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8-Oxo-7,8-dihydroguanine, friend and foe: Epigenetic-like regulator versus initiator of mutagenesis

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ABSTRACT

A high flux of reactive oxygen species during oxidative stress results in oxidative modification of cellular components including DNA. Oxidative DNA "damage" to the heterocyclic bases is considered deleterious because polymerases may incorrectly read the modifications causing mutations. A prominent member in this class is the oxidized guanine base 8-oxo-7,8-dihydroguanine (OG) that is moderately mutagenic effecting G → T transversion mutations. Recent reports have identified that formation of OG in G-rich regulatory elements in the promoters of the *VEGF*, *TNFα*, and *SIRT1* genes can increase transcription via activation of the base excision repair (BER) pathway. Work in our laboratory with the G-rich sequence in the promoter of *VEGF* concluded that BER drives a shift in structure to a G-quadruplex conformation leading to gene activation in mammalian cells. More specifically, removal of OG from the duplex context by 8-oxoguanine glycosylase 1 (OGG1) produces an abasic site (AP) that destabilizes the duplex, shifting the equilibrium toward the G-quadruplex fold because of preferential extrusion of the AP into a loop. The AP is bound but inefficiently cleaved by apurinic/apyrimidinic endoDNase I (APE1) that likely allows recruitment of activating transcription factors for gene induction. The ability of OG to induce transcription ascribes a regulatory or epigenetic-like role for this oxidatively modified base. We compare OG to the 5-methylcytosine (5mC) epigenetic pathway including its oxidized derivatives, some of which poise genes for transcription while also being substrates for BER. The mutagenic potential of OG to induce only ~one-third the number of mutations (G → T) compared to deamination of 5mC producing C → T mutations is described. These comparisons blur the line between friendly epigenetic base modifications and those that are foes, i.e. DNA "damage," causing genetic mutations.

1. Introduction

Reactive oxygen species formed during oxidative stress are electron deficient and readily oxidize proteins, lipids, RNA, and particularly DNA. Oxidative modification of the genomic DNA bases is well documented and can result in mutations responsible for initiation of a number of diseases [1]. The guanine (G) heterocycle is the most susceptible of the four DNA bases to oxidation leading to many products [2]. Chief among these oxidatively modified products is 8-oxo-7,8-dihydroguanine (OG; Fig. 1). Cellular levels of OG in the genome are routinely monitored as a biomarker to assess the extent of oxidative stress to which a cell has been exposed [3]. Moreover, OG is moderately mutagenic, if not repaired, causing G → T transversion mutations that are thought responsible for initiating and driving some cancers [1]. These mutations are a consequence of OG base pairing with A on the Hoogsteen face rather than C on the Watson-Crick face [4]. To counteract mutations from damaged DNA nucleotides, an elaborate DNA repair system has evolved to return modified sites back to the original

Abbreviations: AP, abasic site; APE1, apurinic/apyrimidinic endoDNase 1; *BCL2*, B-cell lymphoma 2; BER, base excision repair; CBP, CREB binding protein; ChIP-Seq, chromatin immunoprecipitation assay with sequencing; *c-MYC*, V-myc avian myelocytomatosis viral oncogene homolog gene; CREB, CAMP responsive element binding protein 1; 5caC, 5-carboxylcytosine; DNMT, DNA methyltransferase; 5fC, 5-formylcytosine; F, tetrahydrofuran; G4, G-quadruplex; G, guanine; Gh, 5-guanidinohydantoin; HIF1-α, hypoxia inducible factor 1 alpha; 5hmC, 5-hydroxymethylcytosine; Ku70, protein encoded by the x-ray repair cross complementing 6 gene; LSD1-2, lysine demethylase 1A and 2A; LIG, ligase; 5mC, 5-methylcytosine; MCF-7, Michigan Cancer Foundation-7 cell line; MEF, mouse embryonic fibroblast; MUTYH, MutY DNA glycosylase; nCaRE, negative calcium response elements; NEIL1-3, endonuclease VIII-like 1–3; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; *NTHL1*, Nth-like DNA glycosylase 1 gene; OG, 8-oxo-7,8-dihydroguanine; OGG1, 8-oxoguanine glycosylase 1; $OGG1^{-/-}$-MEF, mouse embryonic fibroblast with OGG1 knocked out; OG-Seq, 8-oxo-7,8-dihydroguanine sequencing; p300, E1A binding protein P300; POLB, polymerase β; PQS, potential G-quadruplex sequence; Ref-1, redox effector factor 1; RNA pol II, RNA polymerase II; ROS, reactive oxygen species; *SIRT1*, sirtuin 1 gene; SMUG, single-stranded-selective monofunctional uracil-DNA glycosylase 1; Sp, spiroiminodihydantoin; SP1, specificity protein 1; STAT3, signal transducer and activator of transcription 3; TDG, thymine-DNA glycosylase; *TNF-α*, tumor necrosis factor α gene; U, uracil; UNG, uracil-DNA glycosylase; *VEGF*, vascular endothelial growth factor A gene; WT, wild type

