Are healthy smokers really healthy?

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Abstract

Cigarette smoke contains more than 4500 chemicals which have toxic, mutagenic and carcinogenic effects. Strong evidences have shown that current smokers take a significantly higher risk of cardiovascular diseases, chronic obstructive pulmonary disease (COPD) and lung cancer than nonsmokers. However, less attention has been paid to the smoking induced abnormalities in the individuals defined as healthy smokers who are normal with spirometry, radiographic images, routine physical exam and categorized as healthy control group in many researches. Actually, ‘healthy smokers’ are not healthy. This narrative review focuses on the smoking related pathophysiologic changes mainly in the respiratory system of healthy smokers, including inflammation and immune changes, genetic alterations, structural changes and pulmonary dysfunction.

Keywords: Healthy smokers, Respiratory system, Inflammation, Immune changes, Genetic alterations

Background

Cigarette smoke is a multipotent mixture of thousands of components that have toxic, mutagenic and carcinogenic properties. Numerous chemicals are added to the tobacco content, paper, and filter during the manufacturing process. Among the mainstream smoke emissions from cigarettes, polycyclic aromatic hydrocarbons, N-Nitrosamines, nickel, cadmium, chromium, arsenic, misc organic compounds are known carcinogens. Nicotine, carbon monoxide, acrolein and reactive oxidant substances are toxins that can cause immune dysfunction. Moreover, nicotine is the predominant addictive cigarette smoke constituent [1]. Epidemiological studies demonstrate that smoking is a significant risk factor for cardiovascular diseases [2], chronic obstructive pulmonary disease (COPD) [3] and lung cancer [4]. Thus, much work has been done on the molecular and cellular abnormalities in those patients who have a smoking history with smoking related diseases, trying to find the differences in comparison with healthy non-smokers and the possible mechanisms. However, until recently, there has not been much focus on the individuals categorized as healthy smokers who are asymptomatic with normal spirometry, radiographic images and physical exam, appearing to be much healthier than those smokers who have already developed diseases [5]. Interestingly, when compared with subjects who never smoked, the point that asymptomatic smokers with normal physical exam are healthy is much less obvious. Actually, healthy smokers are not healthy. Many studies have detected pathological changes in healthy smokers. In the present paper, we will mainly review the pathophysiologic changes in the respiratory system of smokers with normal physical exam, including inflammation and immune changes, genetic alterations, structural changes and pulmonary dysfunction.

Inflammation and immune changes

The analysis of the induced sputum, expired breath condensate (EBC), bronchoalveolar lavage (BAL) and biopsies of lung tissue from ‘healthy smokers’ show strong evidences of inflammatory and immune changes in the airway.

Induced sputum

The presence of neutrophils in sputum is one of the most common landmarks of inflammatory changes brought by smoking. As early as 1992, Swan et al. [6] found smokers with a greater number of pack years tended to have significantly higher levels of all cytomorphologic components, including neutrophils. Moreover, the neutrophil rating declined over the follow-up in quitters, while it increased among non-quitters. In fact, lots of researches have demonstrated elevated level of both the percentage and absolute number of neutrophils...
in induced sputum from healthy smokers as compared with nonsmokers [7–9].

The phenotype of macrophages in induced sputum is definitely altered by smoking. Domagala-Kulawikan et al. [10] detected increased expression of CD54 and CD71, which had effects on the metabolic activity of macrophages, in induced sputum of smokers compared with nonsmokers. CD54 is an adhesion molecule that mediates adhesion and the cell-cell interactions of macrophages, while CD71 has the function of proliferation and maturation [10, 11]. The up-regulation of CD54 and CD71 indicate besides, the proportion of CD14 positive macrophages in induced sputum, which has been proved to play a role in macrophage activation in infection and inflammatory processes in COPD, was higher in smokers than nonsmokers [10, 12].

Biochemical analysis also showed a lower percentage of CD8+ T lymphocytes and a higher ratio of CD4+/CD8+ T-cells in induced sputum of healthy smokers as compared with that of nonsmokers. After 6 months-cessation, the percentage of CD8+ T-cells increased in quitters and a ratio of CD4+/CD8+ decreased [13]. As the major activity of CD8+ T lymphocytes is the facilitation of the rapid resolution of acute viral infections, the lower percentage of CD8+ T-cells suggests that smokers may have a deficit in cell-mediated immunity in the lung and may explain the increased susceptibility of smokers for viral infections. The alteration of other chemicals or cytokines that represents inflammatory and immune processes in healthy smokers were also found in their induced sputum. Takashashi with his team [14] observed a significant reduction in IL-10 levels and a small number of IL-10-expressing cells in the sputum of patients with asthma and COPD and healthy smokers compared with nonsmokers. The decreased level of IL-10, an anti-inflammatory cytokine with major down-regulatory effects on inflammation, may contribute to the development of chronic airway inflammation among smokers. Both CCL5 and CCR1 were up-regulated on inflammatory cells of induced sputum of healthy smokers compared with nonsmokers [15, 16]. According to one of the latest studies, the level of IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) which were positively correlated with smoking load (pack-years) in induced sputum of healthy smokers were higher than that of nonsmokers [17]. All the three cytokines are important markers of inflammation and play key roles in the persistence of inflammatory process in COPD [18].

