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Review Article

# Oxidatively generated base modifications in DNA: Not only carcinogenic risk factor but also regulatory mark?☆

Marco Seifermann, Bernd Epe*

*Institute of Pharmacy and Biochemistry, University of Mainz, Staudingerweg 5, D-55099 Mainz, Germany*

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ABSTRACT

The generation of DNA modifications in cells is in most cases accidental and associated with detrimental consequences such as increased mutation rates and an elevated risk of malignant transformation. Accordingly, repair enzymes involved in the removal of the modifications have primarily a protective function. Among the well-established exceptions of this rule are 5-methylcytosine and uracil, which are generated in DNA enzymatically under controlled conditions and fulfill important regulatory functions in DNA as epigenetic marks and in antibody diversification, respectively. More recently, considerable evidence has been obtained that also 8-oxo-7,8-dihydroguanine (8-oxoG), a frequent pro-mutagenic DNA modification generated by endogenous or exogenous reactive oxygen species (ROS), has distinct roles in the regulation of both transcription and signal transduction. Thus, the activation of transcription by the estrogen receptor, NF-κB, MYC and other transcription factors was shown to depend on the presence of 8-oxoG in the promoter regions and its recognition by the DNA repair glycosylase OGG1. The lysine-specific histone demethylase LSD1, which produces $H_2O_2$ as a by-product, was indentified as a local generator of 8-oxoG in some of these cases. In addition, a complex of OGG1 with the excised free substrate base was demonstrated to act as a guanine nucleotide exchange factor (GEF) for small GTPases such as Ras, Rac and Rho, thus stimulating signal transduction. The various findings and intriguing novel mechanisms suggested will be described and compared in this review.

## 1. Introduction

The generation of base modifications in genomic DNA is generally an accidental and potentially harmful event. Even at low levels, many types of modification can cause mutations and initiate carcinogenesis. To avoid these consequences, all cells are equipped with powerful repair mechanisms that recognize the modifications and affect their replacement by the original (correct) base. DNA modifications generated by xenobiotic agents are in many cases recognized and repaired by nucleotide excision repair (NER) proteins, which exhibit a broad substrate specificity [1,2]. In contrast, endogenously generated DNA lesions are often recognized and removed by lesion-specific or lesion-selective enzymes in a process known as base excision repair (BER) [3–6]. The removal of the modified base, which leaves a site of base loss (AP site), is followed by single-strand cleavage, either at the 3′-side by a lyase activity associated with some of the damage-recognizing glycosylases or - probably more often and in the regular case - at the 5′-side by the AP endonuclease APE1. Subsequently, a DNA polymerase (e.g. POL-β) and a ligase (e.g. LIG3) get involved to complete the BER process.

A good example for the harmful nature of endogenously generated DNA modifications and the importance of their repair is 7,8-dihydro-8-oxoguanine (8-oxoG).[1] The lesion is generated by reactive oxygen species (ROS), which are produced as by-products in the reactions of many intracellular oxidases and oxygenases, in particular - but not only - in the mitochondrial electron transport chain. 8-OxoG is recognized in eukaryotic cells by the repair glycosylase OGG1, the continuous activity of which keeps the steady-state concentration of 8-oxoG well below 1 per million base pairs under most physiological conditions [7]. Accordingly, a defect in the repair of 8-oxoG in *Ogg1*$^{-/-}$ mice results in an increased steady-state level of 8-oxoG in various organs, which translates into 2–3 fold higher spontaneous mutation rates in the livers and an increased chance for malignant transformation, which is only observed after stimulation of cell proliferation [8–11].

In view of the described risks associated with DNA damage, it is

☆ 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 [129].

* Corresponding author.
*E-mail address:* epe@uni-mainz.de (B. Epe).

[1] 8-Oxo-7,8-dihydroguanine in DNA is abbreviated in the text as 8-oxoG, while the corresponding free base is abbreviated as 8-oxoGua.

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surprising that there are long-known exceptions of the rule that endogenously generated DNA modifications are accidental and harmful. Rather, some of them fulfill important regulatory functions, in analogy to the situation observed in the RNA world for various types of posttranscriptional modification in tRNAs, mRNAs and non-coding RNAs [12–14]. Two well-established examples will be very briefly described in this review, namely the roles of 5-methylated cytosines and uracil in DNA as epigenetic marks and in antibody diversification, respectively. More recently, evidence has been obtained that also 8-oxoG in DNA is not only a risk factor for the induction of mutations and the initiation of carcinogenesis, but may be involved in the initiation of transcription by certain transcription factors and may influence signal transduction after excision by the repair glycosylase OGG1. Moreover, in some cases the generation of 8-oxoG appears to occur locally in certain regions of the DNA by chromatin-associated enzymes, i.e. independent of cellular oxidative stress. The intriguing new mechanisms and the underlying findings will be described and discussed in the main parts of this review. For recent overviews dealing with related topics, see Refs. [15–18].

