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# Oxidatively Induced DNA Damage and Cancer

**Miral Dizdaroglu* and Pawel Jaruga**

*Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA*

## Abstract

Oxidatively induced DNA damage is caused by endogenous and exogenous sources in living organisms. Many resulting DNA lesions are mutagenic and lead to mutations commonly found in cancer. Repairs mechanisms exist to repair this type of DNA damage. Unrepaired and accumulated DNA lesions may lead to carcinogenesis and other disease processes. Defects in DNA repair are associated with cancer. Oxidatively induced DNA lesions accumulate in cancerous tissues, possibly contributing to genomic instability and metastatic potential. Recent evidence suggests that some tumors may even possess increased DNA repair capacity, leading to therapy resistance. DNA repair inhibitors are being developed to target the repair pathways and increase the efficacy of cancer therapy. Oxidatively induced DNA lesions and DNA repair proteins are potential biomarkers for early detection, cancer risk assessment, prognosis and monitoring the therapy. Overall, accumulated evidence suggests that oxidatively induced DNA damage and its repair are important factors in carcinogenesis, and deserve more research to understand and fight cancer.

## Introduction

Oxidative stress that generates oxygen-derived species has been implicated in the pathogenesis of a wide variety of disease processes including carcinogenesis and aging [1,2]. Oxygen-derived species including free radicals, most notably the highly reactive hydroxyl radical (•OH) cause oxidatively induced damage to DNA in living aerobic organisms. These species are formed by normal cellular metabolism and by exogenous sources such as ionizing radiations, UV radiation, redox-cycling drugs and carcinogenic compounds [3]. Hydroxyl radical reacts with DNA constituents at or near diffusion-controlled rates, causing damage to the heterocyclic bases and the sugar moiety by a variety of mechanisms. In addition, ionizing radiation-generated H atom (H•), also a free radical, and hydrated electron ($e^-_{aq}$) add to double bonds of DNA bases, leading to modifications [4]. DNA damage is encountered by cellular repair systems and can be repaired by a number of repair mechanisms [2]. If repair systems of living cells fail, oxidatively induced DNA damage may lead to mutagenesis [1,2,5], and may thus be a significant source of genomic instability, a hallmark of human cancers [6-8]. Experimental and epidemiological evidence suggests that this type of DNA damage may be a major contributor to human cancer [7].

### Mechanisms of oxidatively induced DNA damage

Hydroxyl radical reacts with DNA constituents by addition and abstraction. It adds to double bonds of heterocyclic DNA bases at diffusion-controlled rates with rate constants varying from $4.5 \times 10^9$ $M^{-1}$ $s^{-1}$ to $9 \times 10^9$ $M^{-1}$ $s^{-1}$, and abstracts an H atom from the methyl group of thymine and from each of the C–H bonds of 2'-deoxyribose with rate constants of approximately $2 \times 10^9$ $M^{-1}$ $s^{-1}$ [1,4]. Ionizing radiation-generated $e^-_{aq}$ reacts with DNA bases at diffusion-controlled rates with rate constants varying from $0.9 \times 10^{10}$ $M^{-1}$ $s^{-1}$ to $1.7 \times 10^{10}$ $M^{-1}$ $s^{-1}$, whereas the rates of H• reactions are lower and amount to 1-5 × $10^8$ $M^{-1}$ $s^{-1}$. Because of its electrophilic nature, •OH preferentially adds to the site with the highest electron density. In the case of guanine, addition of •OH to the C4-, C-5 and C-8 positions yields C4-OH–, C5-OH– and C8-OH–adduct radicals [9,10]. Adenine undergoes analogous reactions, yielding at least C4-OH– and C8-OH–adduct radicals [11]. Dehydration of C4-OH– and C5-OH–adduct radicals of adenine and guanine yields purine(–H)• radicals, which are then reduced and protonated to reconstitute adenine and guanine [1,10,12]. C8-OH–adduct radicals of purines undergo one-electron oxidation and one-electron reduction to give rise to 8-hydroxypurines [8-hydroxyadenine (8-OH-Ade) and 8-hydroxyguanine (8-OH-Gua)] and formamidopyrimidines [4,6-diamino-5-formamidopyrimidine (FapyAde) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua)], respectively. Both types of products are formed in the absence and presence of oxygen, albeit with different yields. Reducing agents favor the formation of formamidopyrimidines, whereas 8-hydroxypurines are preferentially produced by oxidizing agents [1].

