Oxidative DNA damage: mechanisms, mutation, and disease

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ABSTRACT **Oxidative DNA damage is an inevitable consequence of cellular metabolism, with a propensity for increased levels following toxic insult. Although more than 20 base lesions have been identified, only a fraction of these have received appreciable study, most notably 8-oxo-2′deoxyguanosine. This lesion has been the focus of intense research interest and been ascribed much importance, largely to the detriment of other lesions. The present work reviews the basis for the biological significance of oxidative DNA damage, drawing attention to the multiplicity of proteins with repair activities along with a number of poorly considered effects of damage. Given the plethora of (often contradictory) reports describing pathological conditions in which levels of oxidative DNA damage have been measured, this review critically addresses the extent to which the in vitro significance of such damage has relevance for the pathogenesis of disease. It is suggested that some shortcomings associated with biomarkers, along with gaps in our knowledge, may be responsible for the failure to produce consistent and definitive results when applied to understanding the role of DNA damage in disease, highlighting the need for further studies.—Cooke, M. S., Evans, M. D., Dizdaroglu, M., Lunec, J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* 17, 1195–1214 (2003)**

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BACKGROUND

Normal cellular metabolism is well established as the source of endogenous reactive oxygen species (ROS), and it is these (normally nonpathogenic) cellular processes that account for the background levels of oxidative DNA damage detected in normal tissue. Electron transport chains all possess the potential to "leak" electrons to oxygen resulting in superoxide formation (reviewed in ref 1). Certain enzyme activities generate superoxide and, via an oxidative burst, ROS are released from phagocytic cells destined to destroy cells infected with viruses, or bacteria, although surrounding tissue can also be affected. Peroxisomes compartmentalize oxidative metabolism leading to reactive products that would otherwise be detrimental to the cell, although under certain conditions these products may be released.

ROS may also be generated by ionizing or ultraviolet radiation. Equally, certain exogenous chemicals may redox cycle following metabolism by the cell, with the subsequent production of electrons that can be transferred to molecular oxygen producing superoxide ($O_2^{\bullet -}$). Irrespective of their origin, reactive oxygen species may interact with cellular biomolecules, such as DNA, leading to modification and potentially serious consequences for the cell.

Mechanisms of oxidative damage to DNA bases

Of the reactive oxygen species, the highly reactive hydroxyl radical ($^{\bullet}OH$) reacts with DNA by addition to double bonds of DNA bases and by abstraction of an H atom from the methyl group of thymine and each of the C-H bonds of 2′-deoxyribose (2). Addition to double bonds of DNA bases occurs at or near diffusion-controlled rates with rate constants from 3 to10×10^9 M^{-1} s^{-1}; the rate constant of H abstraction amounts to 2×10^9 M^{-1} s^{-1} (2). Addition to the C5-C6 double bond of pyrimidines leads to C5-OH and C6-OH adduct radicals and H atom abstraction from thymine results in the allyl radical. Adduct radicals differ in terms of their redox properties, with C5-OH adduct radicals being reducing and C6-OH adduct radicals oxidizing (3).

Pyrimidine radicals yield numerous products by a variety of mechanisms (2, 4–6). Radicals are reduced or oxidized depending on their redox properties, redox environment, and reaction partners (3). Product types and yields depend on absence and presence of oxygen and on other conditions (5, 6). In the absence of oxygen, the oxidation of C5-OH adduct radicals, followed by addition of OH^- (or addition of water followed by deprotonation), leads to cytosine glycol and thymine glycol (Tg; **Fig. 1**) (2, 4–6). The allyl

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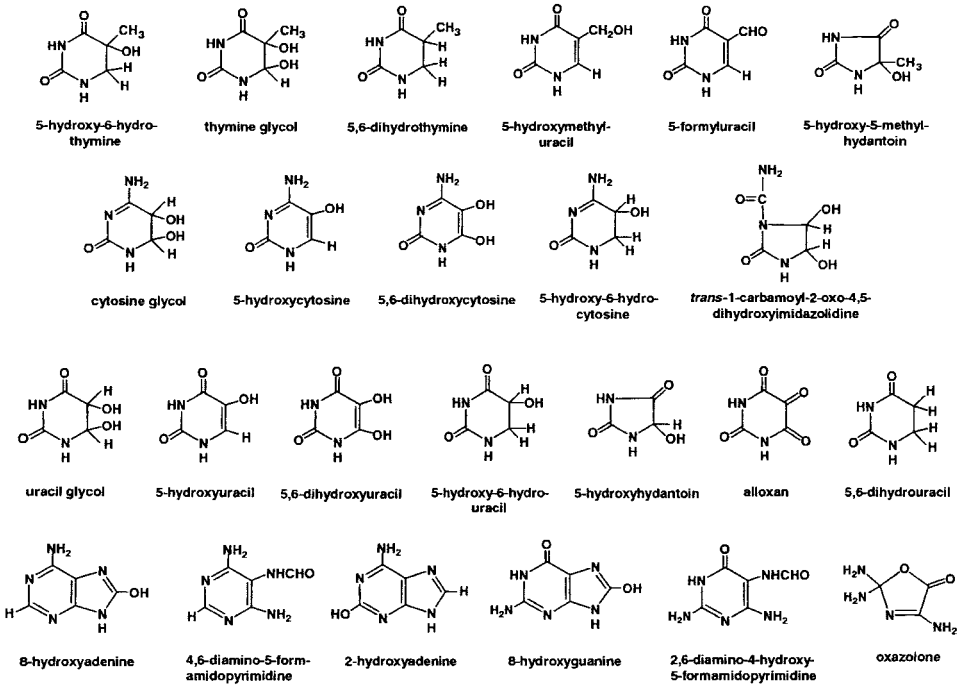

Figure 1. DNA base products of interaction with reactive oxygen and free radical species.

radical yields 5-hydroxymethyluracil. C5-OH-6-peroxyl radicals are formed by addition of oxygen to C5-OH adduct radicals at diffusion-controlled rates. C5-OH-6-peroxyl radicals eliminate $O_2^{\bullet -}$, followed by reaction with water (addition of OH^-) to yield thymine and cytosine glycols (2, 4). Oxygen reacts with the allyl radical, leading to 5-hydroxymethyluracil and 5-formyluracil. Thymine peroxyl radicals are reduced, followed by protonation to give hydroxyhydroperoxides (7), which decompose and yield thymine glycol, 5-hydroxymethyluracil, 5-formyluracil, and 5-hydroxy-5-methylhydantoin (7).

Products of cytosine may deaminate and dehydrate. Cytosine glycol deaminates to give uracil glycol, 5-hydroxycytosine, and 5-hydroxyuracil (Fig. 1) (5, 6, 8–10). However, cytosine glycol, uracil glycol, 5-hydroxycytosine, and 5-hydroxyuracil were all detected in γ-irradiated cytosine, indicating that all these compounds may simultaneously be present in damaged DNA (9). In the absence of oxygen, C5-OH adduct radicals may be reduced, followed by protonation to give 5-hydroxy-6-hydropyrimidines. 5-Hydroxy-6-hydrocytosine readily deaminates into 5-hydroxy-6-hydrouracil. Similarly, C6-OH adduct radicals of pyrimidines may lead to 6-hydroxy-5-hydropyrimidines. These products are typical of anoxic conditions because oxygen inhibits their formation by reacting with OH adduct radicals. By contrast, pyrimidine glycols and 5-hydroxymethyluracil are formed under both oxic and anoxic conditions.

Further reactions of C5-OH-6-peroxyl and C6-OH-5-peroxyl radicals of cytosine result in formation of 4-amino-5-hydroxy-2,6(1H,5H)-pyrimidinedione and 4-amino-6-hydroxy-2,5(1H,6H)-pyrimidinedione, respectively, which may deaminate to give dialuric acid and isodialuric acid, respectively. The detection of 4-amino-6-hydroxy-2,5(1H,6H)-pyrimidinedione and isodialuric acid in DNA suggested that both compounds may simultaneously exist in DNA (11, 12). Oxygen oxidizes dialuric acid to alloxan (9, 11). Alloxan was confirmed as a product using its release from DNA by *Escherichia coli* Nth protein (9). Decarboxylation of alloxan yields 5-hydroxyhydantoin upon acidic treatment. Intramolecular cyclization of cytosine C5-OH-6-hydroperoxide gives rise to *trans*-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine as a major product in cytosine (4, 10). However, this compound is formed as a minor product in DNA (10, 12, 13).