## 2. Methylated cytosines are epigenetic marks

In DNA of vertebrates, more than 70% of the cytosines in CpG sequences are methylated by maintenance and de-novo DNA methyl transferases, viz. Dnmt1 and Dnmt3A and DNMT3B [19]. The resulting 5-methylcytosine (5mC) is often referred to as a fifth DNA base and serves as an epigenetic mark in the silencing of gene expression. The mark is erased by the ten-eleven translocation (TET) family of enzymes, which use molecular oxygen, α-ketoglutarate and Fe(II) as co-factors to oxidize 5mC stepwise to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) [20,21]. 5fC and 5caC are recognized and removed by the thymine-DNA glycosylase (TDG), one of the 11 known mammalian BER glycosylases [20,22,23]. The absence of TDG therefore is embryonic lethal, in contrast to that of the other BER glycosylases [24,25]. The levels of 5fC and 5caC in mammalian tissues are relatively low (in embryonic stem cells approx. 10 and 1.5 modifications per million bp, respectively) [21,26,27], consistent with the assumption that they are mere intermediates in the removal of 5mC. In contrast, up to 15,000 5hmC per million bp are observed in embryonic stem cells and neuronal cells, and it is possible that the lesion has a role as a epigenetic mark that is different from 5mC. Specific "readers" for 5hmC, i.e. proteins which bind to 5hmC, but not to 5mC, remain to be identified, but 5hmC appears to be better correlated with differential gene regulation in tissues than 5mC [28–31].

## 3. Uracils in DNA are involved in antibody diversification

Another well-established DNA base modification with regulatory functions is uracil. Accidentally, it is formed by misincorporation of uracil opposite to adenine or - in proliferating cells much less frequently - by spontaneous deamination of cytosine. The latter reaction results in a U:G base pair with clear mutagenic potential. In both cases, the uracil residues in DNA are rapidly repaired by BER. Among the four glycosylases able to recognize uracil in DNA (called UNG1/2, SMUG1, TDG and MBD4), UNG2 is particularly active in chromosomal DNA during S phase (removing misincorporated uracils), while TDG is expressed in G1 phase only [32] and excises uracil from U:G pairs, in particular in a CpG context [33,34]. SMUG1 and MBD4 probably serve as back-up enzymes, but also fulfill extra functions such as the recognition of 5-hydroxymethyluracil (5-hmU) in the case of SMUG1 and the repair at methylated CpG sequences in the case of MBD4 [35,36]. Interestingly, in the germinal centres of lymph nodes a regulated, non-accidental generation of uracil takes place in the variable regions of immunglobulin genes, which is mediated by the activation induced deaminase (AID) [37–40]. The mechanisms by which other regions of the genome are protected are still under investigation [41,42], but it is clear that UNG2, in combination with mismatch-repair and translesion synthesis enzymes, serves to enhance the spectrum of point mutations that result from the U:G pairs generated by AID [43–45]. It is worth mentioning that this type of active generation of mutations appears to be specific for uracil; a similar role was explicitly excluded for oxidized guanines such as 8-oxoG, which, as already mentioned above, are excised by OGG1 [46].

While the regulatory functions of modified cytosines and uracils in DNA described in this and the previous chapter are well established, indications for similar roles of purine modifications have been rare until recently. An exception is $N^6$-methyladenine, which has long-known divers regulatory functions in many prokaryotes, in particular in their restriction-modification systems. Interestingly, recent evidence points to a role of $N^6$-methyladenine in the regulation of transcription in several eukaryotes [47–49] and even in mammalian cells [50].

## 4. DNA base damage in the context of oxidative stress: bystander or signal transducer?

In the last years, there is accumulating evidence that oxidized purines, in particular 8-oxoG, also have a role beyond that of being accidental and dangerous side products. The analysis of such a function has been complicated by the many regulatory effects associated with oxidative stress, which are observed under all conditions under which an elevated generation of 8-oxoG and related DNA modifications takes place. Obviously, it is difficult to clarify whether the generation of DNA damage is only a side effect of a ROS-mediated signalling or whether the DNA modifications are actively involved in the signalling, as outlined below.

The generation of ROS inside cells has long been regarded as an adverse side effect of various metabolic processes such as mitochondrial respiration, in particular because of the associated (and unavoidable) generation of DNA damage described above. This is supported by the fact that an elevated generation of ROS triggers a protective response, namely an induction of the antioxidant defense system by activation of the transcription factor NRF2. Mechanistically, this redox regulation is mediated by the oxidation of cysteine residues in a NRF2-associated sensor protein called KEAP1 [51]. However, the notion that ROS and their reaction products are just toxic side-products clearly has to be amended. A major reason is that numerous other signal transduction pathways, which are not related to a protective oxidative stress response, are redox-sensitive as well [52]. A classical example is the proinflammatory transcription factor NF-κB, the activity of which is strongly stimulated by ROS and repressed by antioxidants [53,54]. Also, several pathways involving tyrosine kinases are highly redox-sensitive, in particular because certain tyrosine phosphatases are easily inactivated by intramolecular S-S-bond formation [55,56]. The assumption that ROS fulfill a distinct role as second messengers in many of these cases is supported by the existence of professional intracellular generators of ROS, in particular NADPH oxidases such as NOX4, and their receptor-mediated activation [57,58].

The ROS-mediated signalling in the above-mentioned cases proceeds mostly via protein modifications. However, even these low (regulatory) concentrations of ROS are associated with significant DNA damage. For example, an NOX4-mediated generation of SSB and micronuclei can be observed after exposure of cultured cells and perfused mouse kidney to angiotensin [59] and pharmacological doses of the NO donor glyceryl trinitrate give rise to elevated levels of 8-oxoG in various tissues of the treated mice [60], possibly via formation of peroxynitrate [61]. A certain indirect support for the assumption that the DNA modifications generated under these conditions are not just bystanders, but are actively involved in a redox signalling, may be seen in the fact that both OGG1 and APE1 are redox-sensitive. In the case of APE1, which is also known as redox factor 1 (Ref-1), redox activity resides in an aminoterminal domain completely seperated from the