Free Radical Biology and Medicine 107 (2017) 151–158

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

# MTH1 as a nucleotide pool sanitizing enzyme: Friend or foe?☆

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ARTICLE INFO

*Keywords:*
Reactive oxygen species
8-oxo-7, 8-dihydroguanine
8-oxo-dGTP
Base excision repair
OGG1
MUTYH
Carcinogenesis
Neurodegeneration

ABSTRACT

8-Oxo-7,8-dihydroguanine (GO) can originate as 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate (8-oxo-dGTP), an oxidized form of dGTP in the nucleotide pool, or by direct oxidation of guanine base in DNA. Accumulation of GO in cellular genomes can result in mutagenesis or programmed cell death, and is thus minimized by the actions of MutT homolog-1 (MTH1) with 8-oxo-dGTPase, OGG1 with GO DNA glycosylase and MutY homolog (MUTYH) with adenine DNA glycosylase. Studies on *Mth1/Ogg1/Mutyh*-triple knockout mice demonstrated that the defense systems efficiently minimize GO accumulation in cellular genomes, and thus maintain low incidences of spontaneous mutagenesis and tumorigenesis. *Mth1/Ogg1*-double knockout mice increased GO accumulation in the genome, but exhibited little susceptibility to spontaneous tumorigenesis, thus revealing that accumulation of GO in cellular genomes induces MUTYH-dependent cell death. Cancer cells are exposed to high oxidative stress levels and accumulate a high level of 8-oxo-dGTP in their nucleotide pools; cancer cells consequently express increased levels of MTH1 to eliminate 8-oxo-dGTP, indicating that increased expression of MTH1 in cancer cells may be detrimental for cancer patients. *Mth1/Ogg1*-double knockout mice are highly vulnerable to neurodegeneration under oxidative conditions, while transgenic expression of human MTH1 efficiently prevents neurodegeneration by avoiding GO accumulation in mitochondrial genomes of neurons and/or nuclear genomes of microglia, indicating that increased expression of MTH1 may be beneficial for neuronal tissues.

## 1. Introduction

Cellular macromolecules such as lipids, proteins and nucleic acids are always at high risk of being oxidized by one electron oxidants and reactive oxygen species (ROS) including hydroxyl radical (•OH) and singlet oxygen ($^1O_2$). ROS are generated during normal metabolic processes of electron transport in the mitochondria or other metabolic pathways, and are also produced as functional mediators for various biological processes such as host defense, neurotransmission, vasodilation and signal transduction. Production of ROS is enhanced under various pathophysiological conditions, and increased accumulation of such oxidatively generated damage during aging is likely to be a major cause for various types of cellular dysfunctions, causing degenerative disorders and neoplasms [1].

Among the various types of oxidatively generated damage to cellular macromolecules, damage to nucleic acids is particularly hazardous because of the potential alteration or destruction of genetic information in cellular genomes (nuclear and mitochondrial). Accumulated oxidatively generated damage in nucleic acids can result not only in mutagenesis but also in programmed cell death. Mutagenesis can initiate carcinogenesis in somatic cells, and mutations in germ lines cause genetic polymorphisms resulting in gene malfunction or hereditary diseases resulting in degenerative disease [2,3]. In this review, we discuss 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate (8-oxo-dGTP), which is generated in nucleotide pools to precursor nucleotides for DNA replication, and the sanitizing enzyme MutT homolog-1 (MTH1) [2].

## 2. Generation of 8-oxo-7,8-dihydroguanine by ROS and its accumulation in cellular genomes

Among all the nucleobases, guanine is the most susceptible to oxidation by ROS. Exposure of guanine in DNA or free 2′-deoxyguanosine 5′-triphosphate (dGTP) to •OH or $^1O_2$ adds oxygen to the C-8 carbon to generate 8-oxo-7,8-dihydroguanine (GO) or 8-oxo-dGTP, respectively [4–7]. One of the tautomers of GO in equilibrium is 8-hydroxyguanine; however, in neutral solution, GO is the major form (Fig. 1A) [8]. Previous studies showed that dGTP in the nucleotide pool is more susceptible to oxidation than guanine in DNA [6]. DNA polymerases can insert

☆ This article is one of a series of papers on the subject of oxidative DNA damage & repair that have been published as a special issue of Free Radical Biology & Medicine to commemorate the Nobel Prize won by Prof. Tomas Lindahl. A detailed introduction and synopsis of all the articles in the special issue can be found in the following paper by Cadet & Davies [79].

