Genomic Instability in Chronic Airway Inflammatory Diseases

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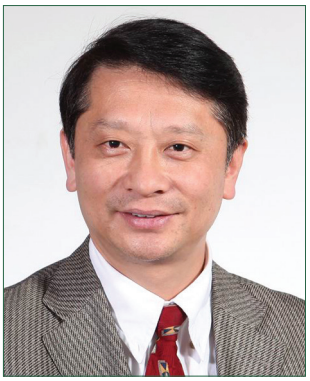

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Chronic airway inflammatory diseases are life-threatening conditions, including bronchial asthma, chronic obstructive pulmonary disease (COPD), and so on. However, as the disease etiology remains largely unclear, current treatments that target chronic airway inflammatory diseases are still not satisfactory. DNA damage response (DDR), regarded as one of the many causes of apoptosis and cell senescence, as well as a factor involved in carcinogenesis, has recently begun to attract attention as a source of chronic inflammation. Considering that COPD and allergic asthma inflammation enhance DNA damage, measures related with DNA repair should be taken so as to reduce the injuries caused by these airway diseases. Small molecule inhibitors specifically against various DNA repair proteins have been developed over the last decade to fight against chronic diseases. Poly(ADP-ribose) polymerase (PARP) inhibitor, for example, has already shown its potential in asthma animal models to block airway inflammation. In this review, we highlight the roles of DDR in chronic airway inflammatory diseases, and try to have a better understanding of these diseases. We also discuss the possibilities of targeting DDR signaling to develop potential novel treatments against these conditions. (*Biomed J 2015;38:117-124*)

Key words: asthma, chronic obstructive pulmonary disease, deoxyribonucleic acid repair, deoxyribonucleic acid damage response, genomic stability

Chronic airway inflammatory diseases are life-threatening conditions, including bronchial asthma, chronic obstructive pulmonary disease (COPD), etc., which impact on the quality of life and healthcare expenditure. Asthma is a complex disease involving multiple interactions of genetic and environmental factors. Patients suffering from asthma range widely in age, from teenage children to elderly people. Over 300 million individuals are affected by asthma worldwide, of which there are at least 35 million patients in the United States alone.[1] COPD is highly prevalent and a significant cause of morbidity and mortality, which affects more than 200 million people globally.[2] It is expected to be the third leading cause of death worldwide in 2020.[3] However, as the disease etiology remains largely unclear, current treatments that target chronic airway inflammatory diseases are still not satisfactory.

Human genomic DNA is constantly exposed to various endogenous and exogenous stress factors, where genome integrity has continuously been threatened. DNA damage constantly takes place under these conditions by free radicals and other reactive compounds produced during metabolism,

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Received: Feb. 11, 2014; Accepted: Sep. 12, 2014

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DOI: 10.4103/2319-4170.143478

errors happening in DNA replication and recombination, ultraviolet (UV) and ionizing irradiation from the environment, some harmful chemicals, and so on. Therefore, cells are often under constant assault by endogenous and environmental DNA damaging agents. It is estimated that approximately 10 DNA double-strand breaks (DSBs) are formed daily in one individual mammalian cell.[4] DNA damage response (DDR), regarded as one of the many causes of apoptosis and cell senescence, as well as a factor of carcinogenesis, has recently begun to attract attention as a source of chronic inflammation.[5-11] Frequent DNA damage may increase the mutation rate and genomic instability, which is in most cases fixed by a variety of DNA repair mechanisms. An essential and first step to initiate DNA repair is to block the cell cycle progression and prevent the cycle events that might aggravate the adverse effects of DNA damage and allow repairing.

Inflammation is a protective response to cellular and tissue damage or injury, and also the main feature of COPD and asthma, the two typical chronic airway inflammatory diseases. However, when this beneficial response occurs in an uncontrolled manner, it causes excessive cellular and tissue damage that results in chronic inflammation and destruction of normal tissue.[12,13] Since human airway is an open system, once exposed to cigarette smoke, gases, or other noxious particles, the epithelial cells are activated to produce inflammatory mediators. Reactive oxygen species (ROS) are one of the most important mediators. Excessive production of ROS is commonly thought to be responsible for a range of respiratory inflammatory diseases, including COPD and asthma. It has been suggested that ROS induces DNA damage in airway inflammation.[14,15]

In this review, we highlight the role of DDR in chronic airway inflammatory diseases, and try to have a better understanding of these diseases at a molecular level. We also discuss the possibilities of targeting DDR signaling to develop potential novel treatments against these conditions.