Expired breath condensate (EBC)

Much attention has been paid to the changes brought by smoking in smokers’ exhaled breath condensate (EBC). Many studies have found lower pH values in EBC, as reflected airway inflammation, in diverse inflammatory airway diseases, including bronchial asthma, bronchiectasis and COPD [19]. In healthy smokers, mean PH values lower than those observed in healthy non-smokers have always been reported. The ECLIPSE study found that EBC PH was significantly reduced both in COPD patients and chronic healthy smokers compared to healthy nonsmokers [20], but there were no differences between COPD patients and healthy smokers. This result is consistent with Koczulla’s [21] and Nicola’s study [22]. Since acidification of the airways reflects airway inflammation, the lower PH value in healthy smokers’ EBC suggest the inflammatory changes in their airways.

Chronic smoking also alters the level of inflammatory markers in EBC of smokers who remain symptomless and seem to be healthy on the surface. Elevated concentrations of IL-6 in EBC, a pro-inflammatory cytokine produced by epithelial cells and macrophages in the airways, was observed in healthy smokers compared to nonsmokers [23]. Higher concentrations of leukotriene (LT)B4, another marker of inflammation, was also detected in EBC of both COPD patients and healthy smokers than in nonsmokers [23]. Garey with his team [24] demonstrated that neutrophil chemotactic activity were significantly higher in EBC of smokers in comparison to non-smokers. This observation was reconfirmed by Corhay after three years and it was in keeping with the fact that neutrophils were well known to be increased in the airways of smokers [25]. Besides, smokers also showed higher TNF-α levels in EBC [26].

In recent years, evidence has emerged that oxidative stress plays a crucial role in the development and perpetuation of inflammation. Higher 8-isoprostane and H2O2 levels in EBC of subjects with COPD and smokers than non-smokers have been reported [27]. Isoprostanes are produced by ROS mediated peroxidation of arachidonic acid. The oxidative stress brought by smoking also promotes the inflammatory process.

Bronchoalveolar lavage (BAL)

The first paper detailing BAL dealt with normal values was published in 1974 [28]. Over the following years, BAL has been used to investigate inflammatory and immune processes in the lower respiratory tract which is able to give us a deeper understanding of pathophysiological changes brought by smoking.

Typically smokers have a decrease of CD4+/CD8+ caused by higher percentage of CD8+ T-cells in BAL as compared with nonsmokers [29]. The same change of T-lymphocyte subsets was also demonstrated in the lung tissue of healthy smokers, but was in contrast with the result detected in induced sputum of smokers that decreased proportion of CD8+ T lymphocytes with increased ratio of CD4+/CD8+ T-cells. One explanation would be the inflammatory microenvironment in airway lumen sampled by induced sputum is different from that in the
BAL and airway epithelium sampled by bronchial biopsies [30]. Another reason would be smoking induced suppression of the trans-epithelial migration of CD8⁺ lymphocytes, increasing their number in the large airway wall, while reducing their number in the airway lumen [31]. Besides, among the subsets of CD8⁺T-lymphocytes, Yu et al. [32] showed a significant trend for greater Tc1/Tc2 ratio in BAL of patients with COPD and smokers compared with nonsmokers. CD8⁺T-lymphocytes who are key inflammatory effector and regulatory cells have been proved to play an important role in the inflammatory process of COPD [33]. CD8⁺T-lymphocytes can be differentiated into cells that synthesize interferon-gamma (IFN-γ) but not interleukin-4 (IL-4) (Tc1 cells) or cells that synthesize IL-4 but not IFN-γ (Tc2 cells) [34]. However, little is known about which subpopulation is mostly involved in the immuno-pathogenesis of COPD. The imbalance of the two phenotypes was actually detected in the BAL of smokers and patients with COPD.