Hydroxyl radical reacts with cytosine and thymine by addition to C5– and C6–positions yielding C5-OH– and C6-OH–adduct radicals, respectively, and by abstraction of an H• from the methyl group of thymine generating an allyl radical. C5-OH– and C6-OH–adduct radicals are oxidized followed by addition of water and subsequent deprotonation to yield cytosine glycol (Cyt glycol) and thymine glycol (Thy glycol) [1]. The oxidation of the allyl radical of thymine yields 5-(hydroxymethyl)uracil and 5-formyluracil. The type of the products and their yields vary depending on the absence and presence of oxygen that reacts with free radicals at diffusion-controlled rates to give peroxyl radicals. Depending on reaction conditions, cytosine products can dehydrate and deaminate, giving rise to products such as 5-hydroxycytosine (5-OH-Cyt), uracil glycol (Ura glycol) and 5-hydroxyuracil (5-OH-Ura) [13]. The formation of the products of DNA bases has been extensively studied in the past. It is beyond the scope of this paper to review all possible reactions. The reader is referred to extensive review articles published previously [1,4,14]. Clustered damage also occurs in DNA by oxidatively induced damage [15-18].

Figure 1 illustrates the structures of the major products of the DNA

***Corresponding author:** Dr. Miral Dizdaroglu, Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA, E-mail: miral@nist.gov

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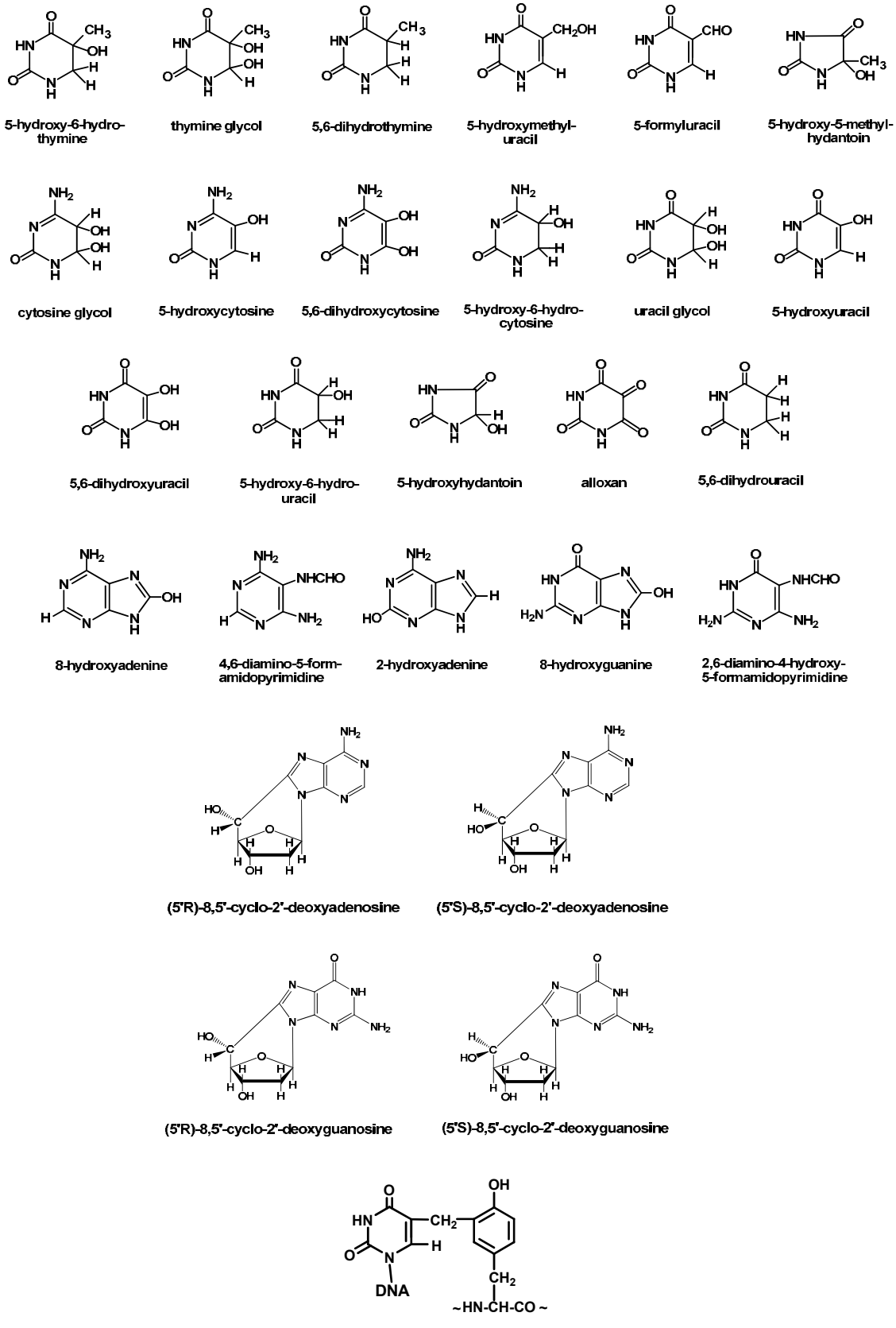


