



Oxidized DNA Base Damage Repair and Transcription

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A New Mechanism for Regulation of Gene Expression in Cancer

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Abstract

The DNA bases in the genome are susceptible to oxidation by reactive oxygen species (ROS). The oxidized DNA base lesions such as 8-oxoguanine (8-oxoG) and thymine glycol are primarily repaired by the base excision repair (BER) pathway. Increasing pieces of evidence suggest that oxidative stress-induced base damages in the gene promoter serve as epigenetics marks to regulate gene expression by recruitment of BER proteins. To shed light on this novel role of oxidative DNA base modifications and BER proteins, in this chapter, we focus on how controlled guanine oxidation in gene promoters and BER proteins 8-oxoguanine DNA glycosylase (OGG1) and AP-endonuclease 1 (APE1) regulate expression of multiple genes that drive cancer progression and metastases.

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Further, we highlight the current studies suggesting a novel role of 8-oxoG and BER in regulating the formation of noncanonical DNA secondary structures such as G-quadruplexes (G4s) to regulate gene expression. Since high oxidative stress in tumor cells creates selective pressure on DNA damage repair pathways to maintain sustained growth, invasion, and metastases via modulating gene expression, such efforts to address the intertwined roles of DNA base modification, BER pathway, and gene expression could broaden cancer-therapeutic strategies.

Keywords

Oxidized DNA base · 8-Oxoguanine · Base excision repair · OGG1 · APE1 · AP site · Histone demethylation · G-quadruplex structure · Gene expression

Introduction

The genome is inherently unstable, and chemical modifications and damaging agents continuously challenge its integrity. Persistent exposure to exogenous and endogenous reactive oxygen species (ROS) generated by metabolic and inflammatory processes can cause DNA base oxidation, one of the most frequent insults to the genome (Winterbourn 2008). ROS comprise of a group of highly reactive molecules such as superoxide anion ($O_2^{\cdot-}$), and hydroxyl radical (OH^{\cdot}) – some potent oxidants which can directly react with DNA and produce oxidized bases (Schieber and Chandel 2014). Oxidation of purines and pyrimidines can generate multiple modified DNA bases (Bauer et al. 2015). ROS can also directly generate single-strand breaks (SSBs) with nonligatable termini. Deoxyribose in DNA is also prone to oxidation, which usually results in base hydrolysis to form an abasic site and/or SSB (Bauer et al. 2015). Among the four canonical nucleotides in DNA, G base has the lowest redox potential, resulting in G being the most susceptible to oxidative modification (Cadet et al. 2014; Fleming and Burrows 2017). Products of G oxidation by ROS include two-electron oxidation product 8-oxo-7-8-dihydroguanine (8-oxoG), which can pair with A during replication and leading to G to T transversion mutation (Grollman and Moriya 1993). Thus, oxidative damage to DNA may lead to mutations accumulation in the genome, and this eventually results in pathological consequences such as cancer (Cooke et al. 2003).

The base excision repair (BER) pathway is the primary mechanism for repairing oxidative, alkylated base lesions, and SSBs in the genome (Lindahl and Barnes 2000; Mitra et al. 2002). BER is an evolutionarily conserved DNA damage repair pathway that is involved in maintaining genomic integrity by removing mutagenic lesions induced due to oxidative stress. BER relies on sequential recruitment and coordinated actions of multiple enzymes, which include DNA glycosylases (such as OGG1), AP-endonucleases (APE1), DNA polymerases, and DNA ligases (Scott et al. 2014). Several studies have demonstrated that in the absence of BER enzymes, cells accumulate mutations and are susceptible to various DNA-damaging agents. Thus, one can envision that BER plays a crucial role in cancer prevention.

Overexpression of BER proteins confers protection of the cell genome from oxidative stress and toxicity to DNA-damaging drugs. Studies have shown elevated levels of APE1 in diverse cancer tissues and its association with chemo and radiation resistance (Abbotts and Madhusudan 2010; Sengupta et al. 2016).

