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DNA damage by oxidative stress: Measurement strategies for two genomes

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Abstract

DNA damage is any modification to the structure of DNA that alters its coding properties and/or interferes with cell processes. One major consequence of oxidative stress is DNA damage, which include base modifications, abasic sites, and strand breaks. A wide variety of tools are available to measure DNA damage, in particular for the nuclear genome. Some of the most widely used tools to measure oxidative DNA damage are the comet assay, PCR-based assays, immunoassays, and mass spectrometry-based approaches. Although the field has made great strides in describing oxidative DNA damage and improving the overall sensitivity of standard techniques, many questions are still unanswered and substantial technical challenges remain. Particularly, differential quantification and description of DNA damage (mitochondrial vs. nuclear) continues to be a challenge and a priority going forward. Technical advancements have allowed us to acquire a great amount of new knowledge in recent years, and as the pace of tool development increases, so will our understanding of DNA damage and its biological consequences for human health and disease.

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DNA damage, Oxidative stress, Mitochondrial DNA, Nuclear DNA, Measurement of oxidative DNA damage.

1. Introduction

DNA damage is any modification to the structure of DNA that alters its coding properties and/or interferes with cell metabolism, such as replication or transcription [1–3]. A major cause of DNA damage is oxidative stress, which is defined as an imbalance between an organism's ability to detoxify the reactive intermediates and the amount of reactive oxygen species (ROS) it faces [4]. Broadly, ROS refers to all physiologically-relevant chemical species capable of reacting with macromolecules, altering their structure directly or indirectly. This includes free radicals, and molecules capable of producing radicals or that are oxidizing agents themselves [5]. Not all ROS affect DNA similarly; superoxide and hydrogen peroxide at physiologically-relevant levels do not readily react with intact DNA (via redox chemistry); however, they can react with other molecules and produce ROS such as hydroxyl radicals, which readily reacts with DNA or any other macromolecule it encounters [6,7].

ROS have been reported to cause a variety of lesions to DNA (such as base and/or sugar alterations, sugar-base cyclization, DNA-protein cross-links, and intra- and interstrand cross-links) which in turn can result in DNA strand breaks; comprehensive descriptions of these lesions and the mechanisms responsible for their formation can be found in (Y Yu et al. [8], J Cadet et al. [9], M Dizdaroglu and P Jaruga [10], and MD Evans et al. [11]). Exogenous agents that can cause DNA damage via oxidative stress include air pollution, ionizing radiation, ultraviolet light, lifestyle (e.g. smoking, diet), and exposure to pesticides and metals [12–14] (see Fig. 1). It is important to highlight that endogenous factors such as spontaneous or enzymatic conversions and ROS from cellular processes (such as mitochondrial respiration and the inflammation response) can also cause DNA damage, particularly when these processes become dysfunctional [15]. This current *opinion article* summarizes the available tools to study oxidative DNA damage, the important outstanding technical hurdles to studying DNA damage to overcome (in particular mitochondrial DNA damage), and potential avenues for future exploration.

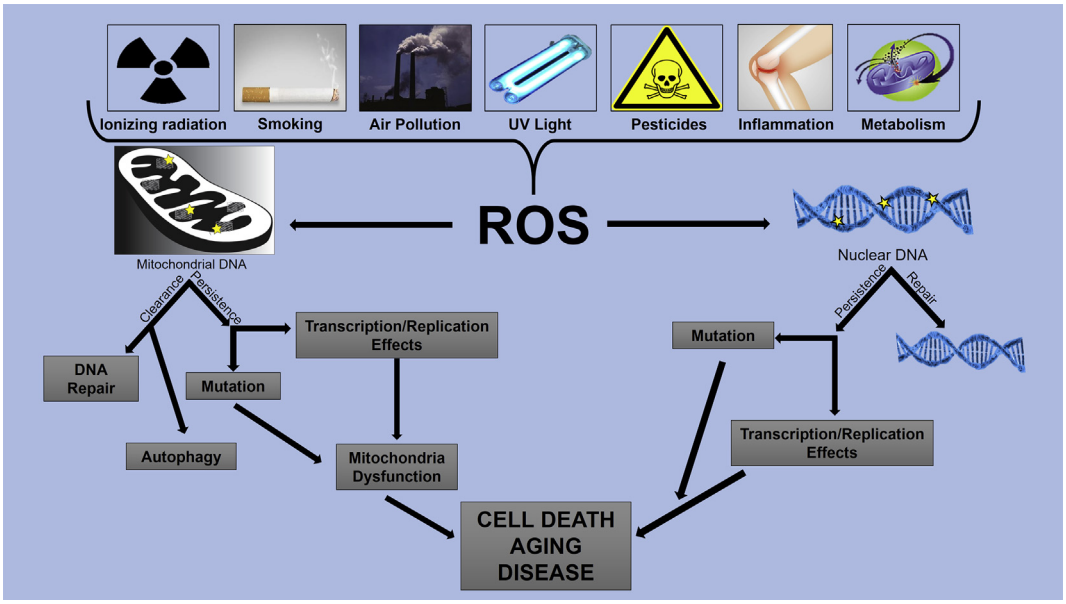
2. Tools to assess oxidative DNA lesions

