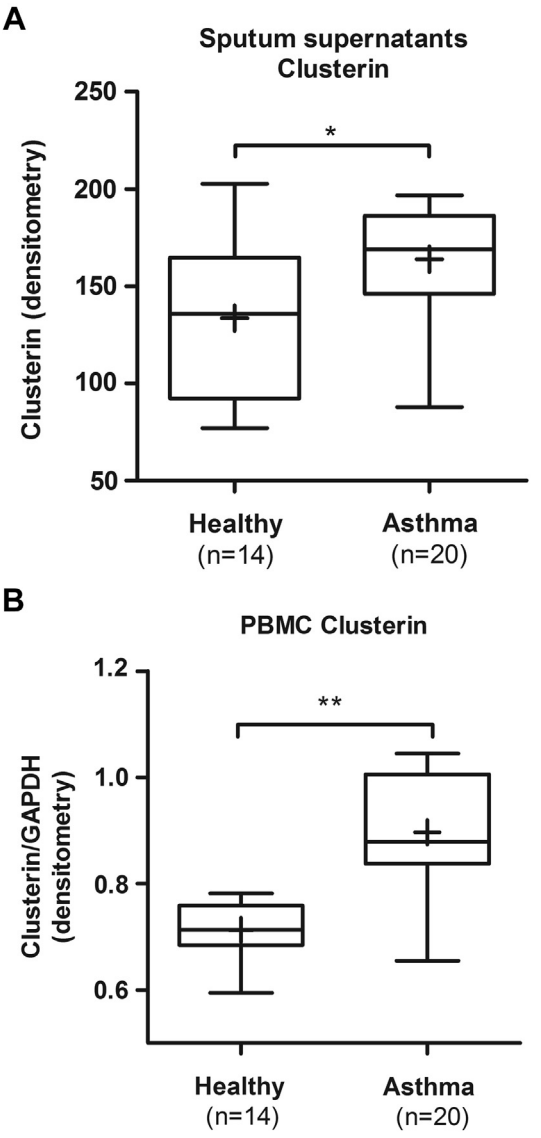

Figure 3. Expression of clusterin in *(A)* supernatants of induced sputum and *(B)* peripheral blood mononuclear cell (PBMC) lysates from patients with asthma or healthy subjects. Box plots indicate the median (*horizontal line inside box*), the interquartile range (*box*), and the smallest and largest values (*whiskers*; not farther away than 1.5 times the interquartile range from the first and third quartiles, respectively). Mean is indicated (+). *$P < .05$; **$P < .01$ by Mann-Whitney test.

Table 2
Levels of clusterin according to sex and smoking history in patient with asthma

	Clusterin (μg/mL)[a]	*P* value
Sex		NS[b]
Male (n = 65)	67.4 ± 15.8	
Female (n = 77)	67.9 ± 13.0	
Smoking		NS[c]
Current smoker (n = 17)	64.4 ± 16.3	
Ex-smoker (n = 45)	70.5 ± 17.5	
Nonsmoker (n = 80)	67.8 ± 13.4	

Abbreviation: NS, not significant.
[a]Expressed as mean ± SD.
[b]Student *t* test.
[c]One-way analysis of variance.

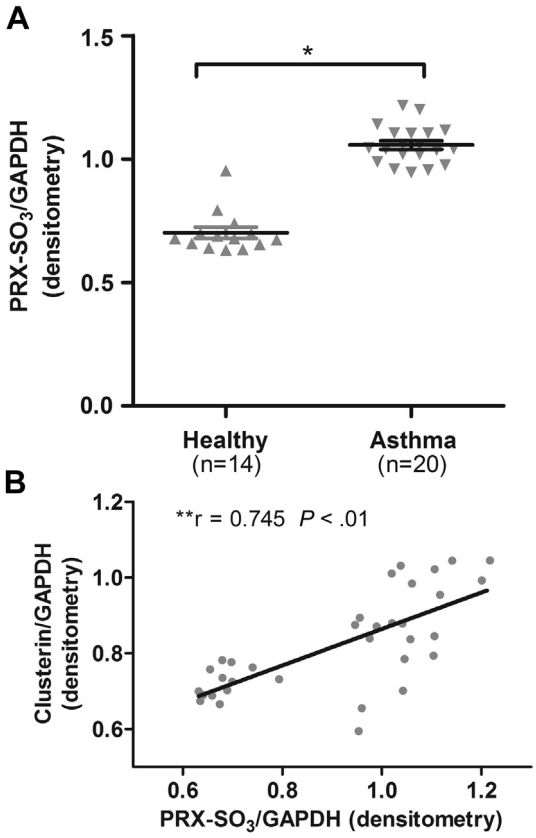

Figure 4. Correlation of clusterin expression with oxidative stress in patients with asthma. *(A)* Expression of hyperoxidized peroxiredoxins (PRX-SO_3) and *(B)* correlation between clusterin and hyperoxidized peroxiredoxins in peripheral blood mononuclear cell lysates from patients with asthma (n = 20) or healthy controls. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