**Figure 1:** Structures of major oxidatively induced DNA base lesions and a thymine-tyrosine cross-link.

bases that have been identified in DNA *in vitro* and *in vivo*.

The sugar moiety of DNA undergoes reactions with •OH by abstraction of an H• from each of the C-atoms, leading to five different C-centered radicals. Further reactions of these 2'-deoxyribose radicals yield a number of sugar products, strand breaks and base-free sites in DNA by a variety of mechanisms [1,4]. Sugar products are either released from DNA or constitute the end groups of broken strands or remain within DNA with both phosphate linkages being intact. Figure 2 shows the structures of the products of the sugar moiety. One unique reaction of the 2'-deoxyribose radicals in purine nucleosides is the attack of the C5'-centered sugar radical at the C8-position of the purine ring within the same nucleoside in the absence of oxygen leading to C5'–C8-intramolecular cyclization [19]. The rate constants for the intramolecular cyclization amounts to $1.6 \times 10^5\ s^{-1}$ and $\sim 1 \times 10^6\ s^{-1}$ for dA and dG, respectively [20,21]. Subsequent oxidation of the N-centered radical leads to 8,5'-Cyclopurine-2'- deoxyadenosine (cdA) from 2'-deoxyadenosine and 8,5'-Cyclopurine-2'-deoxyguanosine (cdG) from 2'-deoxyguanosine. Both *R*- and *S*-diastereomers of each compound are formed. Oxygen inhibits the intramolecular cyclization [22], because it reacts with the C5'-centered radical of the sugar moiety at a near diffusion-controlled reaction with a rate constant of $\sim 1.9 \times 10^9\ M^{-1}\ s^{-1}$ [20]. 8,5'-Cyclopurine-2'-deoxynucleosides represent a concomitant damage to both the base and sugar moieties of the same nucleoside, and thus, are regarded as tandem lesions in DNA. Another unique property of cdA and cdG is that they cause an unusual puckering of the sugar moiety leading to distortion of DNA helix [23,24]. The reaction mechanisms and formation of these compounds *in vitro* and *in vivo* has extensively been reviewed [25].

In addition, •OH reactions with DNA and proteins in chromatin form covalent DNA-protein cross-links by different types of mechanisms. Thus, a thymine-tyrosine cross-link has been identified in mammalian chromatin *in vitro* and *in vivo* by exposure to free radical-generating agents such as ionizing radiation, $H_2O_2$, metal ions and carcinogenic compounds [26-28]. Other types of DNA protein cross-links have also been identified in mammalian chromatin *in vitro*. Figure 1 illustrates the structure of the thymine-tyrosine cross-link. DNA damage products can be measured in DNA by different analytical techniques. Mass spectrometric techniques are the most used ones that provide positive identification and accurate quantification of DNA products. This field has extensively been reviewed in the past by a number of authors [see, e.g., [14,29]].