Given the unavoidable exposure to ROS, cells have evolved strategies to utilize ROS as a biological stimulus or signaling molecule to regulate multiple cellular processes, including cell signaling and transcription. An emerging view in transcriptional regulation is that base oxidation in DNA, which is conventionally thought to be mutagenic and detrimental for cells, has an essential regulatory role in controlling transcription and gene expression. Despite high mutagenic potential of 8-oxoG, GC content in the promoter regions of genes in the vertebrate genome is high (Deaton and Bird 2011). This evolutionarily conserved high GC content has been linked with gene expression (Ba and Boldogh 2018). Indeed, low-resolution mapping of 8-oxoG in mammalian cells by several laboratories found that 8-oxoG is enriched in gene promoters and within potential G-quadruplex-forming sequences (PQSs) (Amente et al. 2019; Fang and Zou 2020). The promoter region of many proto-oncogenes, including *c-MYC*, *KRAS*, and *VEGF*, contains PQS that can fold to noncanonical DNA G-quadruplex (G4) structures to regulate their expression (Fleming et al. 2017a; Pastukh et al. 2015). Over the years, the intertwined roles of the 8-oxoG, BER pathway, and transcriptional regulation have merged as a novel epigenetic mechanism for regulating gene expression. The initial cellular observation of controlled G oxidation in VEGF gene promoter upon hypoxia driving the production of Hif-1 protein was reported by Gillespie's laboratory (Pastukh et al. 2015, 2007). Later, several other laboratories, including ours, have provided evidence that oxidation of bases in these G-rich sequences may serve as critical sensors through which the cells sense the ROS signals, and BER proteins OGG1 and APE1 regulate the transcription of oxidative stress-responsive genes (Fleming et al. 2017a; Pan et al. 2016; Roychoudhury et al. 2020). In this chapter, the roles of oxidative base 8-oxoG and BER enzymes – OGG1 and APE1 in transcriptional regulation – are discussed in detail with an emphasis on how they facilitate the assembly of the transcriptional activators at gene promoters to promote gene expression. Further, we shed light on our current understanding of how oxidative base modification and BER proteins regulate the formation of higher-order DNA secondary structures such as G4s to regulate expression of many oncogenes and promote tumorigenesis.

Oxidative DNA Base Damage Shaping the Mutation Burden in Cancer

Cancer cells are characterized by persistent oxidative stress and high levels of ROS. Out of the several different DNA base derivatives that can arise due to ROS, 8-oxoG is one of the major oxidized bases in DNA that pairs with adenine (A) as well as cytosine (C) during DNA replication, potentially leading to G:C to T:A transversion mutations (Grollman and Moriya 1993) (Fig. 1). Left unrepaired, 8-oxoG can compromise transcription, DNA replication, and telomere maintenance, and being

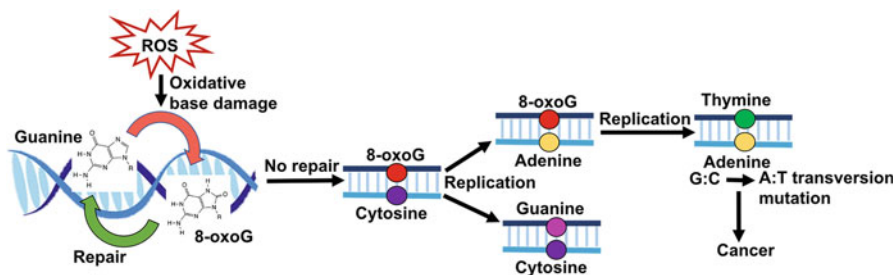


Fig. 1 A schematic representation of how unrepaired oxidative base damage (8-oxoG) in the DNA can lead to mutation in the genome

a highly mutagenic lesion, it has been associated with cellular transformation and cancer initiation. Elevated levels of 8-oxoG and other oxidative lesions have been found in the urine and tumor tissue DNA from patients with various malignancies (Guo et al. 2017). The levels of 8-oxoG are considered as a factor in risk assessment in cancer (Loft et al. 2012). Apart from 8-oxoG, oxidation of G can also result in the formation of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), a major oxidative lesion (Cadet and Wagner 2013).

8-Oxo-7,8-dihydro-2'-deoxyadenosine (8-oxoA) and 4,6-diamino-5-formamido-pyrimidine (FapyA) are the two major products that can arise from the oxidation of A. C can also be oxidized to form 5-hydroxy-2'-deoxycytidine (OH5C), spontaneously on the DNA, or can arise after exposure to ROS generating chemicals (Bjelland and Seeberg 2003; Cadet and Wagner 2013). T can be attacked by free radicals resulting in the generation of different oxidative products. Thymine glycol (Tg) can inhibit replicative polymerases and is highly mutagenic (McCulloch and Kunkel 2008). The 5-methyl group of T can be oxidized to form 5-hydroxymethyluracil (5hmU), which induces transition mutations (Maiti and Drohat 2011).

All these mutagenic events pose a serious threat to the DNA and might lead to genetic instability and malignant transformation. Defective DNA maintenance pathways have been associated with distinct signatures that arise due to these mutations (Alexandrov and Stratton 2014). Though the entire course of malignancy is associated with different mutational processes, there can be specific mutational types that are dominant in a particular type of cancer. For instance, a high proportion of T to G and C to T base mutations was found in the immunoglobulin genes of chronic lymphocytic leukemia and melanoma patients (Alexandrov et al. 2013; Puente et al. 2015; Hodis et al. 2012).