Hydroxyl radical adds to the C4, C5, and C8 positions of purines generating OH adduct radicals. In the case of adenine, at least two OH adducts are formed: C4-OH and C8-OH adduct radicals (14). C4-OH and C5-OH adduct radicals of purines dehydrate and are converted to an oxidizing purine(-H)$^\bullet$ radical, which may be reduced and protonated to reconstitute the purine (15). C4-OH adduct radicals possess oxidizing properties, whereas C5-OH and C8-OH adduct radicals are primarily reductants. On the other hand, different mesomeric structures of these radicals may be oxidizing or reducing, a phenomenon called "redox ambivalence" (14). C4-OH and C5-OH adduct radicals of purines dehydrate and are converted to an oxidizing purine(-H)$^\bullet$ radical, which may be reduced and protonated to reconstitute the purine (15). The rate constants of the dehydration of the C4-OH adduct radicals of guanine and adenine at neutral pH amount to 1.5×10^5 s^{-1} and 6×10^3 s^{-1}, respectively. The guanine radical cation (guanine$^{\bullet +}$) is formed by elimination of OH^- from the C4-OH adduct radical of guanine (k=6×10^3 s^{-1}) and may deprotonate depending on pH to give guanine(-H)$^\bullet$. The radical cation does not hydrate to lead to the C8-OH adduct radical and then to 8-hydroxyguanine (8-oxoguanine, 8-OH-

Gua; Fig. 1) by oxidation; however, it may react with 2′-deoxyribose in DNA by H abstraction ($k<4\times10^3$ s^{-1}), causing DNA strand breaks (16). On the other hand, the hydration of guanine$^{\bullet+}$ in double-stranded DNA forms the C8-OH adduct radical, which gives rise to 8-OH-Gua upon oxidation (17–19). The C4-OH adduct radical of guanine practically does not react with oxygen ($k<10^6$ $M^{-1}s^{-1}$); however, oxygen adds to guanine-(-H)$^{\bullet}$ with a rate constant of 3×10^9 $M^{-1}s^{-1}$. The reaction of guanine(-H)$^{\bullet}$ with oxygen leads to imidazolone and oxazolone derivatives (20–23). However, this was not confirmed by pulse radiolysis and an alternative mechanism was suggested. The C4-OH adduct radical of adenine reacts with oxygen with a rate constant of 1.0×10^9 $M^{-1}s^{-1}$, giving rise to yet unknown products (14).

C8-OH adduct radicals of purines may be oxidized by oxidants including oxygen. In contrast to C4-OH adduct radicals, their reaction with oxygen is diffusion-controlled ($k\cong4\times10^9$ $M^{-1}s^{-1}$) (14). The one-electron oxidation leads to formation of 8-hydroxypurines (7,8-dihydro-8-oxopurines) in DNA (5, 6). However, 8-hydroxypurines are also formed in the absence of oxygen, but to a lesser extent. The oxidation of C8-OH adduct radicals competes with the unimolecular opening of the imidazole ring by scission of the C8-N9 bond at a rate constant of 2×10^5 s^{-1}. The one-electron reduction of the ring-opened radical leads to 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) from guanine and 4,6-diamino-5-formamidopyrimidine (FapyAde) from adenine (5, 6) (Fig. 1). The one-electron reduction of C8-OH adduct radicals without ring-opening may also occur resulting in formation of 7-hydro-8-hydroxypurines. These compounds are hemiorthoamides and may be converted into formamidopyrimidines. 8-Hydroxypurines and formamidopyrimidines are formed in DNA in both the absence and presence of oxygen; however, the formation of 8-hydroxypurines is preferred in the presence of oxygen. Moreover, other experimental conditions profoundly affect the yields of these compounds, such as the presence of reducing or oxidizing agents (5, 6). 2-Hydroxyadenine (2-OH-Ade) is also formed in DNA as a product of adenine by a possible mechanism, including $^{\bullet}$OH attack at the C2-position of adenine, followed by oxidation (24).

Reactions of pyrimidines and purines result in multiple products in DNA, as illustrated in Fig. 1. Most of these modified bases were identified in DNA in vitro and in mammalian cells upon exposure to free radical-generating systems (25). Another reaction of base radicals is the addition to an aromatic amino acid of proteins or combination with an amino acid radical, leading to DNA–protein cross-linking (25). Reactions of $^{\bullet}$OH with the sugar moiety of DNA by H abstraction give rise to sugar modifications and strand breaks. A detailed review of the mechanisms of these reactions can be found elsewhere (2). A unique reaction of the C5′-centered sugar radical is the addition to the C8-position of the purine ring of the same nucleoside. This reaction leads to intramolecular cyclization, then by oxidation to 8,5′-cyclopurine-2′-deoxynucleosides (26, 27). Both 5′R- and 5′S-diastereomers of 8,5′-cyclo-2′-deoxyguanosine (cyclo-dG) and 8,5′-cyclo-2′-deoxyadenosine (cyclo-dA) are formed in DNA (26, 27). (5′R)- and (5′S)-8,5′-cyclo-2′-deoxyguanosines were also identified in human cells exposed to ionizing radiation (28). These compounds represent a concomitant damage to both base and sugar moieties and are considered tandem lesions. Oxygen inhibits their formation by reacting with the C5′-centered sugar radical before cyclization.

Were it not for cellular defenses such as low molecular weight antioxidants, enzymic antioxidants, and DNA repair, levels of such oxidatively modified bases would rapidly represent the majority of bases in DNA. The antioxidant systems have been recognized for many years, and are relatively well defined. In contrast, although it has been some years since repair of oxidative DNA damage was first reported, the last decade has seen a notable increase in research effort directed toward unraveling DNA repair processes.

PREVENTION OF LESION PERSISTANCE: DNA REPAIR

The repair processes for only a relative few of the plethora of modified bases have been studied in detail. However, even for some of the more extensively studied pathways there are still crucial, unanswered questions. The removal of oxidative DNA lesions is certainly important for the limitation of mutagenesis, cytostasis, and cytotoxicity, and, in most cases, oxidative DNA lesions are subject to multiple, overlapping repair processes. This redundancy introduces a fail-safe element to DNA repair such that attenuation or elimination of one repair process does not preclude repair of a particular lesion. Oxidized DNA base lesions are removed by essentially two types of activity: base excision repair (BER), involving removal of single lesions by a glycosylase action; and a more complex process involving the removal of a lesion-containing oligonucleotide, nucleotide excision repair (NER). The identity of the products of these processes are important since their detection in extracellular fluids could allude to the repair pathway(s) operating for a particular lesion. Indeed, in its earliest stages the detection of putative repair activities for a particular lesion have rested on the analysis of the release of oxidized base or deoxynucleoside products from either oxidatively stressed cells or incubation of oxidatively damaged DNA with cell lysates. Although these studies may have ultimately yielded identification of the repair processes for several lesions in human cells, many remain unexplored years after a possible repair process was indicated. It is principally the activity of human DNA repair enzymes that is considered here with reference to those lesions where specific proteins have been identified.

Repair of purine-derived oxidative DNA lesions

The repair of 8-OH-Gua in its many molecular contexts such as DNA, 2′-deoxynucleotides and, more recently, RNA has received considerable research interest. Although many major repair pathways for this lesion have been elucidated, there are still notable gaps in the literature, especially pertaining to any nonglycolytic repair processes. There appear to be several routes in mammalian cells to deal with 8-OH-Gua; in the unlikely event that the repair pathways removing this lesion from DNA or the deoxynucleotide pool are secondary to some as yet unknown function, it would strongly suggest that 8-OH-Gua presents a genuine threat to the integrity of the genome. The formation of 8-OH-Gua in situ in DNA results in an 8-OH-Gua:C pair that is a substrate for the well-characterized OGG1 protein (8-oxoguanine glycosylase 1) (29), which, as the name suggests, liberates 8-OH-Gua via a glycolytic mechanism from double-stranded substrates, relying on an internal Lys residue (30, 31). Another 8-OH-Gua glycosylase (OGG2) also repairs this lesion, but it is antigenically distinct from OGG1 and predominantly removes 8-OH-Gua from 8-OH-Gua:A pairs that may be formed by misincorporation of 8-OH-Gua into nascent DNA (32). This is one route by which misincorporated 8-OH-Gua may be addressed. Two other enzymes, MutY homologue (MYH) and MutT homologue 1 (MTH1), may also be involved. The former enzyme removes adenine, which mispaired opposite 8-OH-Gua. This mispair may arise from either misincorporation of 8-OH-dGTP opposite adenine in the template strand or misincorporation of dATP opposite unrepaired 8-OH-Gua in the template strand, directed during DNA synthesis (33, 34). The removal of misincorporated A allows a more likely replacement by C, offering OGG1 another chance to repair the lesion. Promiscuous removal of Ade from the template strand by MYH would evidently introduce mutations; therefore, the removal of misincorporated Ade specifically from the nascent strand is required, and this appears to be the case (35, 36). The preferential recognition of the lesion in 8-OH-Gua:A mispairs by components of the DNA mismatch repair system MutSα (MSH2-MSH6 heterodimer), an enhancement of MYH activity by the latter, and interactions between these and proliferating cell nuclear antigen (PCNA) could provide some mechanistic insight into the targeting of MYH activity to the template strand at replication foci (35, 37, 38) . In contrast, MTH1 acts at an earlier stage to inhibit erroneous incorporation of 8-OH-Gua into DNA by degrading 8-OH-dGTP to 8-OH-dGMP and pyrophosphate, the former compound being ultimately degraded to 8-OH-dG for excretion (39). This route is one means of producing 8-OH-dG in matrices such as urine, as a product related to DNA repair. However, there is little evidence that 8-OH-dG is a product of DNA repair itself (i.e., released as the deoxynucleoside, rather than the base, from DNA), with only one report alluding indirectly to its formation as a product of repair (40). This brings into focus the question of where 8-OH-dG in blood and urine comes from—repair, diet, or cell death. This is a topic that has been debated at length by several workers in the field, ourselves included, and so far remains largely unsolved. The recent discovery of two new DNA glycosylases, one of which, Nei-like glycosylase 1 (NEIL1), preferentially removes 8-OH-Gua from mispairs with G and A, would indicate yet another route whereby misincorporated lesion is removed from DNA in a transcription- or replication-coupled repair pathway (41). Unlike the OGG proteins, which use an essential internal lysyl residue in the glycosylase action, NEIL 1 uses an amino-terminal prolyl residue in a manner similar to that used by bacterial MutM (FPG protein) or Nei proteins.