Kuschner with his co-workers [35] observed greater concentrations of monocyte chemoattractant protein (MCP)-1 with increased level of IL-6, IL-8 and IL-1β in BAL of control smokers as compared with nonsmokers, moreover, the level of IL-8 and IL-1β were elevated in a cigarette dose-dependent manner. Clara cell 10 kDa protein (CC10), which may have a role in protecting the respiratory tract from oxidative stress and inflammation by inhibiting the expression and/or activity of proteins, such as phospholipase A2, IFN-γ, and TNF-α was found to be significantly decreased in BAL fluids of healthy smokers in comparison with nonsmokers [36, 37]. Hence, a decrease of CC10 levels in the peripheral airways as a result of smoking may be associated with enhanced pro-inflammatory process in the peripheral airways of the smokers. Molecules mediating tissue damage as matrix metalloproteinase (MMP)-9 and MMP-12 had either increased levels and/or enhanced activities in samples from BAL of smokers as compared with nonsmokers [38, 39]. Surfactant protein A and D (SP-A, SP-D), members of the collectin family which play a key role in innate immunity in animal models [40], were decreased in BAL of healthy smokers vs. non-smokers [41]. Therefore, lower levels of SP-D caused by cigarette smoking may weaken lung immunity in healthy smokers.

Alveolar macrophages (AM) are responsible for a broad set of host defense functions including recognition and phagocytosis of pathogenic material and apoptotic cells. Various changes of smokers’ alveolar macrophages have been noted in several studies. The number and proportion of AM in healthy smokers’ BAL are increased as compared with nonsmokers [42, 43]. And AM from smokers differ from those of nonsmokers in that they are slightly larger, and contain more golgi vesicles, endoplasmic reticulum and residual bodies which contain distinctive fiber-like inclusions [44, 45]. Besides the ultrastructural alterations, the function of AM is also changed. Compared with nonsmokers, alveolar macrophages of cigarette smokers has a significantly greater esterase and protease activity with higher resting metabolism and enhanced lysozyme secretion [44, 46, 47]. However, studies showed AM of smokers had impaired phagocytic capability [48]. More interestingly, in contrast with elevated level of IL-6, IL-8 and IL-1β detected in BAL of healthy smokers in comparison with nonsmokers, the decreased capacity of smokers’ AM to release IL-1, IL-6, IL-8 and TNF-α has been observed in many studies and this decreased secretion of cytokines may result in impairment of pulmonary immune responses in smokers with increased incidence of infection [49–52]. One possible explanation would be that these cytokines are produced not only by AM but other cells in BAL. Another reason would be the increased number of AM in BAL which lead to the impaired cytokine secretion by smokers’ AM appearing to be offset. Furthermore, smokers’ AM produces significantly more superoxide anions that may contribute to the lung injury [53, 54]. Cigarette smoke can also change the phenotype of AM. Schaberg T et al. [55] found that much more AM from smokers expressed CD11a, CD11b, CD11c and CD18 as compared with nonsmokers. AM of both healthy smokers and patients with COPD exhibited a unique polarization pattern which was different from nonsmokers’. The analysis from Shaykhiev et al. [56] revealed that M1 polarization related genes which are relevant to inflammation and cell-mediated immunity were down-regulated in AM of smokers and COPD individuals with a smoking history, while M2 related genes closely associated with anti-inflammatory cytokines and molecules implicated in tissue remodeling were up-regulated. Therefore, the result from Shaykhiev et al.’s study is consistent with the previous finding that decreased capacity of smokers’ AM to release pro-inflammatory cytokines, suggesting AM may contribute to smoking related diseases in a non-inflammatory manner.

Biopsies of lung tissue
Histopathological examinations help us find inflammatory alterations in bronchial biopsies of smokers without any symptoms, including vascular hyperplasia, submucosal edema, inflammatory cell infiltrates and goblet cell hyperplasia [57]. An abnormal cellular infiltrate into the airway submucosa of smokers is always reported. Lams et al. [58] found an increase in small-airway neutrophils, total eosinophils and a trend toward an increase in CD 8+ cells in smokers as compared to nonsmokers. Two studies from European countries confirmed a larger number of CD3+, CD8+, CD68+ cells in the bronchial submucosa of smokers compared with nonsmokers [59, 60].
Isajevs et al. [61] demonstrated again a higher level of neutrophils, macrophages and CD8+ cells both in large and small airways of smokers than nonsmokers, but lower than that of subjects with COPD which is consistent with Saetta’s finding [33] that smokers who developed symptoms of chronic bronchitis and chronic airflow limitation had an increased number of CD8+ cells in the peripheral airways as compared with asymptomatic smokers with normal lung function, suggesting this inflammatory process may be under control. Nuclear factor-kB (NF-kB), a transcription factor regulating the expression of many genes involved in inflammation [62], is increased in airways of asymptomatic smokers as compared with non-smokers [61, 63]. Besides, the expression of p65 NF-kB, one of its activated form, is also elevated in the epithelium of smokers with normal lung function and COPD patients that correlated with a greater counts of macrophages, neutrophilic leucocytes and CD8+ T cells in airway walls, when compared to nonsmoking persons [61, 63]. The level of CXCL6 and its receptor, CXCR1 which can induce leukocyte recruitment and activation at sites of inflammation [64] are increased in the epithelium and submucosa of healthy smokers respectively [65]. Moreover, Wang with his team [66] demonstrated the toll-like receptor (TLR)5 expressed mainly on the apical side of the epithelium, was down-regulated in healthy smokers and smokers with COPD, compared to non-smokers. The toll-like receptors are important components of the respiratory epithelium host innate defense and TLR-deficient mice develop exhibit impaired CD4+ T cell response to a flagellated pathogen [67], suggesting suppression of airway epithelial TLR5 may contribute to the increased susceptibility of smokers and smokers with COPD to airway flagellated bacterial infection.