Oxidative DNA Base Damage Repair

Considering the high frequency at which DNA damaging events occur, and the broad spectrum of damages incurred by the cells, it is remarkable that a majority of these DNA lesions are repaired with impressive precision and efficiency by a host of DNA damage repair pathways.

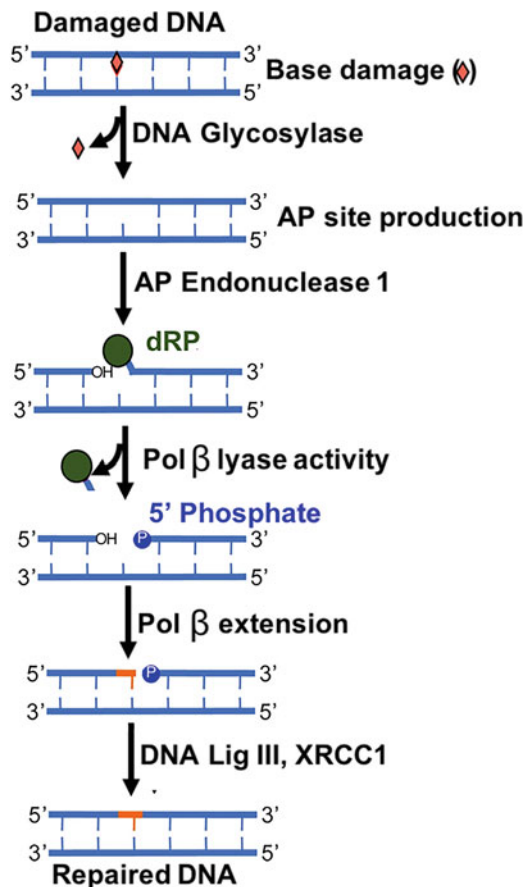
As mentioned before, the BER pathway is the primary mechanism that protects the genome from the deleterious effects of exposure to ROS. Being the most versatile among the excision repair pathways, BER repairs multiple types of modified (oxidized or alkylated) DNA bases as well as DNA single-strand breaks (SSBs) (Hegde et al. 2008; Lindahl and Barnes 2000; Robertson et al. 2009). BER is a highly coordinated multistep cellular process that includes (i) damaged base removal, (ii) 3'- or 5'-end cleaning, (iii) DNA synthesis, and (iv) DNA nick-sealing (Scott et al. 2014). It is generally initiated by a lesion-specific DNA glycosylase, which removes the damaged base. Among 11 human DNA glycosylases, 8-oxoguanine DNA glycosylase 1 (OGG1), NTH1, endonuclease VII-like (NEIL) 1, NEIL2, and NEIL3 are primarily involved in reaping the oxidized base damages from DNA. Once the base lesions are removed, the product apurinic/apyrimidinic (AP) sites or SSBs with nonligatable termini are a substrate for the next enzyme, AP endonuclease 1 (APE1). APE1 cleaves AP site generating 3'-hydroxyl and 5'-deoxyribose-phosphate (5'-dRP) end which is recognized by DNA polymerase β (pol β). Pol β fills the single nucleotide gap by incorporating a complementary base, and finally, DNA ligase III (Lig III) seals the nick (Fig. 2). Of note is that each of these reactions consequently leaves a new intermediate lesion in the DNA, which is harmful if the entire repair process is not completed through nick-sealing by the DNA ligases (Wilson and Kunkel 2000). Thus, BER is dependent on sequential recruitment and coordinated activities of multiple proteins via the formation of a series of transient repair complexes that assemble at the site of the DNA lesion (Hegde et al. 2012).

Apart from the core proteins mentioned above, poly (ADP-ribose) polymerase (PARP) and X-ray repair complementing defective repair in Chinese hamster cells 1 (XRCC1) do not directly participate in the DNA processing but form a scaffold and interact with BER proteins such as APE1 and Lig III which are critical for BER efficiency (El-Khamisy et al. 2003). Furthermore, several other non-BER proteins that are known to be involved in transcription or RNA metabolism have been demonstrated to regulate the activity of BER enzymes (Das et al. 2007). Such auxiliary proteins interact with BER proteins to enhance the overall DNA repair efficiency and coordinate repair and transcription.