In contrast to 8-OH-Gua, the repair of 8 hydroxyadenine (8-OH-Ade) is poorly understood. Although this lesion is reported to be less mutagenic than 8-OH-Gua (3- to 4-fold less mutagenic when assessed in a mammalian system), it has been shown to be a potential target for repair. Possibly, OGG1 removes 8-OH-Ade from 8-OH-Ade:C pairs resulting from misincorporation of 8-OH-dATP into nascent DNA (42). Although the action of OGG1 in this context is not clear, 8-OH-Ade does appear to be released as a possible product of DNA repair from oxidatively stressed cells in culture, at least suggesting the action of a glycosylase for this lesion. Similar to 8-OH-dGTP, MTH1 can also degrade 8-OH-dATP to limit misincorporation into DNA (43). A very recent study has indicated that the Cockayne syndrome B (CSB) protein is important for the repair of 8-OH-Ade, but this importance does not extend to glycolytic removal of the lesion by CSB protein, as this activity is not known for this protein (44). The identity of a specific glycosylase or other activity for 8-OH-Ade is unknown. The occurrence of 2-OH-Ade in DNA is estimated to be very low ($\sim 1/10^7$ normal nucleotides) (45). The repair of this lesion formed in situ (i.e., as 2-OH-Ade:T) appears not to have been reported. However, there are indications that prevention or repair of misincorporated 2-OH-Ade may occur; for example, MYH can remove 2-OH-Ade from a mispair with G and MTH1 can use 2-OH-dATP as a substrate (43, 46).

Purine ring fragmentation products derived from either oxidative attack on guanine or adenine to form formamidopyrimidines are important lesions that tend to predominate under reducing conditions. FapyAde and FapyGua are substrates for NTH1 (Nth or endonuclease III homologue), which repairs many pyrimidine-derived oxidation products by a glycosylase activity (47, 48). FapyGua is repaired by OGG1 (42). Both lesions are reported to be major substrates for NEIL1, which would agree with the substrate preference for the bacterial homologue formamidopyrimidine glycosylase (FPG protein) (41).

That cyclo-dA is a substrate for NER, classically associated with the repair of helix-distorting, bulky adducts, is perhaps not unexpected (49, 50). These lesions are produced in the 5′S and 5′R diastereomeric forms to differing extents, however, it has also been

noted that 5′R-cyclo-dA is more efficiently repaired by NER than the 5′S diastereoisomer. Though not yet experimentally demonstrated, it seems that cyclo-dG would also be a substrate for NER.

There is some evidence for the repair of less bulky oxidative DNA lesions, such as 8-OH-Gua and Tg, by NER. This process is reported to be physiologically feasible: the removal of 8-OH-Gua by NER in cell-free extracts using a synthetic double-stranded DNA substrate appears to occur at rates comparable to those for cyclobutane thymine dimers, a classical NER substrate (51). This is perhaps reflective of a much broader range of substrates for this repair pathway than originally envisioned. However, in a situation where free competition for a substrate between BER and NER is allowed to occur, so-called short patch BER accounts for the majority of 8-OH-Gua repair in human cells, with the remainder due to long patch BER and any contribution by NER is reported to be negligible (52–54). Whether NER is a minor repair pathway for 8-OH-Gua under all circumstances is debatable; it may function preferentially in certain cell types and under specific conditions, perhaps when other mechanisms are compromised (55). It seems reasonable, however, that lesions such as 8-OH-Gua and Tg, which are potentially cytotoxic or mutagenic, should be substrates for multiple DNA repair pathways. What is certain is that the potential products of NER of oxidative DNA damage will be lesion-containing oligomers, typically 24–32 nucleotides long, with those produced for small oxidative DNA lesions at the lower end of this range (51, 56). Potentially these oligomers could undergo intra/extracellular 5′-3′ exonucleolytic digestion to ultimately produce lesion-containing oligomers 6–7 nucleotides long (51, 57). This type of postexcision processing has not been demonstrated for 8-OH-dG-containing oligomers, although there is some tantalizing evidence that 8-OH-dG-containing oligomers may be present in urine, but whether these are reflective of NER is open to debate (58, 59).

Transcription-coupled repair (TCR) directs repair processes to transcriptionally active regions of the genome and may play a role in the removal of small oxidative DNA base lesions such as 8-OH-Gua and Tg (55, 60, 61). Generally, TCR exploits the ability of certain DNA lesions to halt the processivity of RNA polymerase II, although TCR uses some of the same proteins as NER to fulfill its function, TCR is not a sub-pathway of NER, as the nature of the lesion will dictate the actual repair process. The exact role of TCR in the repair of oxidative DNA damage may depend on the experimental system used to examine the phenomenon (60, 62). However, it does seem likely that direction of DNA repair to actively transcribed regions of the genome would be prudent for oxidative DNA lesions, as it would for any other type of DNA lesion.

Repair of pyrimidine-derived DNA lesions

Some repair pathways for pyrimidine-derived oxidative DNA lesions have been examined in detail, approaching or equaling that of 8-OH-Gua. A predominant enzyme involved in the repair of such lesions is NTH1. Studies have revealed that NTH1 has a relatively wide range of substrates, some of which have been mentioned earlier. Certainly, Tg is a prominent substrate for NTH1 (63, 64). Because of its potential helix distorting properties, Tg would be considered amenable to NER, and its repair has been examined in this context. As with 8-OH-Gua, a relatively recent study indicates that ~80% of Tg is removed by short patch BER and the remainder by long patch BER; apparently NER of Tg, if it occurs, is negligible or again may function as a backup repair process (52). Thymine glycol is also reported to be a substrate for the recently described NEIL1 protein, an activity largely detected by the ability of *Nth1*$^{-/-}$ knockout mice to deal with this lesion in the absence of NTH1 (65). Another thymine-derived oxidation product, 5-formyluracil, is a substrate for NTH1 and possibly NEIL1, although the latter finding awaits confirmation (66). Additional known substrates for NTH1 derived from cytosine oxidation are 5-hydroxycytosine (preferentially repaired when paired opposite guanine) and 5,6-dihydroxycytosine (64, 66, 67). The former lesion has been reported to be repaired by NEIL2 (68). The repair of oxidized pyrimidines was until recently dominated by NTH1; however, the discovery of three Nei-like proteins (NEIL1-3) indicates an element of redundancy in the repair of pyrimidines akin to that encountered for oxidized purines (65, 69). This idea for the existence of backup DNA repair pathways comes in part from the presence of repair processes for specific lesions in the absence of the presumed, predominant repair enzyme in knockout mouse models (65). Although substrate specificities of NEIL1 and 2 have received some attention, there still is work to be done fully define the substrate specificity and preference for these proteins (65, 68, 69). The deamination of 5-hydroxycytosine to yield 5-hydroxyuracil (5-OH-Ura) in DNA is reported to be the major substrate for NEIL2, with the 5-OH-Ura:G pairing as the preferred substrate (41, 68). In contrast, because NEIL1 prefers to act on 5-OH-Ura:(A)T, it is suggested that this enzyme is involved in the removal of misincorporated lesion (68). Thus, NEIL1 and NEIL2 may be operating cooperatively to limit 5-OH-Ura persistence in the genome in a manner similar to the actions of OGG1 and OGG2.

In contrast to many oxidatively induced DNA lesions, which can affect coding sequences, DNA structure or RNA polymerase activity, 5-hydroxymethyluracil (5-OHMUra) apparently has little effect with regard to these particular functions (70). 5-Hydroxymethyluracil DNA glycosylase has been known for several years to repair this lesion in double- or single-stranded DNA and is restricted to higher organisms, particularly those that use 5-methylcytosine in the regulation of gene expression. The repair of 5-OHMUra:G pairs predominates in mammalian cells, implying that 5-methylcytosine is the predominant source of this lesion rather than via thymine oxidation (70). A potential precursor to this lesion, 5-hydroxymethylcytosine is also reported

to be repaired by a separate glycosylase activity(71). The identity of 5-OHMUra DNA glycosylase has recently been reexamined: in one case the enzyme was reported to be identical to a recently characterized uracil DNA *N*-glycosylase, hSMUG1; another study failed to confirm this identity, although the protein was isolated from two different sources (72–74).