In the past two decades, numerous studies have shown elevated levels of aforementioned DNA base lesions in precancerous and cancerous tissues [30-41], strongly implicating oxidatively induced DNA damage in the etiology of cancer. As a result, oxidatively induced DNA base lesions have been suggested as potential sentinels for cancer risk assessment and therapy monitoring. The elevated levels of DNA lesions do not necessarily indicate that such DNA damage is responsible for carcinogenic events. However, most of these DNA lesions are strongly mutagenic and may thus be a major contributor to carcinogenesis, as will be discussed below.

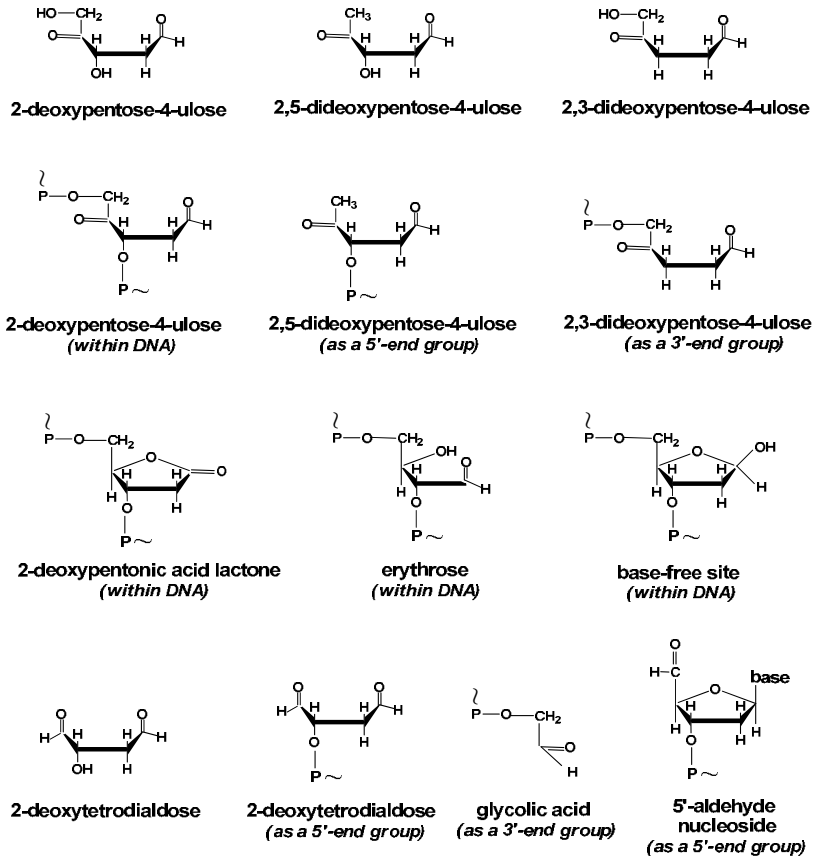


**Figure 2:** Structures of oxidatively induced lesions of the 2'-deoxyribose moiety of DNA.

## Protection of the genomic stability

DNA damage ultimately causes 80 to 90% of human cancers [42]. The genomic instability caused by DNA damage is a hallmark of cancer [8,43]. Living organisms evolved to possess elaborate mechanisms to repair DNA damage to protect the genome from DNA damage for survival. DNA repair is essential for the maintenance of the genomic stability and prevention of disease processes including carcinogenesis [2,44]. As outlined above, a plethora of products are formed in DNA *in vivo* by reactions of •OH and other free radicals. Failure to repair such DNA lesions may lead to mutagenesis, cytotoxicity, cell death and consequently to disease processes such as carcinogenesis and aging. Oxidatively induced DNA lesions are removed from DNA by two major mechanisms, base-excision repair (BER) and nucleotide-excision repair (NER) [2]. Both mechanisms involve multiple steps and enzymes to remove the lesions and subsequently restore the DNA structure. In BER, the enzymes known as DNA glycosylases remove DNA lesions by hydrolyzing the *N*-glycosidic bond between the modified base and the sugar moiety in the first step of this repair pathway, leaving behind an apurinic or apyrimidinic site (AP-site). Some DNA glycosylases are mono-functional and remove the lesion only. Others are also endowed with an associated AP-lyase activity that hydrolyzes the 3′-phosphodiester bond of the AP site by a β- or β-δ-elimination mechanism generating a 3′ α,β-unsaturated aldehyde and 5′-phosphate products, and thus strand breaks [2]. BER is highly conserved from bacteria to humans and is a multiprotein pathway, which is different from NER because of the action of diverse DNA glycosylases rather than a multiprotein complex. BER is sub-divided into two pathways, short-patch and long-patch pathways. A bi-functional glycosylase predominantly