The Interplay Between Posttranslational Modification of BER Proteins and Transcriptional Regulation

Reversible acetylation of histones plays a profound role in epigenetic regulation of gene expression. Many studies have shown the role of acetylation of BER proteins in the regulation of the BER pathway and gene expression (Bhakat et al. 2009; Carter and Parsons 2016). We discovered that the major human glycosylases, OGG1, NEIL1, and NEIL2, which cleave most oxidatively damaged bases, are acetylated at specific lysine (K) residues in cells (Bhakat et al. 2004, 2006; Sengupta et al. 2018). Acetylation of OGG1 at K338 and K341 increased the activity of OGG1 (Bhakat et al. 2006). OGG1 acetylation was enhanced in cells after oxidative stress, suggesting a DNA damage-dependent activation of OGG1 acetylation due to its acetylation. We also showed that NEIL1 is acetylated at multiple K residues (K296,

Fig. 2 A schematic representation of the steps involved in the BER pathway



K297, and K298) located in the intrinsically unstructured C-terminal domain, and acetylation of NEIL1 enhanced its glycosylase activity in vitro (Sengupta et al. 2018). We discovered that human APE1 is also acetylated at multiple K residues located in the unstructured N-terminal region (Bhakat et al. 2003; Lirussi et al. 2012). APE1 is acetylated in chromatin, and acetylation of APE1 enhances its AP-endonuclease activity in vitro and modulates its transcriptional coregulatory functions (Roychoudhury et al. 2017). Notably, while unmodified OGG1 and APE1 are present in cytosol and nucleus, acetylated OGG1 and APE1 are exclusively bound to chromatin (Bhakat et al. 2006; Roychoudhury et al. 2017).

Using unbiased genome-wide mapping of AP sites, a common intermediate in the BER pathway, and binding of acetylated APE1 and acetylated OGG1, we have documented that endogenous oxidative base damages are not randomly located (Roychoudhury et al. 2020). There is a distinct distribution of oxidative damage and enrichment of acetylated OGG1 or acetylated APE1 predominately in the promoter/enhancer regions of the transcribed genes suggesting a connection of

DNA base damage or assembly of repair complexes with the regulation of gene expression. Acetylated APE1-bound regions significantly overlap the regions that bear active enhancer and promoter histone marks (Roychoudhury et al. 2020). Studies have shown the interaction of APE1 with transcription activator or repressor factors such as STAT, HIF-1 α , AP-1, NF- κ B, and HDAC1 in multiple gene promoters (Bhakat et al. 2009; Evans et al. 2000; Xanthoudakis and Curran 1992). Acetylated APE1 was found to be present in the protein complexes that bind to negative calcium response element in gene promoter (Bhakat et al. 2003). Binding of Y-box-binding protein 1 (YB-1) to multidrug resistance gene MDR1 was shown to be dependent on APE1's acetylation (Sengupta et al. 2011, Chattopadhyay et al. 2008). Further, we demonstrated elevated levels of AcAPE1 in diverse cancer types. The loss of APE1 acetylation resulted in altering the expression of hundreds of genes and enhanced tumor cells' sensitivity to chemotherapeutic agents (Sengupta et al. 2016).

8-OxoG and OGG1 as a Novel Epigenetic Regulator for Controlling Gene Expression

Both transcription and DNA repair have intimate transactions with the DNA, and these two processes are often coupled and are perhaps also interdependent and cross-functional. A growing list of DNA damage repair proteins that were thought to function in repair pathways solely is increasingly becoming important in transcriptional regulation as well. 8-oxoG is the most prevalent oxidized DNA base found in the genome. Studies have shown that a controlled or localized oxidation of G in a specific region in the genome, such as promoter/enhancer, can occur in a signal-dependent manner through localized histone demethylation by LSD1, a flavin-dependent monoamine oxidase that generates H₂O₂ (Shi et al. 2005). The locally formed H₂O₂ drives G oxidation in the discrete regions leading to the recruitment of OGG1 and activation BER pathway to promote induction of transcription (Perillo et al. 2008). Furthermore, supporting this, several laboratories found that 8-oxoG is enriched in gene promoters and within G-rich PQS or G4 sequences (see details below). Lastly, the genome-wide mapping of OGG1 (the primary enzyme responsible for removing 8-oxoG from DNA) further supports that oxidative base damage is not random; they are enriched in promoter regions in the mammalian genome (Ba and Boldogh 2018; Roychoudhury et al. 2020).