Deamination of cytosine to uracil is an important promutagenic event in DNA with the potential to produce G:C→T:A transition mutations if not repaired before replication. As with several of the other oxidative base lesions, uracil may arise in DNA from the deamination of cytosine in situ to generate a U:G pair or may be erroneously incorporated by DNA polymerases into DNA, opposite adenine, through use of dUTP. The latter scenario is addressed via a dUTPase activity whose expression/activity is modulated in concert with the cell cycle and the proliferative state of the tissues. One of the earliest DNA glycosylases identified is that which removes uracil from DNA, uracil DNA *N*-glycosylase (UNG or UDG); UNG is reported to repair some oxidized cytosine products such as 5,6-dihydroxycytosine (75, 76). The lack of an obvious mutator phenotype in $ung^{-/-}$ mice led to the suggestion that there is also a backup repair pathway for uracil in DNA (77). Two studies, one based on this $ung^{-/-}$ mouse model, identified single-strand selective monofunctional uracil DNA glycosylase (SMUG1) as a second uracil DNA *N*-glycosylase (73, 77, 78). More recent studies have shown that UNG is probably the major glycosylase that removes misincorporated uracil and deaminated cytosine in single- and double-stranded DNA, particularly in the nucleus (79). In contrast, SMUG1 may have a greater role in the removal of 5-OHMUra from pairings with G or A (79).

While the delicate balance between ROS modification of DNA bases and their repair (**Table1**) is understood to determine the overall level of damage, these processes need to be translated into a cellular context in order to establish the basis by which oxidative DNA damage presents a potential risk in vivo. Some processes other than normal cellular metabolism have been identified that may account for elevated levels of intracellular ROS and oxidative DNA damage.

Impaired/defective repair

Levels of oxidative bases in DNA are the consequence of a balance between lesion induction from radical processes and repair. Clearly, reduced repair will result in elevated lesions and an increased risk of disease. Hence DNA repair capacity has been seen as a potential marker of cancer susceptibility. There is evidence to suggest that exposure of cells to H_2O_2, and perhaps other oxidants, may actually suppress DNA repair in addition to inducing damage (80). As a purportedly important means by which 8-OH-Gua is removed from DNA, human OGG1 (hOGG1) has a major role in the prevention of ROS-induced carcinogenesis. Therefore,

TABLE 1. *Major known repair proteins or pathways for principal oxidative DNA base lesions*

Parent or Lesion[a]	Context[b]	OGG1	OGG2	MYH	MTH1	NTH1	NEIL1	NEIL2	UNG	SMUG1	5-OHMCyt Glycosylase	NER
Ade	:8-OH-Gua			✓								
8-OH-Ade	:C	✓?										
	8-OH-dATP				✓							
2-OH-Ade	:G			✓								
	2-OH-dATP				✓							
FapyAde						✓	✓					
Cyclo-dA												✓
Gua												
8-OH-Gua	:C	✓										✓?
	:A or G		✓				✓					
	8-OH-dGTP				✓							
FapyGua		✓				✓	✓					
Cyclo-dG												✓?
Thy												
Tg						✓	✓					✓?
5-foUra						✓	✓?					
Cyt												
5-OH-Cyt	:G					✓		✓				
5,6-diOHCyt						✓			✓			
5-OH-Ura	:G							✓				
	:A or T						✓					
Ura	:G or A								✓	✓		
5-*MeCyt*												
5-OHMCyt											✓	
5-OHMeUra	:G or A									✓?		

[a] Major known repair proteins or pathways for principal oxidative DNA base lesions. Abbreviations for lesions and enzymes can be found in the text. [b] Base pairing or other context in which lesion is preferred. ✓ = Repair activity reported. ✓? = Repair activity reported but awaits further experimental evidence/evidence of relative importance; see text for details.

inactivation of the *hOGG1* gene could increase the likelihood of malignant transformation. Mapping of the *hOGG1* gene to chromosome 3p25 and identification of chromosome 3p as a frequent site for LOH or deletions in human lung and kidney cancers led researchers to investigate hOGG1 mutations and activity/expression in tumors. Although levels of *hOGG1* mRNA were normal in all tumors examined, sequencing studies revealed that 3 of 40 tumors possessed homozygous mutations, all of which result in an amino acid change in hOGG1 protein (81). Whereas Chevillard et al. (81) did not examine whether these mutations resulted in functional changes in enzyme activity, Kohno et al. (82) described a genetic polymorphism at codon 326 in the *hOGG1* gene that led to differing activities between the isoforms. The authors speculate that interindividual variability in 8-OH-Gua repair could derive from a polymorphic *hOGG1* genotype (82). This suggestion is supported by a report of polymorphisms and alternative splicing of the *hOGG1* gene in human clear cell carcinoma of the kidney, some of which resulted in an impaired or inactive form of hOGG1 (83). However, comparison of *hOGG1* genotype and 8-OH-Gua levels in 34 lung cancer specimens failed to show that polymorphic variation could affect tissue 8-OH-Gua (84), a finding similar to that reported by Hanaoka et al. (85) in an examination of gastric cancers. These findings might be explained by methodological problems that may limit sensitive adduct measurement and/or that hOGG1 is not the sole pathway for 8-OH-dG removal.

Whereas polymorphisms in DNA repair genes generally produce subtle phenotypic differences between isoforms, defects in the NER pathways can have more profound effects as evidenced by xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy, the former condition at least being associated with a predisposition to skin cancer (86). Indeed, reduced expression of some NER genes appears to be associated with increase risk of (lung) cancer (87), and certain NER gene polymorphisms do relate to increased adducts (88). The involvement of NER in the removal of oxidative DNA damage is established; however, as with base excision repair, the effect of NER polymorphisms on oxidative lesion levels and disease risk remains unclear.

Lowered antioxidant capacity

Reduced activities of the antioxidant enzymes catalase, glutathione peroxidase, and superoxide dismutase, with concomitant increased levels of oxidative DNA damage, have been reported in acute lymphoblastic leukemia, seemingly agreeing with the report of Honda et al. (89), who reported elevated levels of urinary 8-OH-dG in leukemia. The effect of lowered antioxidant levels on oxidative DNA damage in vivo was first demonstrated in a study where vitamin C levels of 10 volunteers were depleted and repleted. This work demonstrated that decreasing vitamin C intake from 250 mg/day to 5 mg/day led to a corresponding 50% increase in sperm DNA levels of 8-OH-dG (34.0 fmol/μg DNA +/− 2.4 to 66.90 fmol/μg DNA +/− 8.5, $P<0.01$), although continued depletion resulted in a 248% increase (90). Repletion at 250 mg/day for 28 days led to only a 36% decrease in 8-OH-dG levels. An identical study design, by the same group showed an increase in sperm levels of 8-OH-dG, although depletion had no effect on the 8-OH-dG content of PBMC or urinary 8-OH-Gua. The authors failed to speculate on this discrepancy between the two cell types, though it may reflect the differing requirements of the cells for vitamin C. Such a finding appears to highlight the issue of surrogate measurements, i.e., performing measurements on a cell type, such as PBMC, and extrapolating the results to the perhaps less accessible target cell (reviewed in ref 91).

A locus on chromosome 3p that is frequently subject to loss of heterozygosity (LOH) is the glutathione peroxidase gene (*GPX1*), which encodes for a peroxide scavenging protein. Lung tumors with LOH within *GPX1* displayed reduced enzyme activity, although this did not appear to correlate with tissue 8-OH-dG levels (84), perhaps due to the presence of other antioxidant defense systems that could compensate, a situation similar to that seen for 8-OH-dG repair (see above). A recent review revealed the presence of a sizable number of polymorphisms in genes that encode for antioxidant enzymes, the phenotypic impact of which ranges from having no effect on enzyme activity to a complete absence of gene product (92). From this review it is clear that, as a single factor affecting oxidative stress, polymorphisms in antioxidant defense genes have the potential to play a significant role in the risk of disease development (92).

CELLULAR IMPACT OF OXIDATIVE DNA DAMAGE

The presence of oxidized base lesions in DNA is well established and the number of lesions identified is growing. There is a significant number whose effects on replication and transcription have been described. Some factors influence the effect a particular lesion has on replication and transcription. Not surprisingly, 8-OH-dG is by far the most frequently studied and is often used here as an example of how oxidative lesions may exert their effect.

Formed in situ or misincorporated?

Many oxidative base lesions are mutagenic, irrespective of whether they are formed in situ or arise by misincorporation from the deoxynucleotide pool. For the most part, 8-OH-dG formed in situ results in G→T substitutions; alternatively, 8-OH-dGTP may be misincorporated opposite dA, producing an A→C substitution (93). However, the likelihood for the native form, either in DNA or in the deoxynucleotide pool, to be oxidized can influence what mutations ultimately pre-

dominate. For example, given that nucleotide pool stores of dATP are ~67-fold more easily oxidized than dA in situ in duplex DNA, it is probable that the majority of 2-OH-dA in DNA arises from misincorporation of 2-OH-dATP.