**Genetic alterations**

Epidemiological data has shown that long-term smokers are taking a greater risk of developing COPD and lung cancer as compared with nonsmokers. One of the possible reasons is the smoking induced genetic alterations which modify susceptibility to lung diseases. For smokers, those up- or down-regulation of gene expressions with relevant impaired biological function accelerate the progress of respiratory disorders.

**Genetic alterations in alveolar macrophages**

Human alveolar macrophages, mostly residing on the respiratory epithelial surface, are critical components of the innate immune system. The gene expression of alveolar macrophages has been altered in active smokers when compared with nonsmokers. Table 1 shows up- or down-regulated genes (>2.0 fold change) in AM of smokers reported by at least two different studies [68–72]. The MMPs comprise a family of at least 20 proteolytic enzymes that play an essential role in tissue remodeling. Several studies in animals and humans have provided evidence that MMP12 (human macrophage elastase) is important in airway inflammation and the development of emphysema. For instance, MMP12-knockout mice exposed to cigarette smoke do not develop emphysema [73]. MMP12 up-regulation is also demonstrated to play a critical role in emphysema to lung cancer transition that is facilitated by inflammation [74]. CYP1B1, a member of the P450 superfamily with high affinity for inhaled tobacco carcinogens, is commonly expressed in human lung [75]. Lao et al. [76] found that CYP1B1 Leu432Val polymorphism acted as a risk factor for the carcinogenesis of lung cancer.

**Genetic alterations in airway epithelium**

Airway epithelium, lined by a variety of specialized epithelial cells, represents the first point of contact for cigarette smoke. It not only plays a central role in the barrier function of airway tract, but also responds to environment-induced damage through the release of pro-inflammatory cytokines and chemokines [77]. Genes in functional categories are detected to expressed differentially in the airway epithelium in nonsmokers and smokers. Table 2 displays up- or down-regulated genes in airway epithelium of smokers reported by more than one study [78–83]. Up-regulation of antioxidant-related genes in the airway epithelium of smokers are always reported [78–83], including the glutathione pathway genes (G6pd, GCLC, GPx2, GSR, NQO1), the redox balance genes(ADH7, Akr1b1, Akr1C1, Akr1C2, Akr1C3), the pentosephosphate cycle genes(PGD, TALDO1) and the xenobiotic metabolism genes(CYP1B1). Although catalase and the superoxide dismutase (SOD) contribute a lot to antioxidative defense, the available data suggests that gene expression of catalase and SOD do not differ in the airway epithelium of smokers and nonsmokers [78, 79]. Smoking induced down-regulation of intraflagellar transport gene and cilia-related genes in the airway epithelium of healthy smokers is associated with shorter cilia which affect mucociliary clearance [84, 85]. Healthy smokers have more active MUC5AC-core gene expression compared to the nonsmokers [86]. MUC5AC is one of the major secretory mucins expressed by surface airway epithelial cells. The activated MUC5AC-core gene expression in smokers may lead to mucus hypersecretion. Down regulation of TLR5 and physiological apical junctional complex(AJ/C) gene in healthy smokers may be involved in smoking-related susceptibility to airway infection [66, 87]. Overexpression of ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) which is used as a marker of lung cancer in chronic smokers may represent an early event in the complex transformation from normal epithelium to overt malignancy [88].
Table 1: Up- and down-regulated genes (>2.0 fold change) in alveolar macrophages of ‘healthy smokers’

