Oxidative DNA damage and somatic mutations in chronic obstructive pulmonary disease

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Key words:
- oxidative stress
- DNA damage
- DNA repair
- somatic mutations
- COPD pathogenesis

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OXIDATIVE STRESS AND OXIDATIVE DNA DAMAGE IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Living organisms are continuously exposed to oxidative stress, generated as a result of normal biochemical reactions and also caused by exposure to a variety of external factors related to lifestyle and environment, such as tobacco smoke and air pollution[1]. The lung, in particular, is constantly exposed to a high-oxygen milieu and to exogenous pollutants and toxicants that are carried in the inhaled breath. Because of its large surface area and its associated rich blood supply, the lung is highly susceptible to injury mediated by oxidative stress[1-3]. Oxidative stress has multiple significant effects on lung physiology, including oxidative deactivation of antiproteases and surfactants, mucus hypersecretion, membrane lipid peroxidation, impairment of mitochondrial function, alveolar epithelial injury, remodelling of extracellular matrix, apoptosis and cellular DNA damage (Figure 1)[1,4].

Oxidative stress and chronic inflammation are important features in the pathogenesis of chronic obstructive pulmonary disease (COPD)[5,6]. The oxidative stress in COPD is the result of the increased burden of inhaled oxidants (e.g., cigarette smoke) in addition to increased amounts of reactive oxygen species (ROS) generated by the various inflammatory, immune and epithelial cells of the airways[5,6]. Cigarette smoke, the major aetiological factor in the pathogenesis of COPD, releases thousands of oxidants, free radicals and other chemical compounds, including hydroxyl radicals (-OH) and hydrogen peroxide (H_2O_2). It also recruits immune and inflammatory cells to the lungs, aggravating the oxidant/antioxidant imbalance[2,3,7].

In normal conditions, oxidative stress is combated by multiple antioxidant and repair systems that come into play for the replacement of damaged cells, nucleic acids, proteins and lipids[1,7,8]. The distribution of oxidative damage in the genome depends on the varying susceptibility of the constituent sequences to oxidative attack, and preferential targeting of repair processes. In addition, evidence is accumulating that oxidative damage to non-coding DNA, such as telomeres, microsatellite sequences, promoters and sites of methylation, can also have notable biological consequences, particularly as repair processes directed to non-coding regions are slower[7-9].

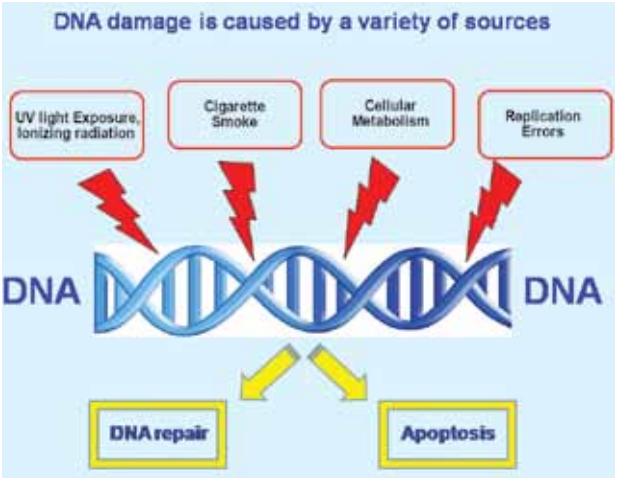

FIGURE 1. Sources of DNA damage: DNA damage is caused by a variety of factors, including UV light or ionizing radiation exposure, cigarette smoke, cellular metabolism by-products, and replication errors. Under normal conditions, oxidative stress is combated by multiple antioxidant and repair systems for the replacement of damaged cells and nucleic acids. When the oxidant burden overcomes the antioxidant repair capacity, the altered cells are removed by apoptosis.

SOMATIC MUTATIONS AND MICROSATELLITE DNA

There are two main categories of genetic mutation; *de novo* and somatic mutations[10]. Mutations that occur only in gametes or that occur just after fertilization are termed *de novo* (i.e., new) mutations. If a mutation occurs in somatic cells, that mutation will be limited to the organism in which it occurred, and will not be passed on to future generations. This type of genetic error is called an acquired or somatic mutation, and it appears in the DNA of individual cells at some time during a person's life. Acquired mutations can be caused by external, environmental factors (ultraviolet radiation, carcinogens, pollutants/toxicants, cigarette smoke) or it can occur if a mistake is made during replication, when DNA copies itself during cell division[10]. Oxidative stress, which is central to the pathogenesis of chronic inflammatory lung diseases, such as COPD, can directly damage lung DNA, and in particular the base composition of the repeated sequences (i.e., microsatellite DNA) especially when coupled with DNA mismatch repair system (MMR) deficiency[8-10].