Alterations in conformation

For lesions that can induce conformational changes in DNA in addition to the structural alterations to the native base itself, the potential for enhancing mutagenicity exists. Illustrative of this is the oxidation of dG to 8-OH-dG in DNA. The native *anti* conformation of dG is maintained; however, when the DNA is made single-stranded, whether it be at replication or transcription, 8-OH-dG can then adopt the energetically more favorable *syn* conformation. This prevents pairing with dC and results in mispairing with dA or T.

Repairability

A further factor affecting the mutagenicity or otherwise of a lesion is the ease with which the lesion is repaired. Increasingly it seems that the repair enzymes have preference for particular lesion:native base pairings. In mammalian cells, the 8-OH-Gua:C pair is effectively repaired (by OGG1), whereas the 8-OH-Gua:A pair is poorly repaired despite established mechanisms to address this mispair (42, 94).

Cell line/polymerase of study

For in vitro studies, mutagenicity can also be affected by the cell line or polymerase chosen in the model system. The former point is well illustrated by 8-OH-dA. Although the mutagenicity of 8-OH-dA in bacterial cells is described as negligible, studies in mammalian cells have demonstrated that rodent pol α and pol β can both misinsert dATP and dGTP opposite 8-OH-dA. The latter point is demonstrated by oxazolone, a major one-electron and hydroxyl radical-mediated oxidation product of guanine (23). Whereas insertion of dAMP by Klenow fragment exo$^-$ and *Taq* polymerase opposite oxazolone occurs, potentially generating G→T transversions, pol β failed to insert any nucleotide generating a stop. The effects of many lesions have not been studied in mammalian cells; given that different polymerases respond differently to different lesions, the mutation spectrum or frequency may alter between bacterial and mammalian cells.

Sequence context

The formamidopyrimidines are major products of hydroxyl radical attack of DNA. Although little is known now about their biological significance, Graziewicz et al. (95) demonstrated that, although less frequently inhibited by oxidized purines than oxidized pyrimidines, DNA synthesis by prokaryotic polymerases was shown to be terminated by both FapyAde and FapyGua. However, this effect was only moderate and depended on the sequence context (95).

Overall its seems that oxidative DNA lesions are best described as weakly mutagenic; for example, 8-OH-dG has mutation frequencies of 2.5–4.8% in mammalian cells although lesion formation, persistence, and accumulation in vivo could give this value greater significance. Indeed, oxidative events are reported to be largely responsible for spontaneous mutagenesis (96). However, mutations are not the only effect of oxidative DNA damage.

Alternatives to mutation

Replicative block

Thymidine glycol occurs mainly as the *cis* isomer, and the mutational specificity of this lesion has been much studied in bacterial and mammalian cells. Even though some mutations have been identified associated with Tg, the general agreement appears to be that Tg does not have a significant mutagenic potential. More recent studies have suggested that rather than being mutagenic, Tg blocks replication one nucleotide before and after the lesion (96).

Deletions

The results of the limited studies investigating the mutagenicity of 5-OHMUra have been conflicting. Although not significantly toxic to a cell, the mutagenicity of 5-OHMUra has been implied by the presence of an enzyme for its repair. Initially described as potentially mutagenic in bacteria and mammalian cells, Chaung and Boorstein (97) furthered such work by reporting that, rather than inducing point mutations, the presence of 5-OHMUra leads to large/intermediate deletions in mammalian cells. However, these deletions do not arise as a result of mispairing or misincorporation; instead, it seems that base excision repair by OHMUra-DNA glycosylase may lead to the deletions (97).

Microsatellite instability/loss of heterozygosity

In normal cells, the length of repetitive sequences of DNA, so-called microsatellites, is constant, but the length of these repeats can be variable in tumor cells. This microsatellite instability (MI) derives from DNA damage and has been linked to some sporadic cancers. The association between oxidative events and MI appears to be increasing, with oxidative DNA damage shown to increase the frequency of microsatellite instability through induction of mutations in the repeat sequences (98) and discontinuous LOH being described as a signature mutational pattern of oxidative DNA damage (99).

Epigenetic effects of oxidative DNA damage

While the mutagenic effects of oxidative DNA damage are largely well recognized, emerging work is broaden-

ing the number of routes by which these lesions may affect the cell, being suggestive of epigenetic effects exclusive of mutation. Central to this work is the finding that when exposed to oxidants, mammalian cells express stress-induced genes or genes encoding antioxidant defenses. Such adaptive responses to oxidative insults are not surprising and are seen with other, nonoxidative insults; however, it remains to be established whether DNA damage itself has any involvement in gene expression or whether this is merely a by-product of ROS generated during stress. Indeed, ROS are themselves intracellular signaling molecules, although whether sufficient levels of ROS can be induced by an oxidative insult, such that gene expression is affected, appears doubtful (100). However, single-strand breaks generated, for example, by 2 Gy of ionizing radiation, would be orders of magnitude greater than endogenous levels and hence a more suitable candidate lesion for signaling (100).

The presence of lesions in the transcribed regions of genes can lead to mutation, but can lesions in nontranscribed regions have an effect? Ghosh and Mitchell (101) demonstrated that the presence of 8-OH-dG in promoter elements can affect transcription factor binding. It appears that that a single 8-OH-dG moiety in the AP-1 transcription factor binding site in the promoter region of genes can prevent transcription factor binding and hence the level of transcription. Being GC-rich, these regions represent a considerable target for ROS. Further support for such a mechanism derives from the substitution of dG for 8-OH-dG in the cognate DNA sequence of the transcription factor SP1 (102). This effect may have pathological consequences, as the interference of Sp1 and NF-κB binding in the kidney and liver of diabetic rats was postulated to be due to ROS-mediated damage to DNA.

These as yet sparsely studied aspects of oxidative damage suggest a potential for oxidants to affect gene expression either through ROS generation or by interfering with transcription factor binding. Such work supports the hypothesis that events at the DNA level, other than mutation, are involved in pathogenesis.

ROLE OF OXIDATIVE DNA DAMAGE IN DISEASE

It is clear that depending on the lesion in question, one consequence of oxidative base lesions persisting in DNA is mutation. For this reason, multiple systems exist to: *1)* prevent lesion formation and, should damage occur, *2)* ensure rapid lesion removal, with the enzyme systems responsible for the latter having much overlap of substrates (see earlier section). DNA mutation is a crucial step in carcinogenesis, and elevated levels of oxidative DNA lesions have been noted in many tumors, strongly implicating such damage in the etiology of cancer.

Carcinogenesis and cancer

Oxidative mechanisms have been demonstrated to possess a potential role in the initiation, promotion, and malignant conversion (progression) stages of carcinogenesis. Given that cumulative cancer risk increases with the fourth power of age and is associated with an accumulation of DNA damage, oxidative DNA damage has been investigated in cancer.

Lesions such as 8-OH-dG are established biomarkers of oxidative stress; coupled with their potential mutagenicity in mammalian cells, this has led to their proposed potential as intermediate markers of a disease endpoint—for example, cancer. Supportive of this proposal are the findings that GC→TA transversions potentially derived from 8-OH-dG have been observed in vivo in the *ras* oncogene and the *p53* tumor suppressor gene in lung and liver cancer. Of course, GC→TA transversions are not unique to 8-OH-dG, whereas CC→TT substitutions in the absence of UV in internal tumors have been identified as signature mutations for ROS.

Numerous studies have attempted to establish a relationship between levels of oxidative DNA damage and cancer. Elevated levels of damage are purported to arise as a consequence of an environment in the tumor low in antioxidant enzymes and are high in ROS generation (103). It has been reported that at least some tumor cell lines can produce significant levels of H_2O_2, without exogenous stimulation, perhaps accounting for the elevated levels of oxidative DNA damage seen. As a result of elevated ROS, transcription factors and their corresponding genes are permanently activated, which, coupled with increased DNA damage, creates a selection pressure for a malignant phenotype seen in cancer (103). Although such studies have furthered the hypothesis that oxidative DNA damage may be an important risk factor for carcinogenesis, it has been argued that the mere presence of 8-OH-dG in DNA is unlikely to be necessary or sufficient to cause tumor formation. There are many pathological conditions in which levels of oxidative DNA damage are elevated (**Table 2**) with no increased incidence of carcinogenesis. This has led us to raise the following issues. *1)* Oxidative DNA damage may be an epiphenomenon to an on-going pathophysiological process, and elevated levels do not have a role in carcinogenesis. *2)* Cause or consequence? The mere presence of elevated levels of damage in tumors does not indicate it was oxidative damage that led to the tumorigenic changes. Elevation in levels may have occurred as a result of well-established characteristics of tumors, e.g., increased metabolism or cell turnover. *3)* For DNA mutations to arise from oxidative damage, the nuclei of undifferentiated, proliferating stem cells must be affected. Given that tissue samples from tumors and normal cells will represent a heterogeneous mixture of differentiated and undifferentiated cells (with the former likely to predominate), current analytical procedures will not reflect lesion levels in the most important target cells. *4)* Not only must the DNA of target

TABLE 2. *Reports of pathological conditions in which oxidative DNA damage has been measured*