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http://dx.doi.org/10.1016/j.freeradbiomed.2016.11.002
Received 12 September 2016; Received in revised form 30 October 2016; Accepted 4 November 2016
Available online 07 November 2016
0891-5849/ 

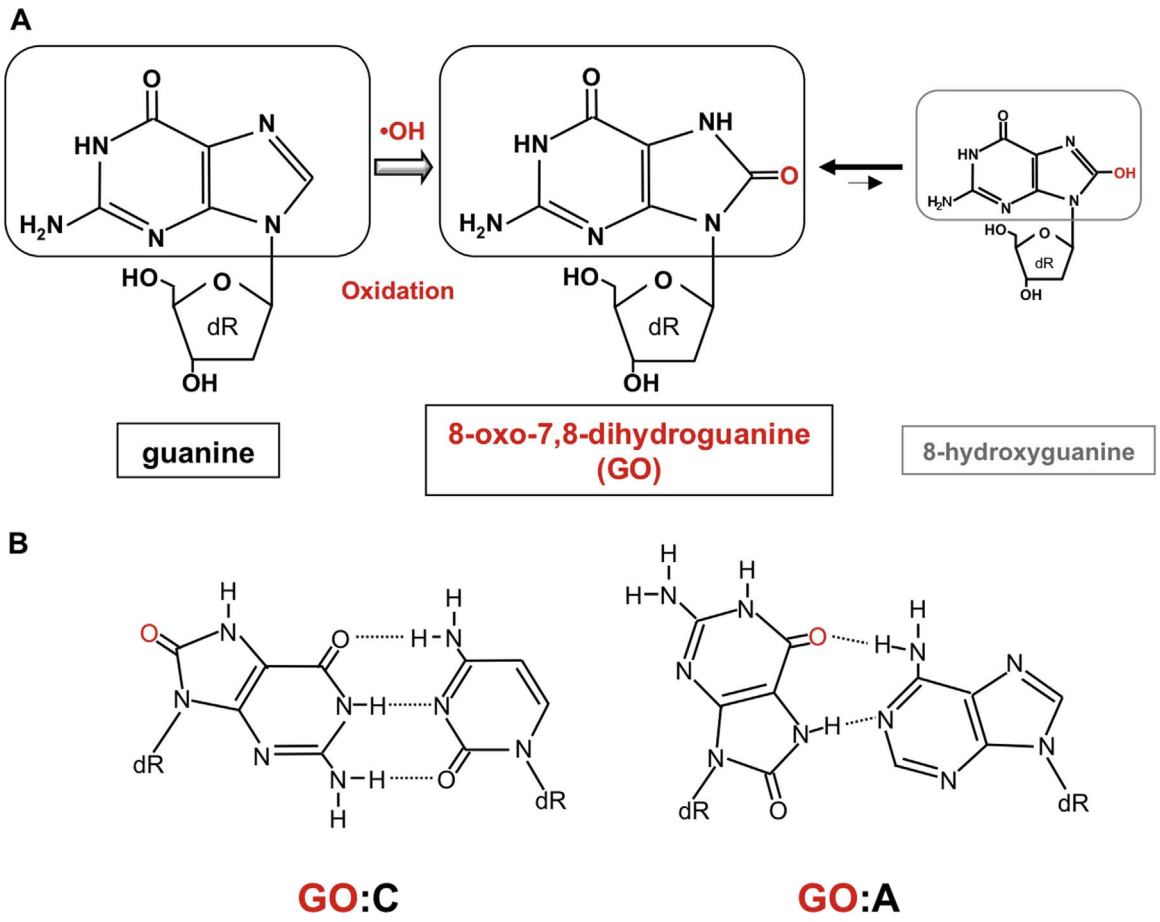


**Fig. 1. Generation of 8-oxo-7,8-dihydroguanine and its altered base pairing property.** (A) Oxidation of guanine by reactive oxygen species (ROS) generates 8-oxo-7,8-dihydroguanine (GO) that is in dynamic equilibrium with 8-hydroxyguanine, a minor tautomer of GO. (B) GO forms a base pair with adenine as well as with cytosine in DNA. dR, 2′-deoxyribose. Modified from Ref. [22].