Types of DNA damage and repair defects in COPD

DNA DSBs

Smoking is known to be highly associated with COPD, which has been reported to be one of the most important environmental factors that cause DNA damage.[16-18] Also, DSBs are among the most dangerous forms of DNA damage caused by smoking.[19] DSBs had been detected in peripheral blood mononuclear leukocytes of COPD patients, and it had been proved that elevated levels of DNA damage are strongly associated with smoking COPD patients than with random COPD patients.[20] When DSBs are induced, the histone H2AX becomes rapidly phosphorylated at serine 139, the so-called γ-H2AX. Following activation by phosphorylation, γ-H2AX serves as a reliable and sensitive indicator for DSBs because it is activated and recruited to the damage loci at a very early stage and can be easily visualized by staining with antibodies.[21-24] Following phosphorylation and recruitment at DSBs, γ-H2AX further recruits other DNA repair proteins, including p53-binding protein (53BP) 1 and BRCA1, resulting in activation of distinctive downstream repair pathways. By immunofluorescence staining of these particular proteins, researchers found that alveolar type I, type II cells, and endothelial cells in patients with COPD showed higher levels of DDR at DSBs than those found in asymptomatic smokers and non-smokers.[25]

Somatic DNA alterations

Microsatellite DNA instability (MSI) has been correlated with a high somatic mutation rate and is associated with deficiency of DNA mismatch repair.[26,27] It was reported in 1999 that MSI was found exclusively in the sputum cells of smokers with COPD, which indicated that genetic alteration might increase susceptibility to COPD.[28] Another study revealed that MSI was found in 38% COPD patients (14 out of 36), while none was found in bronchiectasis or control subjects (non-COPD smokers, healthy subjects).[29] Loss of heterozygosity (LOH) was also observed in epithelial barrier cells of COPD patients. LOH was found in D5S207, D6S344, G29802, and D17s250 microsatellite markers, while MSI was found in D13S71, D5S207, and D6S344.[30] A study aimed to investigate the relationship between MSI in sputum cells and exacerbation frequency, and found that 18 out of 36 patients exhibited MSI in their sputum cells, and patients who exhibited MSI showed significantly increased frequency of COPD exacerbations. In addition, a significantly higher frequency of purulent and severe exacerbation was found in patients exhibiting MSI. These results suggested that somatic mutations could be involved in the pathogenesis of the disease.[31]

Mitochondrial DNA damage

Mitochondrial DNA (mtDNA) contributes to oxidation resistance, which is an important determinant that affects COPD susceptibility. It was reported that mtDNA is about 30-fold more sensitive to exogenous oxidants than nuclear DNA.[32-34] In order to investigate whether mtDNA damage is involved in COPD susceptibility, the frequencies of mtDNA haplogroups and an 822-bp mtDNA deletion in 671 COPD patients and 724 control individuals were analyzed and compared. The results revealed that mtDNA haplogroups A and M7 might be risk factors for COPD, whereas haplogroups D, F, and M9 might decrease risk of COPD. Other evidence revealed that skeletal muscle mtDNA and nuclear DNA

fell significantly after exercise, and the changes were much more obvious in patients with COPD.[35] Investigators had detected a basic site and strand breaks in mtDNA in lung tissue from patients with severe COPD.[36]

Loss of telomeres

It seems that COPD is a disease of accelerated aging, and occurs mostly in elderly people.[37] Oxidative stress, an important factor of COPD, has a significant impact on the rate of telomere loss. Telomere shortening is dramatically accelerated (or slowed) in cells with increased (or reduced) levels of oxidative stress.[38] Shortness of telomere length was indicated to be associated with a 28-fold increased risk of COPD.[39] Telomere dysfunction is one of the major processes perpetuating pulmonary inflammation in COPD.[40] COPD patients were reported to have shorter telomeres in leukocytes, out of 46,396 individuals from the Danish general population. Rode *et al*. found that shortened telomere length was associated with higher risk of COPD, though the association was markedly attenuated after age and multivariable adjustment.[39]