Organ system/disease		Lesion measured	Comments
Blood	Acute lymphoblastic leukemia (ALL)	FapyGua, 8-OH-Gua, FapyAde, 8-OH-Ade, 5-OH-Cyt, 5-OH-5-MeHyd, 5-OH-Hyd[a] (DNA)	• Lymphocyte DNA lesion levels significantly ($P < 0.05$) elevated in ALL vs. control subjects (104).
	Hematological disorders:	8-OH-dG (urine)	• Adult T cell leukemia/lymphoma ($P < 0.05$); lymphoma, acute leukemia, and myelodysplastic syndrome; no significant difference compared to controls.[b]
Brain/nervous system	Parkinson's disease (PD)	8-OH-dG (DNA)	• DNA levels of 8-OH-dG significantly elevated ($P = 0.0002$) in substantia nigra of PD brains (105).
		8-OH-Guo (DNA/RNA)	• Levels of 8-OH-Guo in cytoplasmic DNA and RNA are elevated in substantia nigra neurons of Parkinson's disease patients and (to a lesser extent) in multiple system atrophy-Parkinsonian type and dementia with Lewy bodies (106).
	Alzheimer's disease (AD)	8-OH-dG (DNA)	• DNA levels of 8-OH-dG in AD brain not associated with disease (107).
		8-OH-dG (DNA)	• Higher levels of 8-OH-dG in cortex and cerebellum of AD patients vs. controls (108).
		8-OH-dG (CSF)	• Ventricular cerebrospinal fluid (CSF) DNA levels of 8-OH-dG significantly ($P < 0.05$) elevated and CSF levels of free 8-OH-dG significantly reduced ($P < 0.05$) compared to controls (109).
		8-OH-dG (DNA)	• Significantly higher levels ($P < 0.001$) of 8-OH-dG in lymphocytes from AD patients compared to controls (110).
	Huntington's disease (HD)	8-OH-Gua, FapyAde, 8-OH-Ade, FapyGua, 2-OH-Ade, X, Hx (DNA)	• No difference between lesion levels in caudate, putamen, and frontal cortex from HD brains compared to controls (111).
	Dementia with Lewy bodies (DLB)	8-OH-Gua, FapyGua, 5-OH-Cyt, 5-OHU, 5-HMU, X (DNA)	• Increased levels of lesions in cortical region of brain in DLB patients compared to control tissue (112).
	Multiple sclerosis	8-OH-dG (DNA)	• Significantly elevated levels of 8-OH-dG in plaques, compared to normal-appearing white matter in multiple sclerosis-affected cerebella (113).
	Amyotrophic lateral sclerosis (ALS)	8-OH-dG (plasma, urine and CSF)	• Levels of 8-OH-dG significantly increased in all three matrices, compared to controls; levels of urinary 8-OH-dG increased over a 9 month period and correlated with disease severity (114).
	Friedreich ataxia (FRDA)	8-OH-dG (urine)	• 2.6-fold increase in urinary 8-OH-dG of FRDA patients compared to controls (115).
Breast	Invasive ductal carcinoma	8-OH-Gua, FapyGua, 8-OH-Ade, FapyAde (DNA)	• Levels of lesions, apart from FapyAde, significantly ($P \leq 0.01$, $P \leq 0.02$, and $P \leq 0.05$, respectively) increased compared to calf thymus DNA (116).
	Breast cancer	8-OH-dG (DNA)	• Levels of 8-OH-dG were not significantly elevated in breast cancer tissue vs control, nor were levels associated with expression of oestrogen/progesterone receptors, clinical stage, or histological grade (117).
	Invasive ductal carcinoma	8-OH-dG (DNA)	• Significantly elevated levels of 8-OH-dG ($P < 0.001$) in malignant breast tissue; also levels significantly greater ($P = 0.007$) in estrogen receptor-positive (ORP) vs. ORP-negative malignant tissue (118).
	Primary breast cancer	8-OH-dG (DNA)	• Significantly higher ($P < 0.0001$) levels of 8-OH-dG in tumour vs. nontumor tissue (119).
Cardiovascular disease		8-OH-dG (DNA)	• Strong association ($r = 0.95$, $P < 0.01$) between premature coronary heart disease in men and lymphocyte 8-OH-dG levels (120).
Colon	Colorectal cancer (CRC)	8-OH-dG (DNA)	• Significantly elevated levels of 8-OH-dG ($P < 0.005$) in tumor tissue compared to normal mucosa (121).

TABLE 2. (*continued*)

Organ system/disease		Lesion measured	Comments
Colon (*continued*)		8-OH-dG (DNA)	• Significant correlation between lymphocyte 8-OH-dG levels and colorectal cancer deaths in men (r = 0.91, $P < 0.05$, ref. 120).
Gynaecological	Gynecological cancers	8-OH-dG (urine)	• Levels significantly higher ($P \leq 0.05$) in patients with gynecological cancer compared to control subjects.[b]
	Cervical cancer	8-OH-dG (DNA)	• Levels of 8-OH-dG significantly increased ($P < 0.001$) in low- and high-grade levels of dysplasia, compared to normal, although this did not correlate with human papillomavirus status (122).
Kidney	Renal cell carcinoma (RCC)	8-OH-dG (DNA)	• Levels of 8-OH-dG significantly higher ($P < 0.0005$) in RCC vs. noncancerous tissue (123).
	Transplantation	dTg (urine)	• Significantly elevated levels of dTG after kidney transplantation proposed to be due to ischemia-reperfusion injury (124).
Liver	Haemochromotosis	8-OH-dG (urine)	• No significant difference in levels between patients and control subjects.[b]
	Wilson's disease and primary hemochromotosis	8-OH-dG (DNA)	• 8-OH-dG levels not elevated in liver of hemochromotosis patients and significantly lowered in liver of Wilson's disease (125).
	Chronic hepatitis	8-OH-dG (DNA)	• Liver levels of 8-OH-dG significantly elevated ($P < 0.05$) compared to controls (126).
	HCV	8-OH-dG (DNA)	• Leukocyte DNA levels 8-OH-dG significantly higher than in HBV infection ($P < 0.04$), correlating with clinical diagnosis ($P < 0.025$) (127).
	HCV	8-OH-dG (DNA)	• Significantly elevated levels of liver 8-OH-dG compared to controls ($P < 0.001$; 128).
	HCV	8-OH-dG (DNA)	• PBMC levels of 8-OH-dG significantly elevated ($P < 0.00001$) in HCV-positive patients, compared to controls. 8-OH-dG levels positively correlated ($P < 0.02$) with presence and extent of liver damage (129).
	Hepatoblastoma	8-OH-dG (DNA)	• Positive immunohistochemical staining for 8-OH-dG in liver sections from all 5 patients with hepatoblastoma (130).
	Chronic hepatitis, alcoholic liver disease, primary biliary cirrhosis.	8-OH-dG (DNA)	• Positive immunohistochemical staining for 8-OH-dG in all diseased liver sections; no staining in control liver sections (131).
	Hepatocellular carcinoma (HCC)	8-OH-dG (DNA)	• Significantly ($P < 0.005$) elevated levels of 8-OH-dG in peritumoural tissue compared to tumor tissue in HCC. In contrast, patients with hepatic metastases (non-HCC) or end-stage alcoholic liver disease showed no differences between the corresponding two regions (132).
Lung	Cystic fibrosis	8-OH-dG (urine)	• Urinary levels of 8-OH-dG significantly raised vs. control subjects.[b]
	Squamous cell carcinoma (SCC)	8-OH-Ade	• Levels elevated in tumor tissue of all SCC patients vs. controls,
		8-OH-Gua	- levels elevated in 4/5[c] patients,
		FapyGua	- levels elevated in 3 patients,
		5-OHMe-Ura, 5-OH-Ura, 5-OH-Cyt, 2-OH-Ade	- levels elevated in ⅗ patients,
		5-OH-Hyd, 5,6-diOH-Ura, FapyAde (DNA)	- levels elevated in only ⅕ or ⅖ patients (133).
	Small cell carcinoma	8-OH-dG (urine)	• Elevated 8-OH-dG compared to controls ($P < 0.05$).[b]
	Non-small cell carcinoma	8-OH-dG (DNA)	• No significant differences in 8-OH-dG levels in tumour compared to nontumor tissue (84).