initiates short-patch BER and a mono-functional glycosylase either pathway [45]. The removal of the lesion by a glycosylase is followed by the action of AP-endonucleases, DNA polymerases and DNA ligases that process the AP-sites to fully repair DNA. In the past three decades, numerous prokaryotic and eukaryotic DNA glycosylases have been isolated and purified, and their substrate specificities and excision kinetics have been determined (reviewed in [46]).

DNA glycosylases are generally divided into two families on the basis of structure and sequence homology, the Fpg/Nei family and the Nth superfamily [47,48]. The Fpg/Nei family includes *Escherichia coli* formamidopyrimidine DNA glycosylase (Fpg, also called MutM), which specifically excises FapyAde, FapyGua and 8-OH-Gua from DNA containing multiple lesions [46,49], and *E. coli* endonuclease VIII (Nei), which is specific for the removal of pyrimidine lesions and FapyAde [46]. Another major DNA glycosylase of *E. coli*, endonuclease III (Nth) belongs to the Nth superfamily and possesses an overlapping substrate specificity with Nei, removing pyrimidine lesions and FapyAde [46]. In eukaryotes, three Fpg/Nei homologs, NEIL1, NEIL2 and NEIL3 have been discovered [50-53]. NEIL1 and NEIL3 specifically remove FapyAde and FapyGua from DNA and also some pyrimidine lesions to a lesser extent; however, they exhibit no activity for 8-OH-Gua [50,54-56]. Figure 3 illustrates a comparison of the substrate specificities of mouse NEIL1 and NEIL3 (glycosylase domain) with those of some *E.coli* glycosylases. NEIL2 exhibits no significant sequence homology to NEIL1 and is independent of cell cycle expression [52,57]. This enzyme possesses a unique preference for excision from DNA bubbles generated during transcription and/or replication, and preferentially excises 5-OH-Ura with some lower activity for 5-OH-Cyt and 5,6-dihydrouracil [52]. However, excision of any lesion by NEIL2 from DNA containing multiple lesions has not yet been reported. The Nth superfamily contains 8-hydroxyguanine-

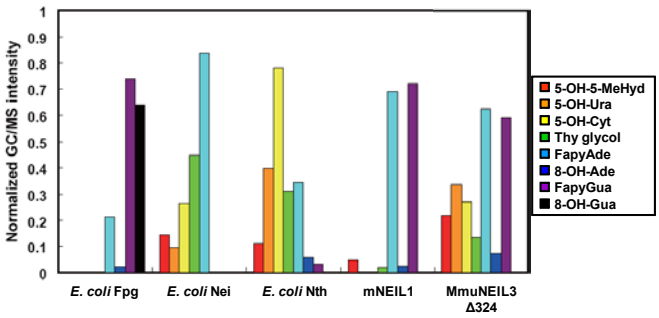


**Figure 3:** Comparison of the substrate specificities of *E. coli* DNA glycosylases with those of mouse NEIL1 and NEIL3. The normalized levels of excised lesions are shown (data from ref. [56]).

DNA glycosylase (OGG1) in eukaryotes, which is a functional homolog of Fpg, and exhibits a strong specificity for excision of FapyGua and 8-OH-Gua, but not FapyAde, unlike Fpg and NEIL1 [58,59]. Different substrate specificities of NEIL1 (mouse and human), *E. coli* Fpg and human OGG1 for purine lesions are clearly demonstrated in Figure 4.