among the regulatory elements in the CLU promoter, which is recognized by heat-shock transcription factor-1 and is a site for activator protein-1.[16,23,24] By acting in synergy, the CLU-specific element and activator protein-1 elements can make the CLU gene promoter particularly sensitive to even minute environmental changes or alterations in the cellular oxidative load of free radicals and their derivatives. Thus, increased CLU levels in biologic samples may sensitively indicate increased oxidative stress, even at very early stages of the inflammatory process. In fact, CLU has been known to show cytoprotective activity because of its antioxidative and antiapoptotic properties.[11–17]

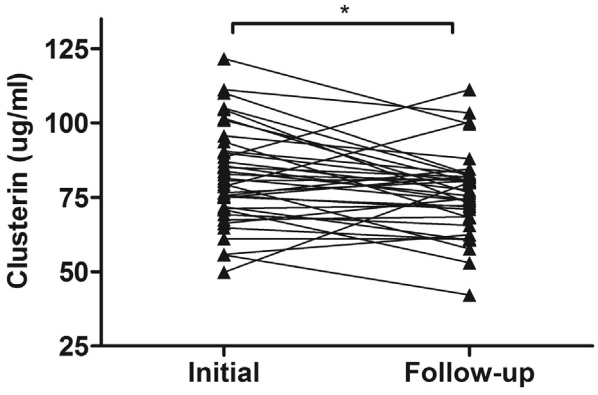

Figure 5. Expression of clusterin in serum of inhaled corticosteroid–naive patients with asthma before and after starting inhaled corticosteroid treatment (n = 37). *$P = .016$ by paired t test.

The CLU levels were higher in the sera, sputum supernatants, and PBMCs of patients with asthma than in samples from healthy controls. The levels of expression of CLU in PBMCs correlated strongly with those of PRDX-SO_3, another biomarker of increased intracellular oxidative stress. These results clearly showed that patients with asthma have high levels of oxidative stress. Moreover, serum CLU concentrations were higher in patients with severe asthma than in those with mild or moderate asthma. Indeed, asthma has been associated with a high level of oxidative stress resulting from an increase in oxidants and a decrease in the antioxidant capacity of various biological samples, including plasma, blood cells, and bronchoalveolar lavage fluid.[4,25–29]

This study showed that serum CLU concentrations were negatively correlated with the percentage of predicted FEV_1. Airway remodeling resulting from chronic airway inflammation and increased oxidative stress is the main cause of morbidity in patients with asthma. Inflammation is linked to tissue remodeling by reactive oxygen species, which can further activate various transcription factors and increase the production of major inflammatory cytokines. Although the pathogenesis of an irreversible decrease in lung function and airway remodeling is unclear, oxidative injury is likely involved. Thus, detection of the burden of oxidative stress in patients with asthma and protection of airways from oxidative injury would be particularly important in the prevention and treatment of severe asthma. Therefore, CLU may be especially important in patients with severe asthma.

This study also found that initial treatment of treatment-naive patients with asthma using ICSs decreased serum CLU concentrations, perhaps by decreasing airway oxidative stress. However, responses in individual patients differed, with some not showing a decrease in serum CLU. Heterogeneity may be due to diverse clinical responses to ICSs, encountered frequently in patients with asthma treated with ICS. Measurements of pulmonary function, including FEV_1, fraction of exhaled nitric oxide, and differential cell counts in induced sputum, can be used to monitor the status of airway inflammation and asthma control. Despite limitations in the accuracy of these biomarkers, many studies using these biomarkers have shown that decreasing airway inflammation is the most important factor in controlling asthma. Thus, detection of persistent airway inflammation and evaluation of its magnitude are crucial for optimizing asthma treatment. The present finding that ICS treatment decreases serum CLU concentrations in some ICS-naive patients indicates that measuring CLU in biologic samples may be useful in monitoring airway inflammation and oxidative stress burden. However, further prospective longitudinal studies are required to show that CLU is useful in assessing whether inflammation is controlled in the airways of patients with asthma.