BER in Hypoxia-Induced VEGF mRNA Expression

The first evidence for a direct connection between oxidized DNA bases, BER pathway, and gene regulation came from the studies by Gillespie's group. A study by Pastukh et al. demonstrated the hypoxia-mediated formation of 8-oxoG in hypoxic response elements (HREs) in the VEGF gene promoter (Pastukh et al. 2007). Hypoxia-induced oxidative stress was shown to promote the assembly of

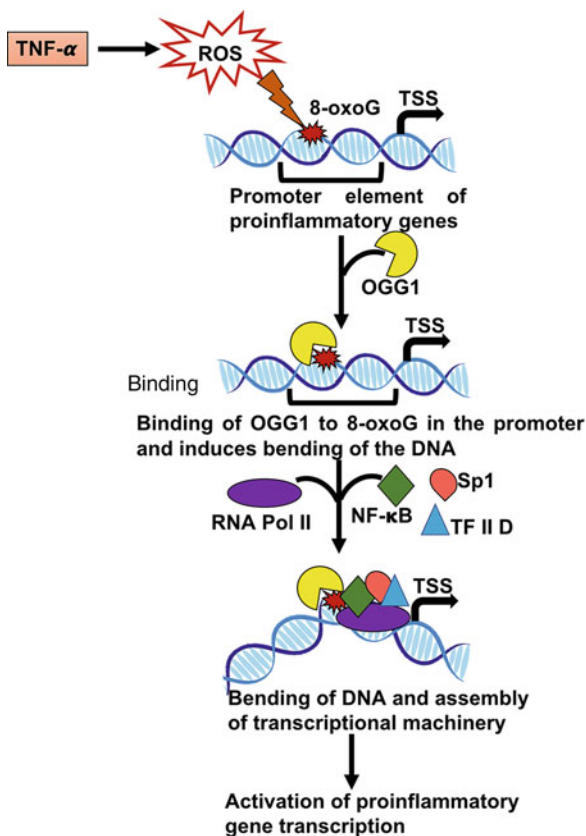
hypoxia-inducible factor-1 (Hif-1 α) and BER enzymes OGG1 and APE1. This recruitment of BER machinery was essential for the assembly of transcription complex on the VEGF HRE and for driving VEGF mRNA expression. Inhibition of the BER pathway resulted in hypoxia-induced accumulation of 8-oxoG and attenuated Hif-1 α and APE1 binding to VEGF HRE sequences and blunted VEGF mRNA expression. Moreover, compared to wild-type HRE luciferase reporter, luciferase activity increased with AP-site containing HRE element in reporter plasmid (Pastukh et al. 2015, 2007). As described above, the AP site is a common intermediate generated after the removal of oxidized bases in the BER pathway. As the BER pathway repairs AP sites, these results suggested that oxidative base lesions in promoters of hypoxia-inducible genes are linked with transcriptional activation. More, recent studies have demonstrated that the signal-induced formation of localized oxidized bases in the promoter regions in many oncogene promoters such as *KRAS*, *c-MYC*, etc., modulates their expression (Fleming et al. 2017b); however, the molecular basis of G oxidation, AP sites formation, and modulation of their gene expression is different in each case (see below).

Role of 8-OxoG and OGG1 in the Regulation of Inflammatory Response Genes

It is well-known that inflammation induces ROS production and that ROS-mediated signaling regulates transcription proinflammatory genes. Previous studies have shown ROS's role in posttranslational modification of TFs such as NF- κ B, and AP-1 (Ba and Boldogh 2018). In a study by Ba et al., it was shown that TNF- α -induced ROS production results in elevating 8-oxoG levels in the genome, including the promoter regions (Ba and Boldogh 2018). Simultaneously, the binding of OGG1 also increased *Cxcl2* expression by facilitating the loading of TFIID, NF- κ B/RelA, Sp1, and p-RNA Pol II (Fig. 3). Depletion of OGG1 reduced transcription factor recruitment and the subsequent TNF- α -mediated innate immune response (Ba et al. 2014).

A master regulator of gene expression, nuclear factor κ B (NF- κ B), is a transcription factor (TF) necessary for the inflammatory response, cell proliferation, and differentiation (Oeckinghaus and Ghosh 2009). Constitutive NF- κ B activity due to the inflammatory microenvironment and the various oncogenic mutations is found in several human cancers. DNA sequences that contain three or more Gs (5'-GGGRNYYYCC-3' where R is a purine, Y is pyrimidine, and N is any nucleotide) are signatures where NF- κ B classically binds. As mentioned before, oxidation of these Gs can lead to the formation of 8-oxoG lesions, which can either change the sequence context of the TF-binding site due to its mutagenicity or directly modulate DNA-protein interactions and thus gene expression. A study by Pan et al. demonstrated that NF- κ B-driven gene expression requires 8-oxoG and OGG1 enrichment in the promoter sequences after TNF- α exposure to the cells. OGG1 bound to 8-oxoG facilitates NF- κ B's binding to the DNA (Pan et al. 2016). Hence, binding of OGG1 to its substrate was shown to function in epigenetic regulation of gene

Fig. 3 Binding of OGG1 to 8-oxoG in promoter sequences bends the DNA and augments proinflammatory gene transcription by facilitating the recruitment of site-specific transcription factors



expression in cells that are under oxidative stress. Additionally, the Tell laboratory proposed that oxidative stress-induced 8-oxoG in the negative calcium responsive elements (nCaRE) in the promoter of sirtuin-1 (*SIRT-1*) gene recruits OGG1 (Antoniali et al. 2014).