| Gene symbol | Description                                             | Regulation | Reference                      |
|-------------|---------------------------------------------------------|------------|--------------------------------|
| PLA2G7      | phospholipase A2, group VII                            | up         | Woodruff et al. [68], Graff et al. [69], Philibert et al. [70] |
| SPP1        | secreted phosphoprotein 1 (osteopontin)                | up         | Woodruff et al. [68], Graff et al. [69] |
| CYP1B1      | cytochrome P450, family 1, subfamily B, polypeptide 1  | up         | Woodruff et al. [68], Graff et al. [69], Philibert et al. [70] |
| ATP6V0D2    | ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d2 | up         | Woodruff et al. [68], Graff et al. [69] |
| SLC7A11     | solute carrier family 7, member 11 (xCT)               | up         | Woodruff et al. [68], Graff et al. [69] |
| MMP12       | matrix metallopeptidase 12 (macrophage elastase)       | up         | Woodruff et al. [68], Graff et al. [69], Heguy et al. [71] |
| FABP3       | fatty acid binding protein 3                           | up         | Woodruff et al. [68], Graff et al. [69] |
| FLT1        | fms-related tyrosine kinase 1 (VEGFR)                  | up         | Woodruff et al. [68], Graff et al. [69], Philibert et al. [70] |
| A2M         | alpha-2-macroglobulin                                  | up         | Woodruff et al. [68], Graff et al. [69], Heguy et al. [71] |
| UCHL1       | ubiquitin carboxyl-terminal esterase L1                | up         | Woodruff et al. [68], Graff et al. [69] |
| S100B       | S100 calcium binding protein B                          | up         | Woodruff et al. [68], Graff et al. [69], Philibert et al. [70] |
| CA2         | carbonic anhydrase II                                   | up         | Woodruff et al. [68], Graff et al. [69] |
| SLC16A6     | solute carrier family 16, member 6 (monocarboxylic acidtransporter)  | up         | Woodruff et al. [68], Graff et al. [69] |
| SSBP3       | single stranded DNA binding protein 3                   | up         | Woodruff et al. [68], Graff et al. [69] |
| TDRD9       | tudor domain containing 9                              | up         | Woodruff et al. [68], Graff et al. [69] |
| C4orf18     | chromosome 4 open reading frame 18 (DKFZp434L142)      | up         | Woodruff et al. [68], Graff et al. [69] |
| DNASE2B     | deoxyribonuclease II beta                              | up         | Woodruff et al. [68], Graff et al. [69] |
| SDC2        | syndecan 2                                              | up         | Woodruff et al. [68], Graff et al. [69] |
| MGST1       | microsomal glutathione S-transferase 1                | up         | Woodruff et al. [68], Graff et al. [69] |
| AGPAT9      | 1-acylglycerol-3-phosphateO-acyltransferase 9          | up         | Woodruff et al. [68], Graff et al. [69] |
| TMTSF4      | transmembrane 7 superfamily member 4 (DCSTAMP)         | up         | Woodruff et al. [68], Graff et al. [69] |
| LIPA        | lipase A, lysosomal acid, cholesterol esterase          | up         | Woodruff et al. [68], Graff et al. [69] |
| CSF1        | Colony-stimulating factor 1                            | up         | Heguy et al. [71], Rose et al. [72] |
| CCR5        | Chemokine (C-C motif) receptor 5                       | up         | Woodruff et al. [68], Graff et al. [69], Philibert et al. [70] |
| CXCL11      | chemokine (C-X-C motif) ligand 11                      | down       | Woodruff et al. [68], Graff et al. [69] |
| CXCL9       | chemokine (C-X-C motif) ligand 9                       | down       | Woodruff et al. [68], Graff et al. [69], Philibert et al. [70] |
| SLC19A3     | solute carrier family 19 (thiamine transporter)       | down       | Woodruff et al. [68], Graff et al. [69] |
| EMR1        | egf-like module containing, mucin-like, hormonereceptor-like 1 (F4/80) | down       | Woodruff et al. [68], Graff et al. [69] |
| CXCL10      | chemokine (C-X-C motif) ligand 10                      | down       | Woodruff et al. [68], Graff et al. [69] |
| PDGFD       | platelet derived growth factor D                       | down       | Woodruff et al. [68], Graff et al. [69] |
| IGF1        | insulin-like growth factor 1                          | down       | Woodruff et al. [68], Graff et al. [69] |
| GBP5        | guanylate binding protein 5                            | down       | Woodruff et al. [68], Graff et al. [69] |
| C8B         | complement component 8, beta                           | down       | Woodruff et al. [68], Graff et al. [69] |
| CD69        | CD69 molecule                                          | down       | Woodruff et al. [68], Graff et al. [69] |
| WDOR69      | WD repeat domain 49                                    | down       | Woodruff et al. [68], Graff et al. [69] |
| TNFSF10     | tumor necrosis factor (ligand) superfamily, member 10 (TRAIL) | down       | Woodruff et al. [68], Graff et al. [69] |
| IFI27       | interferon, alpha-inducible protein 27 (ISG12)        | down       | Woodruff et al. [68], Graff et al. [69] |
| TRHDE       | thyrotropin-releasing hormone degrading enzyme          | down       | Woodruff et al. [68], Graff et al. [69] |
Reduced expression of Notch pathway in both smokers and patients with COPD may be responsible for the abnormal differentiation of the airways [89]. Smoking induced epigenetic changes with corresponding modulation of gene expression are demonstrated both in airway epithelium and alveolar macrophages [90, 91]. Buro-Auriemma with his team [90] identified 204 unique genes differentially methylated in the small airway epithelium DNA of smokers compared with nonsmokers. Cigarette smoking is also associated with genome wide changes in pulmonary macrophage DNA methylation, in particular at the aryl hydrocarbon receptor repressor (AHRR), a known tumor suppressor that may be critical in moderating AHR role in oncogenesis and altered immune function [91].

