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Oxidative DNA damage and repair system

Derya Özsavcı*

Department of Biochemistry, Faculty of Pharmacy, Marmara University, İstanbul, Turkey

Free radicals and other reactive species are produced during aerobic metabolism in the body. ROS occurring in vivo can cause oxidative damage of aminoacids, lipids, proteins and DNA. Excess ROS may induce the formation of oxidative DNA damage, DNA strand breaks, base modifications and chromosomal aberrations. For repair of oxidative DNA damage, human cells are supported by five DNA repair systems. These are: direct reversal, mismatch repair, double-strand break repair, base excision repair (BER) and nucleotide excision repair (NER). DNA repair gene polymorphism accounts for individual variations in DNA repair capacity, thus the genetic background of subjects are the most important implications for prospective disease risks.

Key words: DNA, damage, repair, gene, ROS

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Introduction

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) are produced during aerobic metabolism in the body. Actually in living cells, mitocondria (oxidative phosphorylation), leukocytes (oxidative burst), peroxisomes (degredation of fatty acids) and cytochrome p450 system may contribute to the production of ROS (reactive oxygen species) in normal metabolism.[1] ROS occurring in vivo can cause oxidative damage of aminoacids, lipids, proteins and DNA. Apart from in vivo ROS, DNA can also be damaged through exogenous ROS sources including cigarette smoking, UV and ionizing radiation.[1,2]

In fact, free radicals have two faces in the body. They play as stimulators of signal transduction (e.g Ca^{2+} signaling and protein phosphorylation)[3] and regulatory molecules at physiologic levels whereas, they are highly cytotoxic oxidants at pathologic levels.[1] Excess ROS may induce the formation of oxidative DNA damage, DNA strand breaks, base modifications and chromosomal aberrations.

Available evidence has shown that DNA damage can result from free radical attacks if not repaired, the damage may lead to deteriorated gene expression, development of a number of diseases such as cancer, diabetes, neurodegenerative and vascular diseases and also aging.[4,5] Especially in development of atherosclerosis, ROS have important roles including promoting cell proliferation, hypertrophy, growth arrest, apoptosis and oxidation of LDL.[6] Additionaly, ROS indirectly affect platelet functions through enhanced platelet activation and decreased nitric oxide thus, contribute to atherosclerosis development.[7]

*Correspondence to: Derya Özsavcı
Marmara University, Faculty of Pharmacy,
Department of Biochemistry,
Haydarpaşa 34688 İstanbul, Turkey
Phone: +90 216 414 29 62
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DNA Repair Mechanisms

It is known that antioxidants and antioxidant enzymes are major scavengers of free radicals and have a crucial role in preventing many diseases such as cancer, stroke, neurodegeneration, diabetes and atherosclerosis. Additionally, there are important defense mechanisms against the effects of excess oxidative damage on cell survival. For repair of oxidative DNA damage, human cells are supported by five DNA repair systems. These are: direct reversal, mismatch repair, double-strand break repair, base excision repair (BER) and nucleotide excision repair (NER).

Direct reversal is related to the removal of the damaged parts from DNA, for example, O^6-methylguanine-DNA-methyltransferase can repair O^6-alkylguanine caused by alkylating agents (nitrosoureas, streptozotocin) transferring the alkyl group to a cysteine residue and constituting the correct guanine.

Mismatch repair system repairs the errors that arise during the formation of DNA replication and minor bases (oxidized, alkylated adducts) oxidatively damaged. It also repairs small deletions in the DNA caused by DNA polymerase.

During the ionizing radiation, cross-linking agents (cisplatin) and replication errors, double-strand breaks and DNA cross links are formed leading to chromosomal breaks and cell death. Double-strand break repair system, involving the RAD52 and RAD51 proteins, repairs the above mentioned breaks.[8]

Nucleotide excision repair system (NER) is responsible for the repair of distorted DNA double helix which occurs after exposure to UV light, dietary factors (aflatoxin, polyaromatic hydrocarbons) and cigarette smoking.[8,9]

Base excision repair system (BER) serves to repair apurinic/apryimidinic sites which are mutagenic and cytotoxic DNA lesions. BER system also repairs base deamination, alkylation and oxidation which lead to mispairing and mutations post replication.