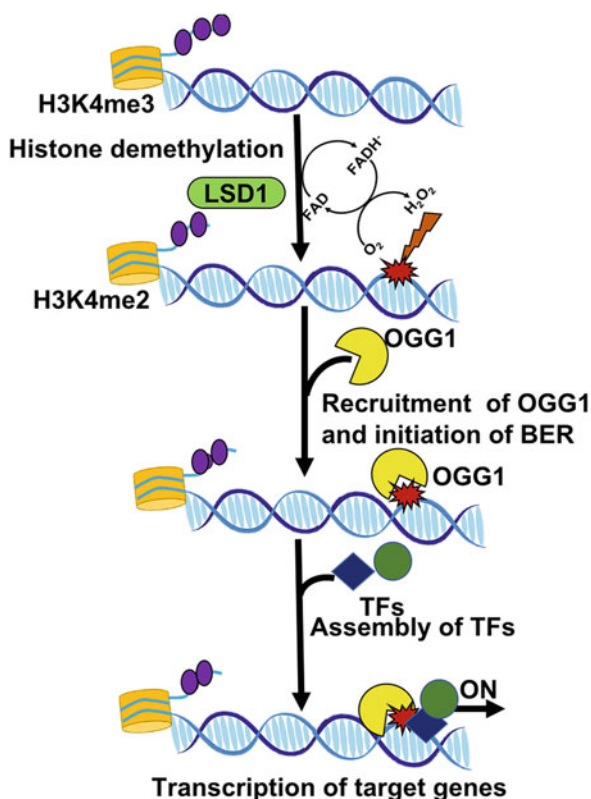
The Ras pathway is often activated in human cancers. Boldogh et al. demonstrated that 8-oxoG is bound by OGG1 at a nonsubstrate site with high affinity (Boldogh et al. 2012). The study was one of the first to document the DNA repair-independent function of OGG1 in modulating cellular signaling.

Histone Demethylation, Oxidation of DNA, BER, and Gene Expression

Unlike histone acetylation, methylation of histone was considered a permanent and irreversible modification. In 2004, Shi et al. demonstrated that LSD1-mediated removal of the methyl group from both H3K4me2 and H3K4me1 in vitro via Flavin adenine dinucleotide oxidation reaction, generates H_2O_2 during demethylation

(Shi et al. 2004). This demethylation resulted in local oxidation of DNA base around promoter or gene body regions and produced 8-oxoG, which is recognized by OGG1. OGG1 initiates the BER pathway, which can regulate transcription and gene expression (Fig. 4). The first direct evidence of histone demethylation and BER's involvement in transcriptional regulation came from the study by Perillo et al. (Perillo et al. 2008). The authors showed that treatment with 17-beta-estradiol induced estrogen receptor recruitment to the estrogen-responsive elements in the promoter and enhancer of Bcl-2 and pS2 genes and formed a chromatin loop between the promoter and enhancer to activate the gene expression. Demethylation of H3K9me2 occurred at the promoter and enhancer sites of these genes, and 8-oxoG could be detected after treatment with estradiol. Importantly, siRNA-mediated knockdown of LSD1 abolished the accumulation of 8-oxoG lesions. Removal of 8-oxoG lesions by OGG1 followed by recruitment of APE1 generates an SSB facilitating chromatin looping, which provides a local signal for the assembly of transcriptional initiation complex to activate gene expression. OGG1 appeared to be required to remove 8-oxoG, and APE1 was necessary to complete the repair process. However, no additional downstream BER proteins were evaluated in this initial study. Downregulation of OGG1 and APE1 was used to confirm the functional role

Fig. 4 LSD1-mediated histone demethylation in gene promoters gives rise to H_2O_2 , which causes local oxidation of DNA base (8-oxoG), leading to the initiation of BER and assembly of transcription factors (TFs) to drive target gene expression



of BER in transcriptional control, and it dramatically reduced the promoter-enhancer loop formation and gene expression.

The same group also demonstrated that DNA oxidation drives Myc-mediated transcription (Amente et al. 2010b). Myc, a transcription factor that is activated in many cancers, contributes to a wide variety of gene regulation involved in cell proliferation, differentiation, and apoptosis (Amente et al. 2010b). They provided a mechanistic framework that links histone demethylation to oxidation of DNA and repairs underlying Myc-activated transcription. They demonstrated that local demethylation of H3K4me2 promotes the recruitment of the OGG1 and APE1 following Myc binding, which produces nicks on the Myc target gene. This subsequently facilitates chromatin loops that bring together RNA polymerase II and Myc. Silencing of OGG1 or APE1 inhibits Myc-mediated transcription (Amente et al. 2010a).