Microsatellites are unique to every individual and are identical in cells from different tissues in the same individual[11-13]. Microsatellites are scattered throughout the genome and are usually located outside of, but occasionally within, coding sequences; they are faithfully replicated in healthy cells, ensuring that coding sequences remain within the appropriate reading frame[11-13]. The insertion or deletion of one or more repeat units can induce frame shift mutations in downstream genes. These mutations serve as highly polymorphic markers that are used for genome mapping in many organisms, including humans[11-13]. The MMR system is very important in the maintenance of microsatellite stability. It is designed to correct single-base mismatches and small insertion/deletion loops that may occur during DNA replication[14,15]. The assessment of microsatellite DNA instability (MSI) in peripheral tissue samples has offered a reliable means for the study of acquired mutations. This is accomplished with the use of microsatellite markers targeting specific chromosomal loci near or in genes that are known or suspected to be implicated in the pathogenesis of a disease[11-13].

MICROSATELLITE DNA INSTABILITY IN COPD

The first studies that revealed somatic mutations in the form of MSI in benign lung diseases, were in COPD[16,17] and later studies were conducted in asthma[18]. In a comparative study between COPD and asthma at the microsatellite DNA level, patients with COPD patients revealed a higher frequency of somatic mutations than those with asthma[19]. In the same, study COPD specific microsatellite sites were identified adjacent to genes related to COPD pathogenesis (e.g. surfactant A, perforin, CD8, TNF)[19].

The relationship between COPD exacerbations and genetic instability was assessed next, and the "frequent COPD exacerbator" was shown to exhibit the highest rates of genetic defects[20]. A possible explanation was that frequent exacerbations characterized by persistent inflammation and increased oxidative burden could lead to greater oxidative DNA damage compared with stable COPD[20].

Another important issue was the exploration of the lung cell subpopulation susceptible to acquired somatic mutations. The hypothesis targeted the lung epithelial barrier cells (LEBCs), since they constitute the outer cellular layer of the bronchial tree and are exposed to numerous host and environmental insults[21]. There was much evidence to suggest that the burden of inhaled oxidants from cigarette smoking, and the increased amounts of reactive oxygen species generated by various inflammatory cells of the airways in COPD could seriously affect the air–lung barrier system as the first line of defence[2-3]. We used immuno-magnetic beads cell separation, verified by

flow cytometry (FC) and microsatellite DNA analysis on sputum and bronchioalveolar lavage fluid (BALF) specimens to identify the cellular subpopulation susceptible to somatic DNA damage in patients with COPD patients and smokers. This was the first study to separate sputum and BAL cellular subpopulations in COPD patients and smokers and it revealed that somatic DNA alterations were exhibited exclusively in the lung epithelial barrier cells of patients with COPD. These findings supported the hypothesis that the persistent inflammation and oxidative burden in COPD could affect the cellular component of the air-lung barrier system leading to oxidative DNA damage and consequent somatic mutations in the lung epithelia[21].

A HYPOTHESIS FOR THE INITIATION OF COPD

Based on the studies described above, we proposed a new model for the initiation of COPD (Figure 2), comprising oxidative DNA damage of the LEBCs and the host immune responses[22].

At the onset of COPD, cigarette smoking affects the air–lung barrier system and, in particular, its cellular component, via repeated oxidative stress leading to oxidative