BER pathway is believed to be the primary defense mechanism against oxidative damage. BER is considered to be the main pathway involved in repair of several types of damage, but NER may be considered as a back-up system. Thus BER removes small DNA lesions, like oxidized or reduced bases, non-bulky adducts and DNA single-strand beraks, whereas NER repairs bulky adducts, crosslinks and oxidative damages.[10]

BER consists of three stages. In the first stage, damage is recognized; in the second stage, damaged bases are removed and apurinic/apryimidinic sites created; and finally these sites are filled with new DNA synthesis. In human cells, there are eleven glycosylases that can detect damaged bases and remove them, creating apurinic/apryimidinic sites. Following this process, apurinic/apryimidinic sites are to be incised so that a single-strand break can be created, either by the glycosylase or by the apurinic/apryimidinic endonuclease enzyme. The most important repair enzymes in the BER pathway are x-ray repair cross-complementing group 1 (XRCC1), 8-oxoguanine DNA glycosylase (OGG1) and apurinic/apryimidinic endonuclease enzyme (APE1).

APE 1, which is one of these endonuclease enzymes, hydrolyzes the phosphodiester backbone 5' to the AP site.[11] Thus, normal 3'-hydroxyl group and abasic deoxyribose 5-phosphate are generated. APE1 also plays a major role as 3'-phosphodiesterase in initiating repair of single-strand breaks resulting from DNA damage by free radicals.[12-14] After hydrolyzation, APE1 produces normal 3'-hydroxyl nucleotide and maintains the DNA repair process.

Genetic (DNA repair gene polymorphism), dietary and lifestyle factors are said to be related to the impairment of DNA repair capacity. Although there are repair mechanisms against the deleterious effects of oxidative damage on cell, this DNA repair process is impaired in several diseases. Genetic background of the individual and inter-individual variations are most important implications for disease risk. Especially, in a number of studies, it was shown the relationship between cancer risk and DNA repair gene polymorphism.[15] It has been reported that, reduced NER capacity is associated with increased risk of skin, lung, head, neck and breast cancer whereas, reduced BER capacity with

lung cancer.[16,17] According to previous studies, APE1 polyymorphism, a T→G transversion, was reported to be related to increased risk of lung cancer. Conversely, it was reported that APE1 genotype was not associated with lung cancer risk in male smokers.[18]

Several studies demonstrated that repair of damage highly depends on the type of DNA lesion. In these studies, it was reported that apurinic/apyrimidinic sites are removed over short times whereas, repair of oxidized bases (8-oxoGuanosin) is a rather slow process.[19] Peroxynitrite mediated DNA strand breaks lead to activation of the nuclear enzyme (poly ADP-ribose) polymerase-1 (PARP-1) which is described as a DNA repair enzyme. According to recent studies, PARP-1 interacts directly with the BER protein XRCC1 and plays a crucial role in p53 activation and regulation after DNA damage.[20]

In the past, oxidative damage was determined with several markers such as malondialdehyde, modified LDL especially oxidized LDL, oxidized histidin and conjugated diens. Recently, other markers have also been used to detect DNA oxidation, for example urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG, or 8-oxodG), specific DNA repair products in urine, has been measured for this purpose. During free radical induced DNA oxidation, oxidatively modified product 8-OHdG is formed, in which hydroxyl group is added to the 8th position of guanine molecule.[21] It has been shown that 8-OHdG plays an important role in carcinogenesis and the increased levels of this product are associated with the size of tumor.[22]

Apart from cancer, 8-OHdG is said to be accumulated in nuclear and mitochondrial DNA in other diseases such as diabetes, rhomatoid arthritis, cronic liver diseases and coronary artery diseases. In the studies carried out with diabetic subjects, 8-OHdG increased and is proposed as a predictor of DNA damage in diabetes mellitus.[23]

Several investigators determined all of these oxidatively modified free bases, nucleosides carrying 8-hydroxylated guanine base, and deoxynucleosides with HPLC, mass spectrometry[24] and ELISA techniques in tissue, plasma and urine samples.[21]