TABLE 2. (*continued*)

Organ system/disease		Lesion measured	Comments
Lung (*continued*)	Lung cancer	8-OH-dG (DNA)	• Lymphocyte DNA levels of 8-OH-dG significantly elevated ($P < 0.05$) compared to controls (134).
	Lung cancer	8-OH-dG (DNA)	• Elevated levels of 8-OH-dG in lung cancer compared to normal lung tissue from control individuals (135).
Skin	Atopic dermatitis	8-OH-dG (urine)	• Urinary 8-OH-dG significantly higher than in controls ($P < 0.0001$) and correlating with disease severity index.[b]
	Psoriasis	8-OH-dG (urine)	• 8-OH-dG levels are not elevated in mild to moderate psoriasis.[b]
	Arsenic-related skin neoplasms	8-OH-dG (DNA)	• Significantly elevated levels of 8-OH-dG ($P < 0.001$) in arsenic-related Bowen's disease, Bowen's carcinoma and actinic keratosis, compared to their corresponding non-arsenic related conditions (136).
Stomach	*Helicobacter pylori* infection	8-OH-dG (urine)	• Subjects without *H. pylori* infection had significantly higher ($P = 0.008$) levels of 8-OH-dG compared to infected patients.[b]
		8-OH-dG (DNA)	• Elevated levels of 8-OH-dG associated with *H. pylori* infection (137).
	Stomach cancer	8-OH-dG (DNA)	• Strong negative correlation ($r = -0.92$, $P = 0.01$) between stomach cancer in women and lymphocyte 8-OH-dG levels (120).
	Gastric adenocarcinoma	8-OH-dG (DNA)	• Significantly higher levels of 8-OH-dG in tumor-adjacent and tumor tissues than in normal tissue ($P < 0.001$) of gastric cancer patients. 8-OH-dG levels also significantly elevated in tissues infected with *H. pylori* compared to noninfected tissues (138).
	Gastric cancer	8-OH-dG (DNA)	• Tissue levels of 8-OH-dG significantly elevated in chronic atrophic gastritis ($P = 0.0009$), intestinal metaplasia (0.035), and *H. pylori* infection (0.001) compared to unaffected controls (139).
Aging		8-OH-dG (urine)	• No correlation between urinary 8-OH-dG output and aging (age range: 35–65 years).[b]
		8-OH-dG (plasma and CSF)	• In all ALS patients and healthy subjects, plasma and CSF levels of 8-OH-dG increase with age (114).
Cancers	Assorted cancers	Tg and dTg (urine)	• No difference in levels of Tg or dTg in neoplastic vs. nonneoplastic urine.[b]
	Assorted cancers: fibrillary astrocytoma (FA; brain); lung cancer (LC); mucinous carcinoma (MC; stomach) ovarian cancer (OC); colon cancer (CRC)		• Lesion significantly elevated ($P \leq 0.05$) in:
		5-OH-5-Me-Hyd	- LC, CRC, OC
		5-OH-Hyd	- FA, LC, CRC, MC, OC
		5-OHMe-Ura,	- LC, MC, OC
		5-OH-Cyt	- LC, OC
		5,6-diOH-Ura	- FA, LC, CRC, MC, OC
		FapyAde	- FA, LC,
		8-OH-Ade	- FA, LC, MC, OC
		Xanthine	- LC, MC, OC
		2-OH-Ade	- FA, LC, MC, OC
		FapyGua	- FA, LC, MC, OC
		8-OH-Gua	- FA, LC, CRC, MC, OC (140)[d]
	Breast, rectal and colon cancer	5-OH-Me-Ura	• Women who develop breast or colorectal cancer have elevated levels of serum autoantibodies to 5-OH-Me-Ura compared to controls (141).
	Assorted cancers	8-OH-dG (urine)	• Elevated levels of urinary 8-OH-dG noted in cancer patients before ($P < 0.01$) and after anti-cancer therapy ($P < 0.001$).[b]

TABLE 2. (*continued*)

Organ system/disease		Lesion measured	Comments
Diabetes mellitus	Non-insulin-dependent (NIDDM)	8-OH-dG (urine)	• Levels of urinary 8-OH-dG significantly higher than controls ($P = 0.001$) associated with high glycosylated haemoglobin.[b]
		8-OH-dG (DNA)	• Elevated levels of 8-OH-dG in muscle DNA of NIDDM patients compared to controls. Significant correlation between mitochondrial DNA deletion (delta mtDNA4977) and 8-OH-dG levels ($P < 0.0001$) and proportional to diabetic complications (142).
	Insulin- and noninsulin-dependent	8-OH-dG (DNA)	• Both groups had significantly higher levels of 8-OH-dG ($P < 0.001$) in mononuclear cell DNA, compared to controls (143).
	Type II	5-OH-MeHyd, 5-OH-Hyd, 5-OH-Ura, 5-OH-Me-Ura, 5-OH Cytosine, Tg, 8-OH-Gua, FapyAde, 8-oxoA, 2-OH-Ade	• PBMC levels of oxidised DNA base products significantly elevated in diabetes patients compared to controls (144).
		8-OH-dG (urine)	• 8-OH-dG levels in 24 h urine collections significantly higher ($P < 0.001$) in diabetic patients than in control subjects.[b]
		8-OH-dG (urine and DNA)	• 8-OH-dG levels in 24 h urine collections and mononuclear cell DNA significantly higher ($P < 0.001$ and $P < 0.0001$) in diabetic patients than in control, nonsmoking subjects.[b]
		8-OH-Gua (serum)	• Diabetic patients possessed significantly higher levels of serum 8-OH-Gua than control subjects.[b]
	Type I and II	8-OH-dG (urine)	• Patients with both type I and II diabetes had significantly higher levels of urinary 8-OH-dG, compared to controls (145).
Down's syndrome (DS)		8-OH-dG (urine)	• Levels significantly increased ($P = 0.00011$) in DS subjects compared to controls.[b]
		8-OH-dG (DNA)	• No significant increase in nuclear DNA 8-OH-dG content of cerebral cortex and cerebellum of DS and Alzheimer's disease patients compared to controls (146).
Fanconi's anemia		8-OH-dG (DNA)	• Leucocyte levels of 8-OH-dG significantly elevated in homozygous Fanconi's anemia patients and their parents compared to age-matched controls (147).
Rheumatoid arthritis		8-OH-dG (urine)	• Levels of urinary 8-OH-dG significantly elevated ($P < 0.001$) compared to control subjects (148).
		8-OH-dG (DNA)	• PBMC levels of 8-OH-dG significantly higher in rheumatoid arthritis patients vs. controls ($P = 0.001$; 149).
Systemic lupus erythematosus (SLE)		8-OH-dG (urine)	• Levels of urinary 8-OH-dG significantly reduced compared to control subjects; presence of 8-OH-dG noted in circulating immune complexes.[b]
		5-OH-Me-Ura	• Titres of serum autoantibodies to 5-OH-Me-Ura significantly elevated in SLE (150).
		8-OH-dG (DNA, serum & urine)	• Attenuated response to vitamin C supplementation in all three matrices.[b]
		8-OH-dG (DNA)	• PBMC levels of 8-OH-dG significantly higher in SLE patients vs. controls ($P = 0.0001$; 149).

[a] FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-OH-Gua, 8-hydroxyguanine; FapyAde, 4,6-diamino-5-formamidopyrimidine; 8-OH-Ade, 8-hydroxyadenine; 5-OH-Cyt, 5-hydroxycytosine; 5-OH-5-MeHyd, 5-hydroxy-5-methyl-hydantoin; 5-OH-Hyd, 5-hydroxy-hydantoin; Tg, thymine glycol; dTg, thymidine glycol. [b] References contained within Cooke et al. (2002). [c] Number of subjects tested, i.e., 4 persons out of 5. [d] Only one patient per cancer apart from lung, where $n = 2$.

cells be affected; to result in a mutation the damage must be within a coding region of the DNA. Issues like these will have to be addressed before the link between oxidative DNA damage and cancer is proven.

As with initiation, much of the data that suggest ROS affects tumor promotion has been derived indirectly: chemical promoters can generate oxidative stress and antioxidants can inhibit promotion; therefore, ROS are involved in promotion. It is possible that the antioxidants themselves, may allow clonal expansion and tumor promotion by protecting initiated cells from excessive oxidant toxicity and apoptosis that would otherwise kill them. Finally, in linking oxidative stress with promotion, it must not be forgotten that biomolecules other than DNA may be oxidatively modified and that these may have a significant effect.

Therefore, although the role of oxidative stress in carcinogenesis appears well established, the extent to which oxidative DNA damage contributes has not been well defined. Nevertheless, it appears that the DNA damage can be more closely associated with initiation events than with promotion, and this may be due to the potential for a multiplicity of mutagenic lesions to be formed in addition to the epigenetic effects described earlier.

Noncancerous disease

Brain

The neurodegenerative conditions Alzheimer's disease, Huntington's disease, and Parkinson's disease have oxidative stress implicated in their pathogenesis (105, 106, 108, 109), although there are reports that levels of oxidative DNA damage are not specifically elevated (107, 111). However, examination of such apparent discrepancies reveal differences in analytical methods and protocols between studies, any one of which may jeopardize consensus. The tendency not to publish essentially negative results means that principally only reports of elevated lesions are described. Supportive of the studies showing elevated lesion levels are data derived from in vitro studies demonstrating that neurotransmitters such as dopamine and serotonin can generate DNA-damaging, free radical species (151, 152). The role of oxidative stress and oxidative damage to biomolecules other than DNA in the pathogenesis of neurodegenerative disease, and Alzheimer's disease specifically, has been supported in several recent reviews of the subject (153), although the greatest significance for the pathogenesis of the disease has been placed on lipid and protein oxidation.