NER is a major versatile repair mechanism for removal of bulky DNA-distorting lesions from DNA such as cyclobutane pyrimidine dimers, benzo[a]pyrene-guanine adducts and guanine-cisplatin adducts [60,61]. NER consists of global genome repair, which is responsible for the repair of the entire genome, and transcription-coupled repair, which preferentially repairs transcribing DNA strands [62-64]. Several syndromes are associated with defects in NER. For example, NER-defective xeroderma pigmentosum exhibits dramatic increase in sun-induced skin cancer. In prokaryotes and eukaryotes, an excinuclease, which is a multisubunit enzyme system, makes dual incisions in the DNA strand to remove an oligodeoxynucleotide containing the lesion. This is followed by the action of polymerases to fill the resulting gap and ligation by ligases to complete the repair. The removed oligodeoxynucleotide contains 12-13 deoxynucleotides in prokaryotes and 24-32 deoxynucleotides in eukaryotes [60,65]. Human excinuclease is also involved in the repair of other lesions that do not distort DNA helix such as AP-sites, methylated bases and mismatches [66]. Repair by NER of oxidatively induced lesions Thy glycol and 8-OH-Gua has been reported [67,68]. 8,5'-Cyclopurine-2'-deoxynucleosides are repaired by NER only, because the 8,5'-covalent bond in these molecules prevents their removal by DNA glycosylases [69-72].

Mismatch repair (MMR) is another major mechanism involved in the removal of nucleotides that are incorrectly paired with a correct nucleotide on the opposite DNA strand during replication [2]. In the case of oxidatively induced DNA damage, 8-OH-Gua mispairs with Ade during replication, forming the Ade●8-OH-Gua mismatch. In *E.coli*, Ade is removed from this mismatch by the DNA glycosylase MutY, facilitating the pairing of 8-OH-Gua with Cyt, which is then repaired by BER [73]. The human homolog of this enzyme MUTYH has been identified [74]. Both enzymes belong to the Nth superfamily. MUTYH is targeted to both the nucleus and the mitochondrion [73,75-78]. MUTYH also removes 2-OH-Ade from opposite all four intact DNA bases [78]. There is a compelling amount of data supporting the evidence that inherited mutations in the *mutyh* gene predispose individuals to colorectal cancer and somatic G→T mutations [73,79-82]. This indicates the importance of MUTYH in preventing human carcinogenesis. No other DNA base lesions in mismatches have been found to be substrates of MUTYH. FapyGua, which is another highly