Gene polymorphisms

HOGG1 Ser326Cys and *XRCC1* Arg399Gln polymorphisms have been shown to contribute to the susceptibility of COPD, where *HOGG1* and *XRCC1* genotypes of 201 COPD patients and 309 controls were determined. The results showed that the risk of COPD is significantly elevated among smokers with *HOGG1* 326Cys and *XRCC1* Arg399Gln.[41] In another study on COPD and gene polymorphisms, which was conducted to find the link between genetic polymorphisms in genes *XRCC1* (Arg399Gln), *OGG1* (Ser326Cys), *XRCC3* (Thr241Met), and *XRCC4* (Ile401Thr) and the level of DNA damage and repair, 51 COPD patients and 51 controls were assessed by comet and micronucleus test, and the results showed that COPD patients with the risk alleles *XRCC1* and *XRCC3* had higher levels of DNA damage.[42] Micronucleus represents a severe form of genomic instability as a result of disrupted faithful segregation of chromosomes during mitosis. *ADAM33*, a disintegrin and metalloprotease domain 33 gene, has been found to be associated with asthma, and declined lung function in COPD, within which seven single-nucleotide polymorphisms (SNPs) had been demonstrated to be associated with COPD in the Mongolian population of China.[43] However, this was not found to be consistent in certain populations such as Caucasians, where no correlation between *ADAM33* polymorphism and COPD was identified.[44] Iron-responsive element-binding protein 2 (IREB2) and mRNA were found to be increased in the lung tissues from COPD patients compared with controls,[45] and evidence suggests that the IREB2 SNPs in association with COPD are SNP rs2568494, rs2656069, and rs12593229.[46] Though COPD is mainly caused by smoking, another investigation found that IREB2 may affect COPD independent of smoking.[47]

DNA damage repair response

DNA repair mechanisms are versatile tools for cells to correct damaged DNA, which include base excision repair, nucleotide excision repair, DSB repair, and cross-link repair.[48] Efficient repair of damaged DNA, particularly DSBs, is essential for the maintenance of chromosomal integrity,[49] as DSBs are among the most serious forms of DNA damage caused by smoking. Impaired DNA repair efficiency is common in COPD. This may be due to the lack of DNA damage repair in both bronchial epithelium and connective tissue induced by heritable genetic polymorphisms.[50,51] Generally, DSBs are repaired either by homologous recombination or by non-homologous end-joining (NHEJ) pathway; in the latter pathway, six distinct proteins (Ku70, Ku80, XRCC4, DNA ligase IV, Artemis, and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) play a major role.[52,53] The damage sites are initially recognized by γ-H2AX, which will extend up to several thousand nucleosomes from the actual loci of the DSB and may mark the surrounding chromatin for recruitment of the proteins that are required for the following downstream DDR signaling transduction and repair.[54] XRCC5, also known as Ku80, is an ATP-dependent DNA helicase mapped to chromosome 2q35 and contains 21 exons spanning about 97 kb. *XRCC5* is identified as a potential COPD susceptibility gene, by combining data from COPD genetic association studies conducted in four independent patient samples.[55-57]

Poly (ADP-ribose) polymerase-1 (PARP-1) is a monomeric nuclear enzyme present in eukaryotes, and its primarily role is to act as a sensor of DNA damage.[58-60] To facilitate DNA repair on damaged DNA loci, PARP-1 becomes highly activated.[61,62] The activated PARP-1 transfers ADP-ribose units from NAD^+ to a protein acceptor to produce AD-ribose polymers, which will lead to rapid decline of cellular NAD^+ concentrations and pose a large demand on cellular ATP stores for re-synthesis of NAD^+.[63] The level of NAD^+ in cells is considered to play a key role in the control of many fundamental cellular processes.[64] Under conditions of energy crisis, cells undergo necrotic death, further amplifying the inflammatory response.[65] In a patient–control study, 37 stable COPD patients and 21 age-matched healthy volunteers were enrolled. PARP-1 activation was tested by immunofluorescent detection of PAR polymers in peripheral blood lymphocytes. The level of PAR polymer-positive lymphocytes was found to be higher in COPD patients than in healthy controls, and trolox equivalent antioxidant capacity of deproteinized plasma, plasma uric acid, as well as blood NAD^+ of stable COPD patients were significantly reduced compared to controls. In