Inflammation/infection

The association between inflammation and oxidative stress is well documented (154, 155), with studies of inflammatory conditions or infections reporting elevated levels of 8-OH-dG: hepatitis (126), hepatitis C infection (127), and atopic dermatitis (Table 2). Bactericidal species ($O_2^{\bullet -}$ and H_2O_2), generated from the respiratory burst of invading neutrophils, macrophages, and eosinophils damage surrounding tissue, initiating further radical reactions and potentially oxidative stress. Chronic inflammation and hence oxidative stress have been closely linked to the pathogenesis of such autoimmune diseases as rheumatoid arthritis (149) and systemic lupus erythematosus (148), with radical production resulting not only in connective tissue damage, but also modified biomolecules being exposed to the systemic circulation, postulated to be the antigen driving autoantibody production (156). Mechanistically, chronic inflammation can be closely linked to carcinogenesis (reviewed in ref 157), although there is little evidence to suggest that patients with chronic inflammatory diseases such a systemic lupus erythematosus have an increased rate of cancer development (158). Nevertheless DNA damage does occur in cells cocultured with activated phagocytes (157), with lymphocyte DNA from patients with RA, SLE, vasculitis, or Behcet's disease containing elevated levels of 8-OH-dG; furthermore, lymphocytes from RA and SLE patients are more sensitive to the cytotoxic effects of hydrogen peroxide (149). Such damage may fulfill initiation; tumor promotors have been reported to recruit inflammatory cells that, with their potential to generate ROS, may provide the appropriate stimuli to lead to promotion (159).

Cardiovascular disease

Whereas there is growing evidence for the involvement of ROS in atherosclerotic plaque development, the role of DNA damage in this chronic inflammatory disease is less clear. In fact, there are relatively few reports examining levels of oxidative DNA damage in cardiovascular disease, but perhaps one of the most striking results was that reported by Collins et al. (120), in which examination of a mixed European population revealed a strong association ($r=0.95$, $P<0.01$) between premature coronary heart disease in men and lymphocyte 8-OH-dG levels. However, this work has been criticized on a methodological basis (160). Furthermore, it is not clear why elevated levels of 8-OH-dG in lymphocytes should be associated with premature coronary heart disease or whether lymphocyte lesion levels reflect those in the target tissue (i.e., blood vessels of the heart). It may be that given the inflammatory nature of atherosclerosis and the fact that lymphocytes spend the majority of their lifetime in peripheral tissue rather than in the systemic circulation, the cells may be exposed to the oxidizing species associated with inflammation. Nevertheless, in the absence of this information, such a finding may bring into question the use of lymphocytes as a surrogate tissue. In an animal model of atherosclerosis, increased levels of 8-OH-dG and repair-associated proteins in macrophage-derived foam cells (both associated with diet-induced hypercholesterolemia) were shown to be reduced during lowering of dietary lipid (161). The same group showed a similar

result in human plaque-associated cells (162). Humans with *GSTM1* null genotypes are reported to possess higher levels of 8-OH-dG in the smooth muscle cell DNA from atherosclerotic lesions (163). These reports are further evidence for the presence of DNA damage in cardiovascular disease, but fail to determine whether or not this is an epiphenomenon.

Transplantation (ischemia-reperfusion injury)

Urinary levels of Tg were significantly elevated in rats that had undergone liver transplantation. It appeared that the greater the levels of Tg output, the better the viability of the graft. Elevated levels of urinary dTg were also reported by Thier et al. (124) in six patients following kidney transplantation, which the authors proposed to be due to ischemia-reperfusion or reoxygenation injury. This is supported by studies by Loft et al. (164) whose work in pigs described substantial oxidative insult to DNA post-transplantation, as indicated by elevated urinary 8-OH-dG. Such injury is a significant factor affecting morbidity and mortality after bypass and transplantation surgery, hemorrhagic or septic shock, myocardial infarction, and multiple organ failure. During the period of ischemia, xanthine dehydrogenase is converted to xanthine oxidase. Upon reperfusion, there is a "burst" of xanthine oxidase activity that, rather than transferring electrons to NAD^+, transfers them to oxygen, generating superoxide. Endogenous levels of xanthine dehydrogenase vary from organ to organ and hence ischemia-reperfusion injury might be more relevant to some tissues than others (1). Human leukocytes appear to sensitive to the genotoxic effects of ischemia-reperfusion (165) and therefore represent a potential surrogate tissue in which to study the effects of ischemia-reperfusion that have affected a less accessible tissue.

Aging

Broadly speaking, theories of aging are grouped under two categories: damage accumulation aging and developmentally programmed aging. However, an emerging hypothesis described as the free radical theory of aging appears to have adopted elements of the former theories. The basis of the theory described by Harman (166) suggested that aging occurs through the gradual accumulation of free radical damage to biomolecules. The failure of antioxidant defenses to scavenge all radical species, evident from the increasing background levels of damage with age, will result in the insidious accumulation of damage and gradual loss of function (comprehensively reviewed in ref 167). Illustrating this point is the report of an age-related increase in serum 8-OH-dG in apparently disease-free individuals over an age range of 15–91 years (168). Although this same trend was not evident in the urinary 8-OH-dG output of infants, a gradual increase was noted over the first month postpartum that mirrored the velocity growth curve (169). Nevertheless, numerous studies have reported the accumulation of 8-OH-dG, and hence other lesions, with age both in vivo and in vitro in nuclear and mitochondrial (mt) DNA (170). Indeed, damage to mitochondria has received a great deal of interest as lesions or mutations in mtDNA could drastically alter the function this oxygen radical-producing organelle (reviewed in ref 171).

The accumulation of lesions can be explained in part by the discovery that DNA repair capability correlates with species-specific life span. Furthermore, repair activity appears to decline with age, allowing the persistence of damage and a subsequent increase in replication errors, although in the case of 8-OH-dG the numerous repair processes involved may largely compensate for such a decline and the age-related decline may only affect slowly proliferating tissues with high oxygen metabolism, such as liver (172). Hamilton et al. (173) noted age-related increases in 8-OH-dG in nuclear and mitochondrial DNA derived from a variety of rats organs, concluding this to derive from a tissue's increased sensitivity to oxidative damage, rather than decreased repair. Whereas antioxidant status is also likely to be a factor in establishing basal levels of damage, age appeared to have no effect of the activity of major antioxidant enzymes (catalase, glutathione peroxidase, Mn and CuZn superoxide dismutase; ref 173). Similarly, in a human study no differences were noted in superoxide dismutase, glutathione peroxidase, catalase and ceruloplasmin, uric acid, or bilirubin levels between the 35–39, 50–54, and 65–69 year age groups. Although in this study there did appear to be a significant difference in repair capacity of PBMC after ex vivo hydrogen peroxide challenge between the 65–69 and 35–39 year age groups (P=0.013) (174).

The significance of lesion accumulation could lie with another hypothesis, the somatic mutation theory, which states that an accumulation of DNA mutations not necessarily derived from ROS leads to degenerative senescence. However, Holliday (175) suggested that because aging is a multicausal process, DNA damage and mutation, though important, were unlikely to be responsible for all the pathophysiological changes seen. Overall these findings appear consistent with the observation that high metabolic rate equates to short maximum life span potential and faster aging; although the experimental evidence is not conclusive, the hypothesis for free radicals in aging remains compelling.

CONCLUSIONS

Demonstrating a link between defects in repair of oxidative DNA damage and a propensity for disease has not been easy. Experiments with single-gene knockout mice have been rather disappointing, with mice thus far displaying few ill effects. Combined gene knockouts such as *OGG1* and *CSB* have been more promising, with elevated tissue levels of 8-OH-dG, although a pathological consequence of this has yet to be reported. It is worth noting that whereas NER might be thought of as

a backup for glycosylases in the repair of oxidative DNA damage, the relative contribution of each repair process may vary from tissue to tissue. Such a hypothesis might support findings that suggest that defects in the NER of oxidative lesions in xeroderma pigmentosum account for the accumulation of damage and increased frequency of internal cancers and, in certain cases, neurological degeneration characteristic of this disease.

Nevertheless, it is not unreasonable to speculate that given the multiple pathways for its repair, oxidative DNA damage is likely play an important role in disease. Indeed, it seems that ROS and oxidative DNA damage are omnipresent in disease; for researchers this means there is no limit to the conditions in which oxidative stress may be studied. However, the mere presence of damage is not proof of a causative link, although given the close link between ROS formation and oxidative DNA damage and the importance of DNA damage and mutation in carcinogenesis, it is not a large leap of intuition to link oxidative DNA lesions and cancer. With this accepted, it is nevertheless difficult to account for why elevated ROS/DNA damage in other diseases does not in itself lead to malignancy. The basis of this apparent contradiction and the failure of current studies to definitively establish the significance of oxidative DNA damage in disease may lie with the numerous factors operating simultaneously in pathogenesis. It would be unrealistic for a single experiment to be expected to consider all these factors, particularly as new factors are continually being identified and the importance of existing factors reevaluated. Clearly a great deal of work remains to be completed in defining the exact roles of oxidative DNA damage in the pathogenesis of disease; with this established, it might be possible to determine how modulation of repair might be useful in disease prevention and therapy. FJ

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