The main types of DNA damage (or lesions) generated by oxidative stress are base modifications, abasic sites (i.e. base loss) and DNA strand breaks. The ideal tool and technique to measure DNA damage varies based on which lesion is of interest. In the following subsections we describe some of the current tools available and their applications, and pros and cons for each method. Brief descriptions of the major tools to measure DNA damage can be found in Table 1.

2.1. Base modifications and abasic sites

One of the major products of DNA oxidation is the base modification 8-oxoguanine (8-oxo-G, also known as 8-hydroxyguanine or 8-OH-dG), yet there are well over

Fig. 1



Causes and consequences of oxidative DNA damage. Reactive oxygen species (ROS) from a variety of endogenous and exogenous sources can have damaging effects on DNA. The fate of this damage has important consequences for the cell; if not repaired (or removed, an option for mitochondrial DNA), it can lead to transcription/replication issues, cellular dysfunction, mutations, aging, disease, or eventually cell death.

20 additional base lesions that have been identified [16]. Several methods have been historically used to study 8-oxo-G and other oxidative base modifications. Gas chromatography (GC) and liquid chromatography (LC), in conjunction with electrochemical detection (ECD) or mass spectrometry (MS), have been widely used to detect oxidative base modifications in DNA. These methods involve hydrolyzing the DNA to nucleosides, in effect measuring 8-hydroxy-2′-deoxyguanosine (8-OH-dG or 8-oxo-dG). Currently, LC with tandem MS (MS/MS) is the gold standard to measure oxidative DNA base lesions in biological samples. Its strength lies in the fact that it provides structural information to identify specific lesions with great accuracy and sensitivity.

However, in addition to the many advantages of using mass spectrometric approaches, these techniques have important drawbacks. The preparation of DNA for these assays (which involves isolating DNA followed by enzymatic digestion) is known to cause artifactual oxidative lesions; indeed, these spurious lesions can increase the lesion frequencies by several fold [9,17]. This important challenge has rendered these assays useful only in the context of elevated lesion burden, and not for steady-state lesion measurements. Also, the high amounts of DNA needed (around 30 μg) makes it technically difficult to measure lesions in mitochondrial DNA (mtDNA) given its low abundance per tissue mass [9]. For mtDNA, this technique would also require separating it from nuclear DNA (nucDNA), a challenging process given that isolating mitochondria can cause artifactual oxidative lesions even in the presence of antioxidants [18]. Furthermore, it is difficult to obtain pure mtDNA (whether it is extracted from isolated organelles or directly from whole cell lysates); that is, mtDNA not contaminated with nucDNA or other cellular components. Recently, B Ma et al. [19] utilized LC-nanoelectrospray ionization (NSI)-MS/MS to measure 8-oxo-dG and 8-oxo-dA lesions and observed a higher number of lesions in mtDNA compared to nucDNA; however, the levels of 8-oxo-dG reported are very high compared to what has been previously reported using LC-ECD (albeit performed in different tissues) [20]. This discrepancy highlights the difficulty in using chromatography-based methods for mtDNA lesion quantification.

Immunoassays can also be used for detecting specific lesions such as 8-oxo-G, 5,6-dihydroxy-5,6-dihydrothymine, and 5-hydroxymethylcytosine, as they utilize antibodies that have been generated against those lesions [21–23]. These antibodies have traditionally been utilized in enzyme-linked immunoabsorbent assays (ELISAs), radioimmunoassays (RIA) and even for immunohistochemistry purposes in tissue sections. Despite their ease of use, a major concern with this approach is the lack of specificity towards the oxidized lesion versus the undamaged base, making it impossible to evaluate physiological levels of oxidative lesions at their current detection capabilities [17].