**Structural changes**

Since 1957, numerous studies have proved the structural changes of airway epithelium brought by smoking [92]. An increase in thickness of the epithelium with an elevation in size and number of goblet cells, a decrease in the length of cilia, loss of cilia and occurrence of cells with atypical nuclei were revealed in both tracheal and bronchial epithelium of smokers compared with nonsmokers [92–94]. Besides, the percentages of individuals exhibiting precancerous lesions including basal cell hyperplasia and squamous metaplasia, increased with the habit of cigarette smoking [93, 95]. Robust structural changes in airway epithelial mitochondria induced by cigarette smoke were detected, such as fragmentation, branching and quantity of cristae [96]. While the changes as a consequence of tobacco mentioned above could be reversible. Bertram and Rogers [97] demonstrated that the structural recovery occurred in bronchial epithelium in people who stopped smoking for over two years. Healthy smokers’ cilia was much shorter than non-smokers’ which may affect the mucociliary clearance [84].

### Table 1

| Gene symbol | Description | Regulation (HSa/NSb) | Reference |
|-------------|-------------|----------------------|-----------|
| MYB         | v-myb myeloblastosis viral oncogene homolog | down | Woodruff et al. [68], Graff et al. [69], Philibert et al. [70] |
| ARHGAP24    | Rho GTPase activating protein 24 | down | Woodruff et al. [68], Graff et al. [69] |
| TRPC6       | transient receptor potential cation channel, subfamily C, member 6 | down | Woodruff et al. [68], Graff et al. [69] |
| ITHIHS      | inter-alpha (globulin) inhibitor H5 | down | Woodruff et al. [68], Graff et al. [69] |

*aHS: healthy smokers, bNS: nonsmokers*

### Table 2

| Epithelium | Gene symbol | Description | Regulation (HSa/NSb) | Reference |
|------------|-------------|-------------|----------------------|-----------|
| SAE/LAE    | G6pd        | glucose-6-phosphatedehydrogenase | up | Carolan et al. [78], Hackett et al. [79] |
| SAE/LAE    | GCLC        | Glutamate-cysteineligase, catalytic subunit | up | Carolan et al. [78], Hackett et al. [79], Spira et al. [82] |
| SAE/LAE    | GPx2        | Glutathioneperoxidase 2 | up | Carolan et al. [78], Hackett et al. [79], Harvey et al. [80], Turetz et al. [81], Spira et al. [82], Zhang et al. [83] |
| SAE        | GSR         | Glutathionereductase | up | Carolan et al. [78], Hackett et al. [79] |
| SAE/LAE    | ADH7        | Alcoholdehydrogenase 7, mu or sigmapolyepptide | up | Carolan et al. [78], Hackett et al. [79], Harvey et al. [80], Turetz et al. [81], Spira et al. [82] |
| SAE/LAE    | AKR1B1      | aldo-keto reductasefamily 1, memberB1 | up | Carolan et al. [78], Hackett et al. [79], Harvey et al. [80], Turetz et al. [81], Spira et al. [82], Zhang et al. [83] |
| SAE/LAE    | AKR1C       | aldo-keto reductasefamily 1, memberC | up | Carolan et al. [78], Hackett et al. [79], Harvey et al. [80], Turetz et al. [81], Spira et al. [82], Zhang et al. [83] |
| SAE        | TXNRD1      | Thioredoxinreductase 1 | up | Carolan et al. [78], Hackett et al. [79], Spira et al. [82] |
| SAE        | PGD         | Phosphogluconatedehydrogenase | up | Carolan et al. [78], Hackett et al. [79] |
| SAE        | TALD01      | transaldolase 1 | up | Carolan et al. [78], Hackett et al. [79] |
| SAE/LAE    | CYP1B1      | cytochrome P450, family 1, subfamilyB, polyepitope I | up | Carolan et al. [78], Hackett et al. [79], Harvey et al. [80], Spira et al. [82], Zhang et al. [83] |
| SAE/LAE    | CX3CL1      | Chemokine(C-X3-C motif)ligand 1 | down | Harvey et al. [80], Turetz et al. [81], Spira et al. [82] |
| SAE/LAE    | ALDH3A1     | Aldehydedehydrogenase 3family, memberA1 | up | Harvey et al. [80], Turetz et al. [81], Spira et al. [82] |
| SAE/LAE    | NQO1        | NAD(P)Hdehydrogenase, quinone 1 | up | Harvey et al. [80], Spira et al. [82], Zhang et al. [83] |
| LAE        | SLIT        | slit homolog (Drosophila) | up | Turetz et al. [81], Spira et al. [82] |
| SAE        | UCHL1       | Ubiquitin carboxyterminal, esteraseL1 | down | Harvey et al. [80], Spira et al. [82] |