Regulatory Roles of G Oxidation and BER Proteins in G4 Structure Formation in the Genome to Regulate Gene Expression

G-quadruplexes (G4s) are DNA secondary structures that can form when four Gs associate through Hoogsteen hydrogen bonds to form a planar tetrad. Stacking of two or more tetrads forms a G4 structure (Bochman et al. 2012; Burge et al. 2006). G4 DNA structures in key regulatory regions in the genome, such as promoters, replication origins, etc., regulate multiple biological processes, including transcription, replication, and telomere maintenance (Hansel-Hertsch et al. 2016, 2017). There is a growing consensus among Cynthia Burrows' laboratory, ours suggesting that 8-oxoG and BER pathway plays a pivotal role in G4 structure formation in the genome to regulate gene expression (Fleming et al. 2017a, b; Roychoudhury et al. 2020). G4s are overrepresented in regulatory regions such as gene promoters, 5' and 3' untranslated regions, replication initiation sites, and telomeres (Fleming et al. 2019; Hansel-Hertsch et al. 2016). The promoter region of many proto-oncogenes, including *c-MYC*, *KRAS*, and *VEGF*, which are activated by oxidative stress, contains PQS that can fold to G4 to regulate their expression. Through biochemical, cellular, and genetic approaches, Burrows' laboratory showed that 8-oxoG or AP sites in the PQS of VEGF and endonuclease III-like protein 1 (NTHL1) promoter activate transcription of a reporter gene expression (Fleming et al. 2017a). OGG1 and APE1 are required for the induction of transcription. Further, it was found that oxidative modified PQS close to the transcription start site (TSS) can activate transcription when the damaged base and repair chemistry occur in the coding strand (Fleming et al. 2019). On the other hand, modification of PQS in the template strand turned off transcription. Thus formation of 8-oxoG under oxidative stress conditions functions as a signaling or epigenetic mark to promote G4 fold, thus leading to transcriptional activation. This also implies that G oxidation is an epigenetic modification, and G4-forming sequences serve as sensors for oxidative stress.

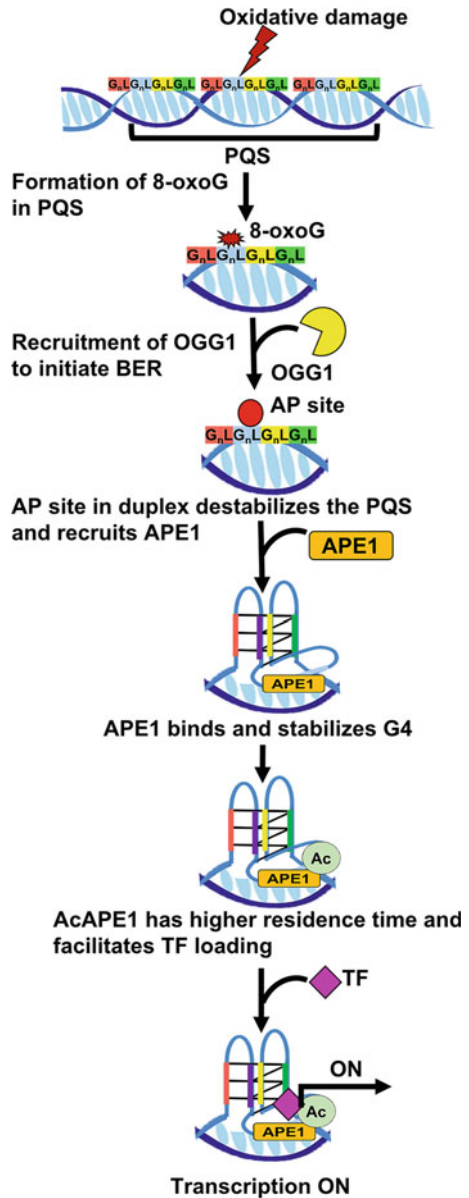
Similarly, in a separate study, Redstone et al. showed that oxidative modification of PQS in the proliferating cell nuclear antigen (PCNA) gene promoter could

turn on its transcription (Redstone et al. 2019). PCNA overexpression has been detected in malignancies ranging from colorectal cancer to breast cancer. The study showed the possible mechanism of PCNA gene activation during oxidative stress conditions. Briefly, the G-rich PQS promoter element of the PCNA gene can undergo oxidation to produce 8-oxoG. This event directs DNA repair via the BER pathway toward the promoter for initiating events that ultimately induce transcription of the gene.