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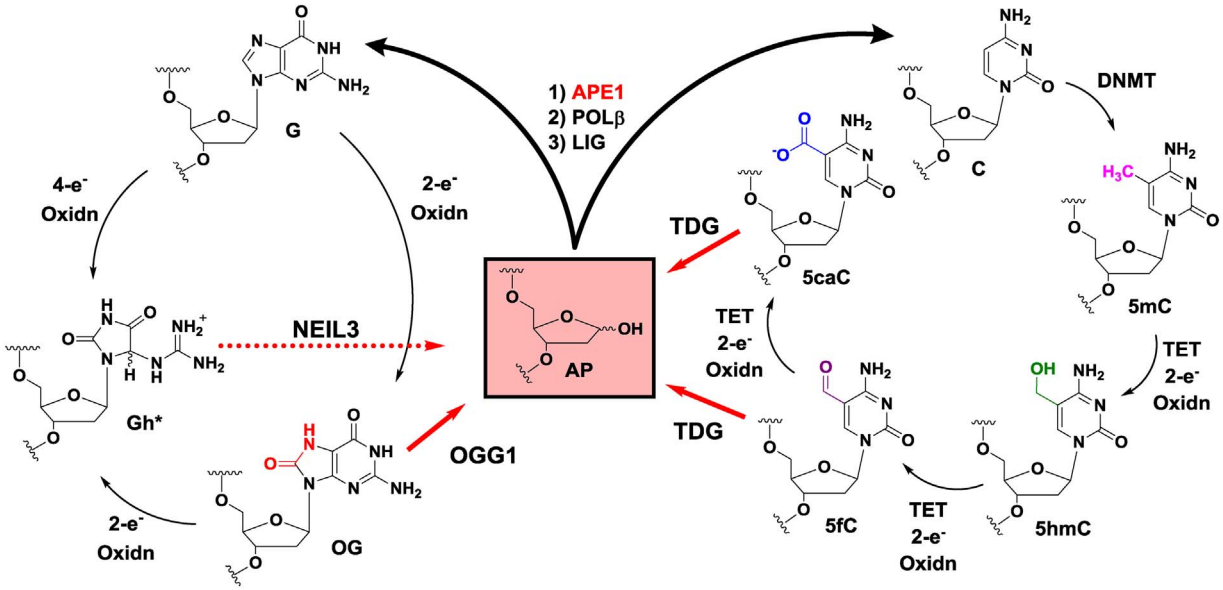

Fig. 1. Comparison of the G oxidative modification cycle with the C methylation and oxidative modification cycle to illustrate the centrality of the abasic site (AP) to return the sequence back to the original active state. *For the sake of brevity, the 4-electron oxidation product of G, or 2-electron oxidation product of OG yielding 5-guanidinohydantoin (Gh) is shown; the other 4-electron product spiroiminodihydantoin (Sp) is not shown [7]. The yields of Gh and Sp show strong dependency on the reaction conditions and context, favoring Gh in duplex DNA oxidations or reactions at pH < 6 and favoring Sp in single-stranded and G-quadruplex DNA oxidations or reactions at pH > 7 [8,9].