Reduction–oxidation homeostasis is important for maintaining normal cellular function, and antioxidants are critical for regulating this homeostasis. Many intracellular molecules, including the peroxiredoxins (PRDXs), have antioxidant activity. PRDXs can be distinguished from conventional antioxidants by their transformation into PRDX-SO_3 in the presence of excess H_2O_2, a transformation that is irreversible and produces functionally inactive molecules.[30] Thus, increased cellular PRDX-SO_3 indicates a high burden of cellular oxidative stress and has been associated with asthma severity.[20] To investigate the association between CLU expression and intracellular oxidative stress in patients with asthma, the authors analyzed the intracellular expression of CLU and PRDX-SO_3 in PBMCs of patients with asthma and found that they were strongly correlated, further confirming that CLU is associated with increased cellular oxidative stress.

This study found that serum CLU concentrations increased with aging, a finding consistent with studies showing that the CLU

increase in elderly subjects was due to progressive failure of homeostasis and/or to prolonged exposure to a stressful environment, which are associated with the aging process. Indeed, antioxidants have been found to decrease with age.[31] This decreased antioxidant capacity may lead to increased oxidative stress in the lungs, increasing CLU expression and airway hyper-responsiveness and decreasing lung function. This is supported by the higher prevalence of asthma in elderly than in younger populations, although direct evidence of these steps is required.

There are some limitations to this study. Although there was a positive correlation between disease activity or severity of asthma and CLU expression levels in the biological samples, the strength of the correlation was modest at best. Such a modest correlation could have resulted for multiple reasons. First, asthma is a heterogeneous disease and there are many different subtypes or endotypes with distinct pathophysiology.[19,32] Thus, it is hypothesized that there could be some subtypes in which CLU may have a strong correlation with disease activity and be a useful biomarker indicating the burden of oxidative stress in those patients. Further studies are needed to investigate the value of measuring CLU levels in each subtype of asthma. Second, clusterin expression could have been affected by various clinical conditions, such as inflammatory and infectious diseases.[13] Although all patients with asthma in the present study did not have any other active diseases, the patients might have had other clinical conditions that could have affected the level of CLU expression.

Altogether, the authors conclude that the increase in CLU levels in patients with asthma and its ease of measurement in common biologic samples suggest that CLU may be a promising biomarker of asthma severity and oxidative stress burden in patients with asthma. Moreover, as an early biosensor of oxidative stress, CLU may be useful in the prompt assessment of airway inflammation status. Further studies are required to determine additional pathophysiologic roles of CLU in the disease process and its role as a biomarker for assessing longitudinal asthma control.