In an independent study by Cogoi et al., it was found that 8-oxoG formation in the PQS of the KRAS oncogene promoter positioned from 148 to 116 relative to the TSS, turned on the transcription (Cogoi et al. 2018). In this study, the authors demonstrated that 8-oxoG in the G4 region of KRAS promoter recruits OGG1. OGG1 removes 8-oxoG from the G4 motif in duplex, but when folded, it binds to the G4 in a nonproductive way. Recruitment of MAZ and hnRNP A1, the two nuclear factors that are essential for KRAS transcription to the KRAS promoter, is enhanced by 8-oxoG (Cogoi et al. 2018). Thus, this oxidative lesion in the G4 region was demonstrated to be a novel player in oncogene expression regulation, adding to other pieces of evidence of the epigenetic potential of oxidative base damage and its associated repair pathway in controlling gene expression.

Although these studies mentioned above have established that an oxidized G or AP site in PQSs of many oncogene promoters such as *KRAS*, *MYC*, and *VEGF* modulates their expression, the requirement of G oxidation, AP sites, and APE1 in the spatiotemporal regulation of gene expression in cells was mostly unknown. We have recently demonstrated a mechanistic framework for the role of oxidized DNA-base-derived AP sites and APE1 in the formation and stability of G4 structures (Roychoudhury et al. 2020). We showed that endogenous oxidized DNA-base-derived AP sites in G4 sequences and subsequent recruitment of APE1 drive the spatiotemporal formation and stability of G4 structures to regulate *KRAS* and *MYC* oncogene expression. Mapping of the genome-wide APE1 binding and G4 structures revealed that the binding of APE1 is predominant in PQS sequences and is nonrandom. Furthermore, it was shown that APE1 supports the formation of G4 structures and G4-mediated gene expression. Binding of APE1 to G4 sequences promotes G4 folding, and the acetylation of APE1 stabilizes G4 structures by enhancing its residence time. APE1 subsequently facilitates transcription factor loading to the promoter, leading to G4-mediated gene expression. We postulated that cellular oxidants could oxidize G bases within a PQS into 8-oxoG, which is excised by OGG1 to generate an AP site. Unlike 8-oxoG paired with C, an AP site significantly impacts the thermal stability of duplex DNA (Fleming et al. 2017a). We proposed that the generation of an AP site in a PQS destabilizes the duplex and shifts the equilibrium to form an unstable G4. Subsequent binding of APE1 results in the stabilization of a G4 structure, and acetylation of APE1 by p300 enhances its affinity/interaction to stabilize G4 structure and facilitates the loading of TFs to activate gene expression (Fig. 5). This study highlighted a novel role of oxidized DNA bases and APE1 in controlling the formation of G4 structures to regulate transcription (Fleming et al. 2017a; Roychoudhury et al. 2020).

Fig. 5 Schematic representation linking the role of endogenous oxidized DNA base (8-oxoG) in gene regulatory elements and APE1 in controlling the formation of G4 structures to modulate transcription of target genes



Thus, all these studies collectively demonstrated that oxidative base modifications such as 8-oxoG in the gene promoters that contain PQS might serve as epigenetic marks and serve as a common ground to coordinate the initial steps of DNA repair and the assembly of transcriptional machinery to launch adequate alteration of expression of the gene.

Conclusion

Over the years, studies from several laboratories have supported the view that oxidative DNA modifications such as 8-oxoG in the promoter/enhancer regions could serve as an epigenetic mark to regulate gene expression. Additionally, the BER pathway has been deemed necessary for modulating DNA G4 structures and histone demethylation-mediated epigenetic regulation, which are distinct from its well-established role in maintaining genome stability. Defects in BER may affect the formation of G4 structures and reduce promoter-enhancer formation affecting many oncogene gene expressions. Although several studies discussed above support the concept that action of BER in gene promoters guided by oxidative modification of G regulates transcription of several genes, some obvious questions warranting answers are (a) How a nonselective oxidant such as H₂O₂ generated during LSD1-mediated histone demethylation induces specific G oxidation in promoter/enhancer sequences to regulate gene expression? (b) Do oxidative base modifications other than 8-oxoG also function as an epigenetic regulator, and are they often required for transcription of oxidative stress-dependent genes? In the context of G4s, future studies are required to understand whether promoter PQSs serve as sensors of oxidative stress to modulate transcription of downstream genes through direct oxidation of DNA bases in the promoters under stressed conditions. Given the fact that tumor cells are highly transcriptionally active and have elevated levels of oxidative stress and BER enzymes, addressing these questions would help illuminate the complex interplay between oxidative DNA damage, BER, and transcriptional regulation in cancer.

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