8-oxo-dGTP into DNA opposite either adenine or cytosine with varying efficiency [9–11]. Thus, the presence of GO in DNA has two distinct origins: one is derived from 8-oxo-dGTP in the nucleotide pool and the other generated by direct oxidation of guanine in DNA [7].

GO in DNA can adopt both *anti* and *syn* conformations with respect to the 2′-deoxyribose moiety and form stable base pairs with cytosine and adenine (Fig. 1B) [12]. In the presence of GO in DNA or the nucleotide pool, transversion mutations (guanine to thymine or adenine to cytosine) can be induced (Fig. 2) [13].

## 3. Cellular defense mechanisms to avoid 8-oxo-7,8-dihydroguanine accumulation in cellular genomes

In mammalian cells, 8-oxo-dGTP is hydrolyzed to 8-oxo-dGMP and pyrophosphate mainly by an oxidized purine nucleoside triphosphatase encoded by *MTH1*, also known as *nudix hydrolase 1* (*NUDT1*) (Fig. 2) [14,15]. MTH1 protein is primarily localized in cytoplasm, with some portion of MTH1 protein in the nucleus and mitochondria [16]. There are several nudix hydrolases that can hydrolyze 8-oxo-dGTP to 8-oxo-dGMP or 8-oxo-dGDP, although their contribution to sanitize the nucleotide pool is not yet confirmed [17,18]. DNA polymerase cannot utilize 8-oxo-dGMP as its substrate, and thus once MTH1 hydrolyzes 8-oxo-dGTP, its incorporation into DNA is prevented. It has been shown that 8-oxoGMP, the degradation product of 8-oxoGTP by MTH1, cannot be reutilized, since guanylate kinase, which has the potential to phosphorylate both GMP and dGMP, is inactive on 8-oxoGMP, and probably on 8-oxo-dGMP [19]. This may be another reason why MTH1 efficiently prevent GO accumulation in DNA.

GO paired with cytosine in DNA is excised by GO DNA glycosylase, which is encoded by the *OGG1* gene, and then replaced with guanine through base excision repair (BER) (Fig. 2) [20]. The *OGG1* gene encodes both nuclear and mitochondrial forms of OGG1 protein by alternative splicing [21,22].

Adenine can be incorrectly inserted into the nascent DNA strand opposite GO in the template DNA strand during DNA replication, and MutY homolog (MUTYH) with adenine DNA glycosylase excises the adenine. During BER, after the adenine is excised, either cytosine or adenine can be inserted opposite GO in the template strand. If cytosine is inserted, OGG1 can excise the GO, allowing replacement of it with guanine. However, if adenine is inserted again opposite the GO, the process of BER initiated by MUTYH must be repeated (Figs. 2, 3A) [23,24]. The MUTYH gene also encodes nuclear and mitochondrial forms of MUTYH protein by alternative splicing [25]. The nuclear form of MUTYH interacts with components of the replication machinery, including proliferating cell nuclear antigen (PCNA), MutSα (MSH2/MSH6 heterodimer), and replication protein A (RPA) [26–28], thus ensuring specific recognition of adenine in the nascent strand inserted opposite GO in the template strand during replication [24].

## 4. Deficiency in MTH1, OGG1 or MUTYH increases spontaneous mutagenesis and carcinogenesis

*Mth1*-knockout (KO) mice exhibited an increased occurrence of spontaneous tumorigenesis, especially in liver, lung and stomach, within 18 months after birth, and the percentage of *Mth1*-KO mice with liver tumors (38%) was significantly higher than that in male wild-type mice (13%) [29]. *Mth1*-KO mice also exhibited slight increases in both spontaneous mutation frequency [30] and GO content in genomic