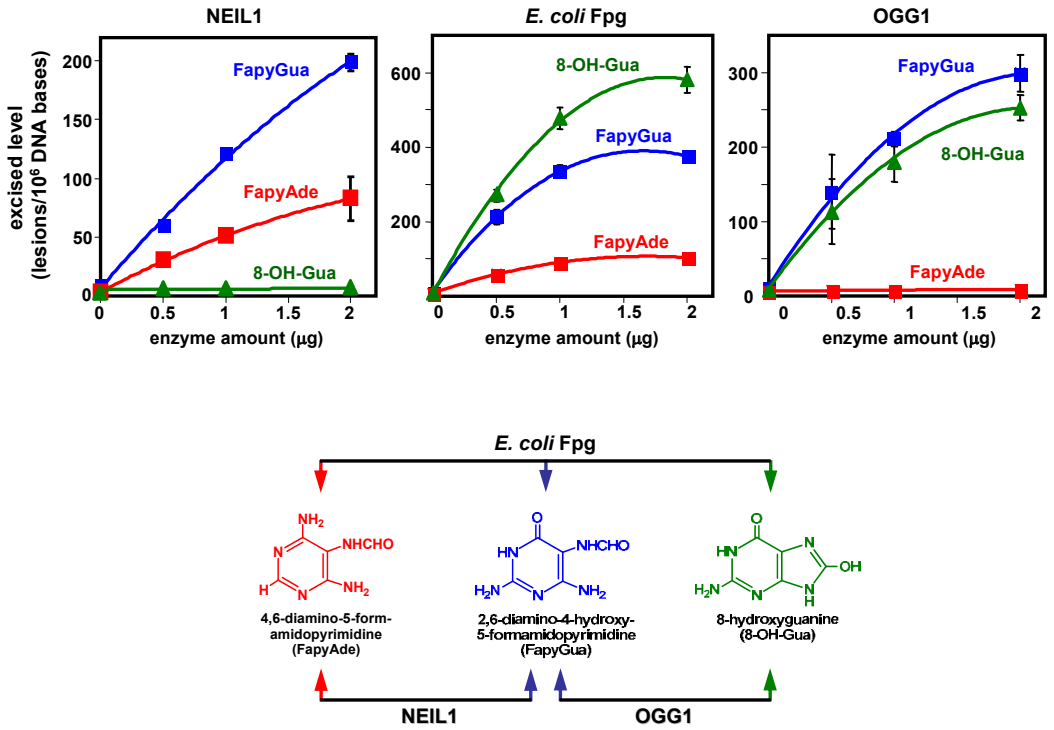


**Figure 4:** Comparison of the substrate specificities of *E. coli* Fpg, mouse NEIL1 and human OGG1. The uncertainties are standard deviations (data from refs. [49,54,58]).

mutagenic Gua lesion and gives rise to G→T mutations (see above) associated with MUTYH and colorectal cancer, should be investigated for its potential to be a substrate of MUTYH and its role in colorectal cancer.

Repair in the nucleotide pool also occurs, hydrolyzing oxidized 2'-deoxynucleoside triphosphates to monosphates thus functionally eliminating them before they can be inserted into DNA by DNA polymerases [83-85]. The MutT protein of *E. coli* hydrolyzes 8-OH-dGTP to 8-OH-dGMP and prevent its incorporation into DNA [83]. 8-OH-dGMP cannot be rephosphorylated by guanylate kinase. A mammalian homolog of this enzyme with the same function has also been discovered and named MTH1 protein [84,86]. Human MTH1 and *E. coli* MutT possess some sequence homology and similar molecular mass [87]. *Mth1*$^{-/-}$ mice have been found to be prone to spontaneous carcinogenesis with many tumors found in lungs and livers [87,88]. MTH1 also hydrolyzes 8-OH-dATP and 2-OH-dATP [89], pointing to an important role of human MTH1 protein in prevention of mutagenesis and consequently carcinogenesis. Another type of oxidatively induced damage to DNA is DNA single- and double-strand breaks, which present a threat to the genomic integrity. Single strand breaks are repaired in a fashion similar to that discussed in the case of BER. However, repair of double-strand breaks is more complex and occurs by either homologous recombination or non-homologous end-joining mechanisms [90,91].

## DNA repair and cancer

Unrepaired and accumulated DNA lesions may have detrimental consequences in living organisms. Resulting increase in mutation rate, i.e., mutator phenotype, can lead to increased genetic instability, which is a hallmark of cancer [6,8,43]. Genetic instability may affect gene expressions involved in many cellular processes such as DNA replication, DNA repair, apoptosis, etc. [92]. Single GC→AT transitions are the most frequent mutations that accumulate in human tumors [93]. 60% of cancer cell lines have somatic mutations in DNA repair genes (for example, see http://www.sanger.ac.uk/genetics/CGP). Germline mutations and polymorphisms in DNA repair genes are also linked to predisposition to cancer [2,8,43,44,94-96]. Thus, DNA repair plays a major role in carcinogenesis and many other disease processes. A variety of cancers are associated with defects in DNA repair [8,92,94-102]. Numerous studies demonstrated an association of OGG1-Cys$^{326}$, a polymorphic form of OGG1 with the risk of esophageal, colon, orolaryngeal, lung, gastric, cervical, gallbladder, head, neck, kidney and bladder cancers [103-118]. Mutant forms OGG1-His$^{154}$ and OGG1-Gln$^{46}$ have been discovered in a human gastric cancer cell line and human kidney tumors, respectively [104,119]. Moreover, low OGG1 activity has been shown to constitute a risk factor in lung, head and neck cancers [120-124]. It should be pointed out that OGG1-Cys$^{326}$, OGG1-His$^{154}$ and OGG1-Gln$^{46}$ exhibit lower activity for the excision of FapyGua and 8-OH-Gua from DNA than wild type OGG1 [58,59]. This fact may indicate a role for these mutagenic lesions in carcinogenesis.