*aHS: healthy smokers, bNS: nonsmokers, cSAE: small airway epithelium, dLAE: large airway epithelium*
Pulmonary dysfunction

Smoking is regarded as the major contribution to pulmonary dysfunction, and this deterioration of lung function in smokers is far in excess of that predicted by age [99]. The ventilation of the upper zones of the lungs was significantly less than that of the lower zones in smokers, suggesting the upper zone abnormalities found in the group of smokers were consistent with the development of early emphysema [100]. Although, parameters of pulmonary function test of healthy smokers are within the normal range, some abnormalities are detected by pulmonologists when compared with lifelong non-smokers. Reduced forced expiratory volume in one second (FEV1), peak expiratory flow (PEF) and the ratio of FEV1 to forced vital capacity (FVC, FEV1/FVC), decreased diffusing capacity for CO and forced expiratory flows at high lung volume, increase in total lung capacity (TLC), the ratio of residual volume (RV) to TLC (RV/TLC) and the ratio of functional residual capacity (FRC) to TLC (FRC/TLC) were demonstrated in smokers [101–104]. Forced expiratory time for the last 0.5 l of the forced vital capacity was significantly higher in the heavy smokers (those who had smoked a lifetime total of more than 10,000 cigarettes) than the nonsmokers [102]. A linear association between smoking years and reduced level of FEV1 and FVC was reported [105] and the decline in FEV1 can also be detected in teenage smokers [106]. Moreover, smoking cessation not only stopped the smoking-induced fast decline in lung function, but even led to some reversal toward nonsmoking values [107]. Frette with his team [108] found that smokers who quit before age 40 had an age- and height-adjusted FEV1 that did not differ from that of never smokers in either men or women. In summary, these findings confirm the deleterious effect on lung function of smokers whose spirometry values are within normal range and prove a beneficial effect of quitting smoking at an early age.

Systemic effects

Systemic inflammation

Numerous studies have shown significantly increased level of white blood cell [109–111], TNF-α [26, 112] and C-reactive protein [113, 114] in serum of asymptomatic smokers with normal lung function, providing direct evidence of systemic inflammation in smokers. However, most of researches found no differences in the subsets of T-lymphocytes which mediate abnormal intrapulmonary inflammation and have been identified as a key component in the development and the progression of COPD in peripheral blood [29, 109, 115]. But, Miller et al. [116] detected the decreased level of CD4+ T lymphocytes and increased level of CD8+ T lymphocytes in peripheral blood from heavy smokers which may need further studies.

Oxidative damage

Many researches have confirmed the systemic oxidative damage and overall decrease in antioxidant activity in smokers as compared with nonsmokers. Antwerpen et al. [117] demonstrated that smoking was associated with significantly increased phagocyte-derived ROS-generation. The mean plasma malondialdehyde (MDA) level, a parameter of lipid peroxidation caused by the oxidants, was higher both in healthy smokers and smokers with COPD than in healthy nonsmokers [118]. Besides, after 4 week smoking cessation, significant decreases in MDA were detected [119]. Significantly lower CuZnSOD and Se-GSH-Px activities have been reported both in teenage and adult smokers than non-smokers [120, 121]. Concentrations of serum antioxidants, such as folate, vitamin C and vitamin E, have been proved to be lower in chronic smokers [122, 123], confirming again smoking induced damage to the oxidant defense system.

Endothelial dysfunction

Oxidative damage brought by smoking is closely related to endothelial dysfunction. Guthikonda with his colleagues [124] demonstrated xanthine oxidase contributes importantly to endothelial dysfunction caused by cigarette smoking. Hirai et al. [125] found that the impaired endothelial dysfunction in smokers could be improved by the antioxidant, vitamin C. In fact, lots of studies have proved endothelial dysfunction measured by flow-mediated dilation (FMD) in healthy smokers [126, 127]. Moreover, Mendes with his team [128] found that impaired airway vascular endothelial function might precede endothelial dysfunction of other areas in healthy smokers. Celermajer et al. [126] demonstrated an inverse relation of FMD and lifetime cigarette dose smoked and former smokers had higher FMD values than current smokers, indicating potentially reversible smoking induced endothelial dysfunction.