In the different studies performed with different gene polymorphisms including APE1, it has been shown that there is correlation between various polymorphisms and lung cancer. Ruyck K. et al found a significant correlation between cigarette smoking and the "XRCC1 Arg399Gln and the XPD Lys751Gln" polymorphisms.In the same study, a significantly decreased lung cancer risk was found in individuals heterozygous for the XRCC1 Arg194Trp, the XRCC1 Arg280His or the OGG1 Ser326Cys polymorphisms.[10] Although some studies have reported that there is no association between APE1 Asp148Glu polymorphisms and lung cancer risk,[25] Ruyck K. et al showed that the APE1 Asp148Glu polymorphism may develop genetic predisposition to lung cancer.[10]

It has been shown that detection of DNA damage is a useful biomarker not only in carcinogenesis but also in atherogenesis. It was found that leukocyte 8-OH-dG is significantly higher in patients with atherosclerosis. 8-oxo-dG is strongly mutagenic and causes to increased frequency of spontaneous GC → AT transversion mutations in repair deficient cells.[4] Wim Martinet at al. demonstrated that in the atherosclerotic plaque, there are increased DNA repair enzymes involving base excision repair (Ref-1, PARP-1) and nonspecific repair pathways (p53, DNA-PK). They also showed that levels of 8-oxo-dG in human atherosclerotic plaques increased compared to the underlying media or nonatherosclerotic arteries.[6]

In another study carried by Barouch L. et al, it was reported that cardiac monocyte apoptosis increased in leptin deficient *ob/ob* (naturally occurring leptin deletion) and leptin resistant *db/db* (nonfunctional leptin receptor) obese mice where also DNA damage increased and long-term survival decreased. Occuring cardiac monocyte apoptosis may be associated with development of cardiovascular outcomes in the future. Furthermore, the DNA damage marker, 8-oxoG, was elevated whereas, the DNA repair marker, MHY glycosylase, was decreased in old *ob/ob* and *db/db* mice.[26]

On the other hand, several studies suggested that the expression of the apurinic/apyrimidinic endonuclease Ref-1 protein (APE/Ref-1 protein)

was preserved in patients with heart failure. This DNA enzyme is responsible for preserving the genomic stability by repairing the apurinic/apyrimidinic sites, its downregulation precedes the cell death during the neuronal apoptosis.[27] It was also demonstrated that in advanced heart failure, preserved expression of this BER enzyme may be important to prevent apoptosis in nonischemic cardiomyopathy.[28]

As another damage protein, DNA damage protein 34 (GADD34), is upregulated in cellular stress and believed to mediate DNA repair and restore protein synthesis. Morton et al. indicated that GADD34 immunoreactivity was downregulated in tissue displaying ischaemic damage.[29]

It is known that elevated oxidative stress is an important characteristic of hypercholesterolemia-induced atherosclerosis. Actually, cholesterol induced plaque formation is related to DNA damage. Several studies using immunohistochemical method indicate that 8-oxoG levels were increased both in macrophage-derived foam cells and ferric nitriloacetate- induced carcinogenesis. It was found that the number of DNA strand breaks was higher in the hypercholesterolemic animals using alkaline single-cell gel electrophoresis. Elevated levels of DNA repair enzymes (poly[ADP-ribose] polymerase 1, p53, phospo-p53 [phosphorylated at Ser 392]and XRCC1 [x-ray repair cross-complementing 1] were observed in atherosclerotic plaque.[19]

Conclusion

DNA repair gene polymorphism accounts for individual variations in DNA repair capacity, thus the genetic background of subjects are the most important implications for prospective disease risks. As it shown that oxidative damage and repair increase significantly in human atherosclerotic plaques, thus good investigation and maintaining this equilibrium is crucial for the nature of disease. To date, we have not experienced any study related the importance of APE1 poymorphism in atherosclerosis but in accordance with our results, it seems that APE1 polymorphism is one of important predictors for atherosclerotic diseases. Although genetic polymorphism seems to be one of the major factors underlying the ethiopathology of several disorders involving atherogenesis, gene-gene interactions and other environmental factors should be considered in further studies.

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