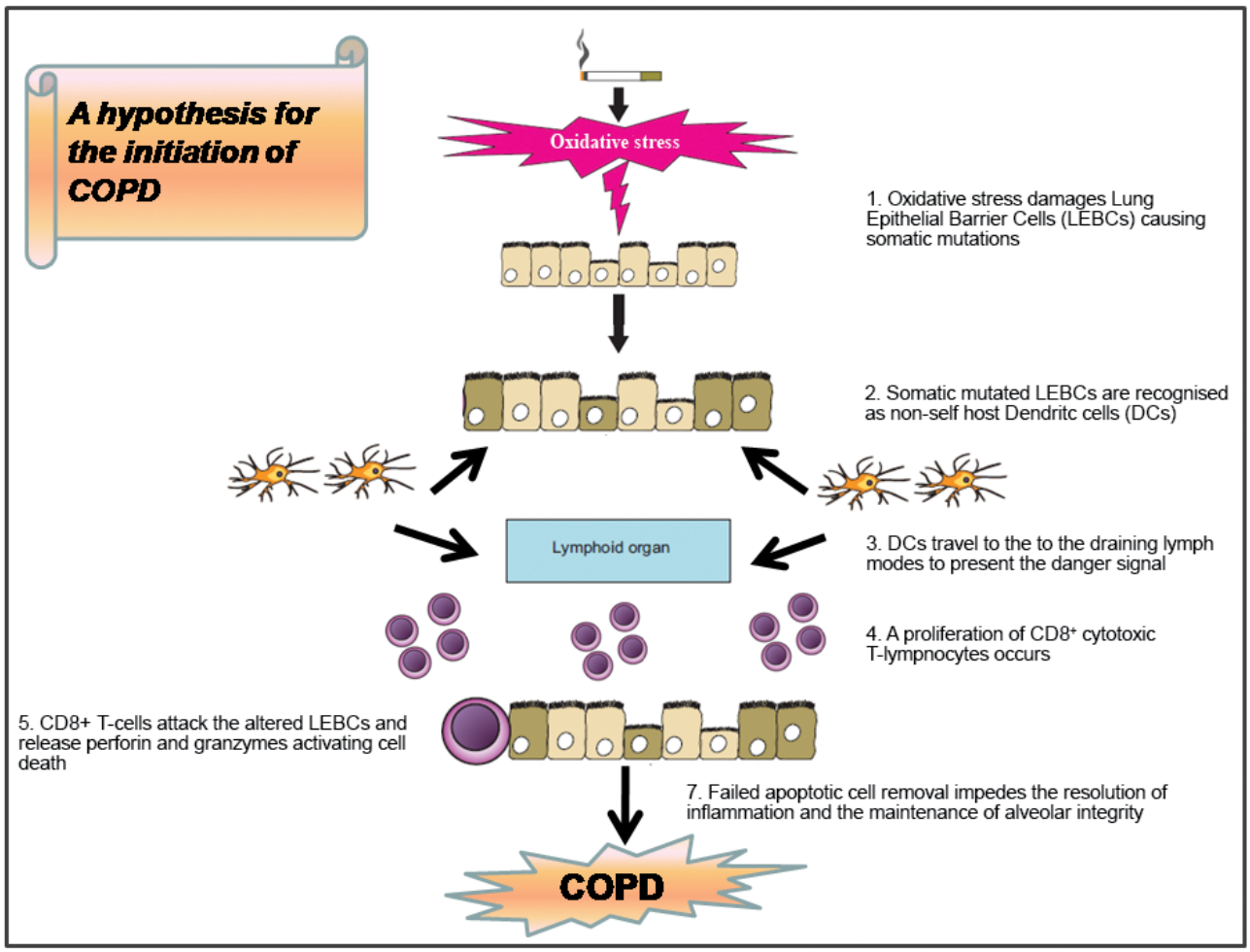

FIGURE 2. A hypothesis for the initiation of COPD: At the onset of COPD, cigarette smoke injures the lung epithelial barrier cells (LEBCs) via repeated oxidative stress, and affects the DNA auto-repair ability, leading to acquired somatic mutations. Affected LEBCs, by emitting danger signals, constitute an indirect way of activating and polarising the dendritic cell (DC) network. Lymphoid organs respond with a proliferation of CD8+ cytotoxic T-lymphocytes which attack the affected LEBCs and release perforin and granzymes, activating cell death. Failed apoptotic cell removal (efferocytosis) impedes the resolution of inflammation and the maintenance of alveolar integrity, leading to COPD.

DNA damage[2-4,9]. Oxidative stress damages the DNA of the LEBCs, leading to acquired somatic mutations, and further suppresses key genes of the DNA MMR system, impairing the DNA auto-repair ability[9,21]. Somatic mutations of the LEBCs are an indirect way of activating and polarising the dendritic cell (DC) network, by danger/alarm signals expressed by the altered cells[22]. In addition, smoke-induced changes in pulmonary DCs lead to the development of inappropriate immunological responses to antigens[23].

In the "danger model", antigen-presenting cells, such as DCs, can be activated by danger/alarm signals that are produced by self-injured cells after exposure to pathogens, toxins, mechanical damage, oxidative stress and cigarette smoke[24,25]. Polarised DCs travel to the draining lymph nodes carrying this endogenous alarm signal, produced by the damaged LEBCs, and they present it to the naive T-lymphocytes, inducing a proliferation of CD8+ cytotoxic T-lymphocytes[22,25]. The CD8+ T-cells migrate to the sites of the initial insult and, by releasing perforin and granzymes, attack the altered LEBCs, activating cell apoptosis cascades[22,25,26].

Disruption of the balance between apoptosis and replenishment of lung structural cells has been reported in COPD[27-29]. This phenomenon was confirmed by the detection in lung biopsies of increased expression of the pro-apoptotic p53 in the type II pneumocytes (PN II) of patients with COPD compared with smokers without COPD[30]. We also evaluated the expression of surfactant protein-A (SP-A), a lectin with multiple functions in innate host defense, regulation of the inflammatory process and clearance of apoptotic and necrotic cells in the lung. We found a decreased ratio of SP-A positive PN II to total PN II in patients with COPD patients compared with smokers without COPD. This ratio was also found to be correlated with the degree of airway obstruction[31]. The decreased levels of SP-A in COPD may thus contribute to the increased prevalence of respiratory infections, impaired efferocytosis (removal of apoptotic bodies), and abnormal tissue remodelling[31,32]. In earlier studies, we had observed genetic instability in the G29802 microsatellite marker, located adjacent to SP-A gene, in patients with COPD[19,20].

In conclusion, oxidative DNA damage contributes to the molecular pathogenesis of COPD. Somatic acquired mutations in COPD result from the oxidative DNA damage and the inefficient DNA repair machinery. The varying efficiency of DNA repair across the genome may be viewed as analogous to inter-individual variation in repair efficiency, which is a potential determinant of disease susceptibility.

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