canonical nucleotides [5]. Repair of OG is achieved by base excision repair (BER) that initiates removal of OG when base paired with C by the action of 8-oxoguanine glycosylase 1 (OGG1) in mammals (Fig. 1); in contrast, when OG is incorrectly base paired with A, MutY DNA glycosylase (MUTYH) removes the A allowing a second chance for a polymerase to insert C opposite OG for further action by OGG1 [5]. Following removal of OG by OGG1, an abasic site (AP) is formed that is a substrate for apurinic/apyrimidinic endoDNase I (APE1) to cleave the 5'-phosphodiester linkage yielding a nick in the DNA (Fig. 1) [5]. The repair process is completed by polymerase β (POLB) that removes the sugar fragment at the nick site followed by inserting the correct G nucleotide, and finally ligase (LIG) seals the nick to return the DNA back to its native state (Fig. 1) [5]. This dynamic process of G oxidation to OG followed by DNA repair has been estimated to occur up to 10^5 times per cell per day [6].

The long-standing view has been that OG is mutagenic and detrimental to cellular processes such as transcription. For instance, the presence of OG in template strands can stall the advancement of RNA pol II [10], and initiation of OG repair causes polymerases to stop [11], thus ascribing a role to OG as a transcriptional repressor. However, there are a few notable examples of oxidative stress leading to increased OG formation in the genome in tandem with increased gene expression. This has been documented in livers from mice with infection-induced colitis [12], and rat pulmonary artery endothelial cells exposed to hypoxic conditions [13]. Observations like these led our laboratory and others to inspect how the *VEGF* [14], *TNFα* [15], and *SIRT1* [16] genes respond when G is oxidized to OG in their promoters. The most interesting finding in these cellular studies showed that OG can increase gene transcription via the BER pathway [14–16]. These results identify an intertwining of DNA repair with gene activation that is a phenomenon gaining appreciation [17]. Therefore, oxidative modification of G to OG may have regulatory and possibly epigenetic-like features in cells that are responding to oxidative stress. This perspective will discuss the discovery that OG can stimulate transcription via BER activity. These results provide the background for a comparative discussion between OG as a possible epigenetic-like DNA modification vs. the traditional 5-methylcytosine (5mC) epigenetic modification. Additionally, the ten-eleven translocation (TET) proteins oxidize 5mC in a stepwise fashion to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) (Fig. 1) in genes poised for activation by BER removal after being silenced by 5mC [18–21]. The observation of oxidative modification to DNA bases in the form of OG or oxidized 5mC highlights a possibility that base oxidation is a DNA-based mechanism for gene activation. Finally, the ability of OG to regulate gene expression vs. its ability to cause mutations will be discussed.

2. Initial reports that OG is epigenetic-like

A few initial reports proposed that OG, if present in key regions of the genome, could impact cellular processes. For instance, synthetic oligonucleotides with OG in protein transcription factor binding sequences found this modification negatively impacted factor binding affinity. This effect was demonstrated in the consensus sequences of specificity protein 1 (SP1) [22], nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [23], and CAMP responsive element binding protein 1 (CREB) [24]. The work with the CREB transcription factor in the Strauss laboratory led them to propose that OG might be epigenetic by decreasing protein binding resulting in OG as a transcriptional repressor [24]. The Olinski laboratory quantified OG in heterochromatin vs. euchromatin from porcine thymus DNA to find that the transcriptionally active euchromatin DNA harbored more OG [25]. Their observation of OG concentrations varying throughout the genome led them to speculate that OG might be an epigenetic modification. Lastly, Park, et al. developed a method to demonstrate that G oxidation to OG could occur site specifically *in vivo* under oxidative stress conditions leading them to propose OG as an epigenetic modulator [26]. These observations of OG as a regulatory modification (i.e., epigenetic-like) were all lacking in cellular experiments demonstrating G oxidation to OG can form in critical regions of the genome and impact transcription.

3. OG activates mRNA synthesis by facilitating promoter G-quadruplex formation

The vascular endothelial growth factor A (*VEGF*) gene harbors a G-rich promoter element critical for regulation of mRNA synthesis [27]. The G-rich element is located between positions −86 and −56 relative to the transcription start site (TSS) in the coding strand (Fig. 2A); further, this region is bound by three equivalents of the SP1 transcription factor [27]. Cellular regulation of *VEGF* by SP1 has been documented,