addition, the levels of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-8, and soluble intercellular adhesion molecule-1 (sICAM-1), were found to be increased in COPD patients. Collectively, these data indicated involvement of PARP-1 activation in the pathophysiology of COPD.[66]

DDR in asthma

Asthma, a truly complex disease, is currently considered as a chronic respiratory disorder associated with airway inflammation. Allergic asthma is characterized by increased levels of eosinophils, immunoglobulin (Ig) E, and multiple cytokines [including IL-4, IL-5, IL-9, and tumor necrosis factor (TNF)-α].[67,68] From the perspective of its pathogenesis, asthma is a result of complicated interactions between genetic and environmental factors, which induce airway inflammation and remodeling.[69] Surprisingly, damages in nuclear DNA are correlated with asthmatic inflammation; for example, it was reported that the frequency of spontaneous sister chromatid exchanges (SCEs) was increased in asthmatic patients (AP) compared with control subjects, indicating elevated levels of homologous recombination repair in damaged chromatids.[70] In addition, DNA damage levels in lymphocytes increased significantly in children with asthma.[71] This damage might be related to increased oxidative stress. A study of DNA damage in children with asthma demonstrated that DNA damage parameters and glutathione (GSH) levels were decreased after treatment, which implicated anti-asthmatic therapy could control asthmatic syndrome and, at the meantime, reduce mutation risks in pediatric patients.[72]

Abnormality of cell cycle regulation in asthma

Faithful DNA replication and repair need precisely regulated cell cycle control. Since the asthmatic bronchial epithelium is characterized by widespread damage, researchers postulated that this is associated with deregulation of cell cycle profiles. Expression of $p21^{waf}$, a cyclin-dependent kinase inhibitor, was shown to be increased in asthmatic bronchial epithelium.[73,74] Analysis of bronchial biopsies from 6 normal subjects without asthma, 14 subjects with mild asthma, and 10 subjects with severe asthma by immunohistochemical staining showed that $P21^{waf}$ expression was significantly higher in asthmatic versus non-asthmatic epithelium.[74] Increased levels of $P21^{waf}$ were found not only in adult asthma patients but also in pediatric asthma patients. Immunostaining of intact bronchial epithelium from 23 asthmatic children (7 controls, 7 moderate asthma patients, and 9 severe asthma patients) showed that $p21^{waf}$ expression was significantly higher in asthmatic children than in healthy controls.[75] $P2^{1waf}$ over-expression was reported to influence cell cycle checkpoint activation, cell proliferation, and survival. Following p53 activation, p21 induction might, in turn, affect DNA repair response that contributes to airway inflammation and remodeling.[73,76]

Defects of DNA repair in asthma

Increased levels of DNA damage in asthma patients are not only a result of high intensity of allergic stress, but also due to reduced ability to fix damaging problems. PARP, a poly-ADP ribose polymerase, is involved in a number of essential cellular processes, including DNA repair and programmed cell death.[77] The widely studied PARP-1 has been implicated in the regulation of distinct biological activities including base excision DNA repair. PARP-1 plays truly a determining role in cell survival in response to DNA damage,[78] and may include damages induced in asthma. Furthermore, increased activation of PARP-1 depletes the cellular stores of NAD and ATP in conditions involving massive DNA damage, which directly induces irreversible cytotoxicity and potential cell death.[79] It was reported that over-activation of PARP by oxidative stress-induced massive DNA damage may exacerbate inflammation.[80] The polymerase chain reaction (PCR)-based restriction analysis of 112 stable asthma patients and 180 normal controls revealed that PARP-1 762 V allele had 5 times higher risk of susceptibility to asthma than those without the allele, and PARP-1 762AA genotype conferred only a 3.4-fold reduction in risk while VA genotype conferred an even greater level of protection.[81]