Reduced number of circulating endothelial progenitor cells (EPCs) is reported [129]. The number of EPCs is also indicated to be correlated with endothelial function as measured by FMD [130]. Furthermore, Michaud’s study [131] demonstrated the impairment of EPC differentiation and functional activities brought by smoking and this impairment might be associated with lower serum antioxidant levels. A recent study also identifies that epigenetic regulation of DNA damage and senescence are closely related to the endothelial progenitors’
dysfunction in both smokers and COPD patients [132]. While, smoking induced effect on circulating EPCs was reversible. Kondo et al. [133] observed that the recovery of EPC levels was greater in light smokers than in heavy smokers, suggesting the significance of smoking cessation.

Effects of cardiovascular, nervous-mental, endocrine and reproductive system
Compared with nonsmokers, smokers have significantly elevated risk factors for cardiovascular diseases. Although, healthy smokers’ heart rate, blood pressure and level of serum lipid and lipoprotein are in the normal range, increases in heart rate and blood pressure are detected as compared with nonsmokers [134, 135], they also have higher serum concentrations of cholesterol, triglycerides, very low density lipoprotein cholesterol, and low density lipoprotein cholesterol and lower serum concentrations of high density lipoprotein cholesterol and apolipoprotein A1 [136]. Endothelial dysfunction and elevated level of white blood cell in healthy smokers mentioned above also contribute a lot to the onset of cardiovascular diseases [137] and become an independent risk factor for all atherosclerotic cardiovascular diseases [138].

Among the healthy smokers who don’t have any metal illnesses including alcohol or drug abuse/dependence with normal brain MRI results, abnormalities are still detected in their nervous-mental system. Hao with his team [139] found that neural function was less synchronized in the right inferior frontal cortex and more synchronized in the left superior parietal lobe in chronic smokers compared to nonsmokers, indicating lacking of control over reward-related behavior and smoking urges respectively. Significantly greater rate atrophy over 2-years than nonsmokers in multiple brain regions associated with the early stages of Alzheimer Disease were found in healthy, cognitively-intact elderly smokers [140]. Furthermore, chronic smokers were demonstrated to have a worse visual memory and poorer sleep quality compared with lifelong nonsmokers [141]. Jiménez-Ruiz C.A. et al. [142] found that 10.2% of healthy smokers had high dependence on nicotine evaluated by the Fagerstrom Test for Nicotine Dependence (FTND) which is a quantitative scale used commonly for the definition of nicotine dependence. Actually, tobacco dependence itself is not only a bad habit, but a chronic disease [143].

Attvall et al. [144] demonstrated that smoking could impair insulin action and lead to insulin resistance in healthy smokers even their blood glucose was normal. Smoking cessation improving insulin sensitivity in healthy middle-aged men were also reported [145]. Besides, detrimental effects on reproductive system brought by smoking are detected. Mostafa with his colleagues [146] found that smoking had negative effects on sperm motility, viability, DNA fragmentation, seminal zinc levels, and semen reactive oxygen species levels, even in healthy fertile smokers.

Conclusion
The biochemical analysis of the induced sputum, expired breath condensate, bronchoalveolar lavage, biopsies of lung tissue and peripheral blood from healthy smokers are strongly enough to prove the exist of local and systemic inflammation. Genetic alterations detected in the lung tissue of healthy smokers may contribute to smoking-related susceptibility to lung diseases, such as emphysema and lung cancer. The structural changes in respiratory system, as well as the decline in lung function as compared with nonsmokers demonstrate again smoking induced negative effects on healthy smokers which accelerate the onset of respiratory disorders. Therefore, healthy smokers who are normal with spirometry, radiographic images and routine physical exam are not really healthy. Smoking cessation as an early intervention may lead to some reversal toward the better health of lifelong nonsmokers.

Abbreviations
BAL: Bronchoalveolar lavage; COPD: Chronic obstructive pulmonary disease; EBC: Expired breath condensate; EPCs: Endothelial progenitor cells; FEV1: Forced expiratory volume in one second; FMD: Flow-mediated dilation; FTND: Fagerstrom Test for Nicotine Dependence; FVC: Forced vital capacity; HS: Healthy smokers; LAE: Large airway epithelium; MCP-1: Monocyte chemotactrant protein-1; MDA: Malondialdehyde; MMP: Matrix metalloproteinase; NF-kB: Nuclear factor-kB; NS: Nonsmokers; PEF: Peak expiratory flow; RV: Residual volume; SAE: Small airway epithelium; TLC: Total lung capacity; TLR: The toll-like receptor; TNF-α: Tumor necrosis factor alpha

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Availability of data and materials
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Authors’ contributions
ZZ drafted the whole manuscript. HP performed “Systemic effects” Part and participated in revising this manuscript and checking the grammar and spelling errors. PC contributed to designing the structure of this review and gave the final approval of the version to be published: All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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