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References

[1] Rahman I, Adcock IM. Oxidative stress and redox regulation of lung inflammation in COPD. *Eur Respir J*. 2006;28:219–242.
[2] Gill R, Tsung A, Billiar T. Linking oxidative stress to inflammation: Toll-like receptors. *Free Radic Biol Med*. 2010;48:1121–1132.
[3] Dozor AJ. The role of oxidative stress in the pathogenesis and treatment of asthma. *Ann N Y Acad Sci*. 2010;1203:133–137.
[4] Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med*. 1996;154:1055–1060.
[5] Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med*. 1999;160:216–220.
[6] Ochs-Balcom HM, Grant BJ, Muti P, et al. Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. *Eur J Clin Nutr*. 2006;60:991–999.
[7] Ercan H, Birben E, Dizdar EA, et al. Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. *J Allergy Clin Immunol*. 2006;118:1097–1104.
[8] Cho YS, Moon HB. The role of oxidative stress in the pathogenesis of asthma. *Allergy Asthma Immunol Res*. 2010;2:183–187.
[9] Zoubeidi A, Ettinger S, Beraldi E, et al. Clusterin facilitates COMMD1 and I-kappaB degradation to enhance NF-kappaB activity in prostate cancer cells. *Mol Cancer Res*. 2010;8:119–130.
[10] Zoubeidi A, Chi K, Gleave M. Targeting the cytoprotective chaperone, clusterin, for treatment of advanced cancer. *Clin Cancer Res*. 2010;16:1088–1093.
[11] Trougakos IP, Gonos ES. Regulation of clusterin/apolipoprotein J, a functional homologue to the small heat shock proteins, by oxidative stress in ageing and age-related diseases. *Free Radic Res*. 2006;40:1324–1334.
[12] Shin YJ, Kim JH, Seo JM, et al. Protective effect of clusterin on oxidative stress-induced cell death of human corneal endothelial cells. *Mol Vis*. 2009;15:2789–2795.
[13] Trougakos IP, Gonos ES. Chapter 9: oxidative stress in malignant progression: the role of clusterin, a sensitive cellular biosensor of free radicals. *Adv Cancer Res*. 2009;104:171–210.
[14] Strocchi P, Smith MA, Perry G, et al. Clusterin up-regulation following sublethal oxidative stress and lipid peroxidation in human neuroblastoma cells. *Neurobiol Aging*. 2006;27:1588–1594.
[15] Carnevali S, Luppi F, D'Arca D, et al. Clusterin decreases oxidative stress in lung fibroblasts exposed to cigarette smoke. *Am J Respir Crit Care Med*. 2006;174:393–399.
[16] Jin G, Howe PH. Regulation of clusterin gene expression by transforming growth factor beta. *J Biol Chem*. 1997;272:26620–26626.
[17] Jun HO, Kim DH, Lee SW, et al. Clusterin protects H9c2 cardiomyocytes from oxidative stress-induced apoptosis via Akt/GSK-3beta signaling pathway. *Exp Mol Med*. 2011;43:53–61.
[18] Kim TB, Park CS, Bae YJ, Cho YS, Moon HB. Factors associated with severity and exacerbation of asthma: a baseline analysis of the Cohort for Reality and Evolution of Adult Asthma in Korea (COREA). *Ann Allergy Asthma Immunol*. 2009;103:311–317.
[19] Kim TB, Jang AS, Kwon HS, et al. Identification of asthma clusters in two independent Korean adult asthma cohorts. *Eur Respir J*. 2013;41:1308–1314.
[20] Kwon HS, Bae YJ, Moon KA, et al. Hyperoxidized peroxiredoxins in peripheral blood mononuclear cells of asthma patients is associated with asthma severity. *Life Sci*. 2012;90:502–508.
[21] Bae YJ, Kim TB, Moon KA, et al. Vascular endothelial growth factor levels in induced sputum and emphysematous changes in smoking asthmatic patients. *Annals Allergy Asthma Immunol*. 2009;103:51–56.
[22] Barnes PJ. Reactive oxygen species and airway inflammation. *Free Radic Biol Med*. 1990;9:235–243.
[23] Michel D, Chatelain G, Herault Y, Brun G. The expression of the avian clusterin gene can be driven by two alternative promoters with distinct regulatory elements. *Eur J Biochem*. 1995;229:215–223.
[24] Wong P, Taillefer D, Lakins J, Pineault J, Chader G, Tenniswood M. Molecular characterization of human TRPM-2/clusterin, a gene associated with sperm maturation, apoptosis and neurodegeneration. *Eur J Biochem*. 1994;221:917–925.
[25] Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Garg ML. Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. *Lipids*. 2000;35:967–974.
[26] Vachier I, Damon M, Le Doucen C, et al. Increased oxygen species generation in blood monocytes of asthmatic patients. *Am Rev Respir Dis*. 1992;146:1161–1166.
[27] Chanez P, Dent G, Yukawa T, Barnes PJ, Chung KF. Generation of oxygen free radicals from blood eosinophils from asthma patients after stimulation with PAF or phorbol ester. *Eur Respir J*. 1990;3:1002–1007.
[28] Kelly FJ, Mudway I, Blomberg A, Frew A, Sandstrom T. Altered lung antioxidant status in patients with mild asthma. *Lancet*. 1999;354:482–483.
[29] Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet*. 1994;343:133–135.
[30] Kim SY, Jo HY, Kim MH, et al. H2O2-dependent hyperoxidation of peroxiredoxin 6 (Prdx6) plays a role in cellular toxicity via up-regulation of iPLA2 activity. *J Biol Chem*. 2008;283:33563–33568.
[31] Mecocci P, Polidori MC, Troiano L, et al. Plasma antioxidants and longevity: a study on healthy centenarians. *Free Radic Biol Med*. 2000;28:1243–1248.
[32] Xie M, Wenzel SE. A global perspective in asthma: from phenotype to endotype. *Chin Med J*. 2013;126:166–174.