Future development of therapy

In conclusion, severity of DNA damage is a pivotal factor in the development of chronic airway inflammatory diseases (COPD and asthma), which may serve as a molecular link in these diseases.[82] In certain conditions, DNA damage triggers airway inflammation, and therefore causes COPD or asthma, while on the other hand, chronic inflammation may also enhance the levels of DNA damage. Currently, anti-inflammatory treatment is still a major strategy to manage airway inflammatory diseases. It does not seem to be sufficient. Measures related to DNA repair should be taken so as to minimize the injuries caused by these airway diseases. Small molecule inhibitors, especially against various DNA repair proteins, have been developed over the last decade to fight against chronic diseases like cancer. Some of them have shown promising results in killing tumor cells with minimum effects on normal tissues, as little damage occurred in healthy conditions. It remains to be seen if it would be plausible to extend this idea in the management of chronic inflammatory diseases.

PARP inhibitors, for example, have already shown their potential in COPD and asthma patients or animal models in blocking airway inflammation. For instance, it was shown that PARP inhibitor could attenuate lipopolysaccharide (LPS)-induced cytokine (TNF-αand IL-6) release from leukocytes of patients with COPD.[83] An *in vitro*

study also found that flavone (a PARP-1 inhibitor) could reduce LPS-induced IL-8 production in pulmonary epithelial cells, leading to a hypothesis that PARP-1 inhibitor could have beneficial effects in COPD by preservation of cellular NAD^+ levels and attenuating inflammatory conditions.[84] Studies based on PARP-1 knockout mice as well as specific PARP inhibitors have indicated that inhibition or genetic ablation of PARP-1 protects asthma mice model from oxidative stress-induced inflammation.[85] In murine models, PARP-1 plays a critical role in the pathogenesis of asthma-related lung inflammation. PARP-1 deficiency leads to increased production of the Th1 cytokines IL-2 and IL-12, but prevents recruitment of eosinophils by modulating Th2 cytokines, particularly by regulating IL-5 production.[86] Further investigation was carried out to investigate the action of PARP-1 inhibitor (HYDAMTIQ) in the process leading from asthma-like events to airway damage. In the ovalbumin (OVA)- induced asthma model, HYDAMTIQ treatment could reduce lung histological abnormalities, lung oxidative level, and also the lung content of pro-inflammatory cytokines (TNF-α, IL-1β, IL-5, IL-6, IL-18).[87] These findings support the idea that PARP inhibitors could have a therapeutic potential to reduce chronic airway inflammation, airway damage and remodeling in asthmatic patients.

Another PARP family protein, PARP-14, was also shown to be correlated with asthma. PARP-14 was reported to act as a transcriptional switch for IL-4–dependent signal transducer and activator of transcription 6 (STAT6).[88] IL-4–activated STAT6 is involved in Th2 response and promoting the asthmatic condition,[89-93] thus STAT6 is an attractive therapeutic target for asthma. Further investigation revealed that PARP-14 and its enzyme activity aid in the differentiation of T cells toward a Th2 phenotype by regulating the binding of STAT6 to the Gata3 promoter.[94] So, it might be a potential new therapy for allergic asthma targeting PARP-14. Exact functions of PARP protein are still very much unknown. It is, therefore, very much needed to clarify whether it is the DNA repair roles of the PARP family proteins that play key functions in these conditions.

Although great progress has been made in the last decades, it is still largely unclear how DDR impacts on chronic airway inflammatory diseases. To eventually improve treatments and alleviate the suffering of patients, more investigations would be required to further fully enhance our understanding of DNA damage response signaling and regulation in chronic airway inflammatory diseases.

Acknowledgments

We are grateful to Drs. Chao Cao and Hongbin Zhou for critical reading of this manuscript. This work was supported by National 1000 Talents Program for Young Scholars (SY), National Natural Science Foundation of China (31370901), the Research Funding from Zhejiang Provincial Department of Education (Y201329936), the Fundamental Research Funds for the Central Universities, and the State Key Program of National Natural Science Foundation of China (81130001).

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