Mammalian NEIL1 has been isolated and characterized by a number of laboratories [50,51,53-55,57,125-131]. Activation of NEIL1 depends on cell cycle. NEIL1 is expressed predominantly during the S-phase and thus likely to be associated with replication-associated and/or transcription-coupled repair [50,132,133]. Similar to *E. coli* Nei and Fpg, this enzyme contains a "zinc-less finger" motif [127]. Human NEIL1 and mouse NEIL1 possess identical substrate specificities and efficiently excise FapyAde and FapyGua from DNA containing multiple lesions with somewhat reduced activity for pyrimidine lesions and with

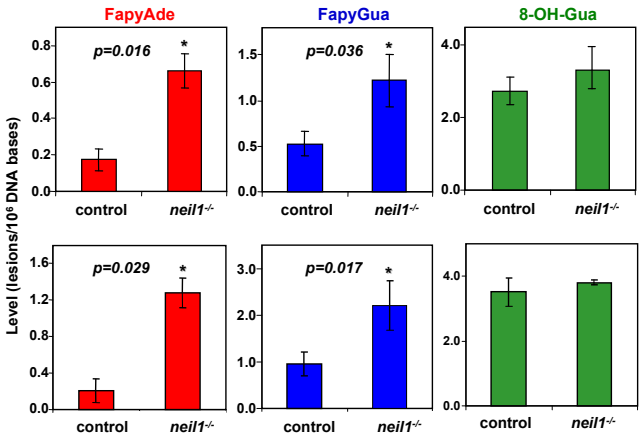


**Figure 5:** Levels of FapyAde, FapyGua and 8-OH-Gua in livers (upper) and kidneys (lower) of control and *neil1*$^{-/-}$ mice. Stars denote statistical significance ($p$<0.05). The uncertainties are standard deviations (data from ref. [137]).

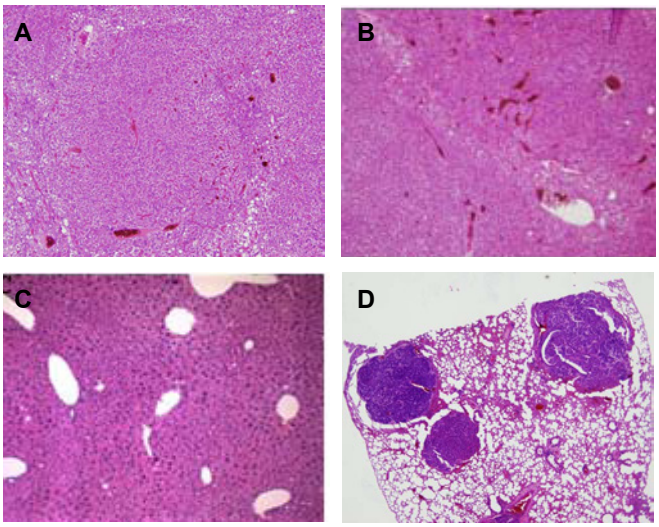


**Figure 6:** Histological sections of liver tumors, pre-malignant livers and lung tumors of knock-out mice. A: Nodular hyperplasia of hepatocytes in *neil1*$^{-/-}$ mice; B: hepatocellular carcinoma in *nth1*$^{-/-}$ mice; C: Severe hepatocellular dysplasia in *neil1*$^{-/-}$/*nth1*$^{-/-}$ mice; D: lung tumors in *neil1*$^{-/-}$/*nth1*$^{-/-}$ mice (from ref. [137]).

no activity for 8-OH-Gua unlike Fpg and OGG1 [50,54,55,131]. NEIL1 is located to both the nucleus and mitochondrion [129], suggesting the involvement of this enzyme in the overall maintenance of the genomic integrity. Four polymorphic variants of NEIL1, NEIL1-Cys$^{82}$, NEIL1-Asp$^{83}$, NEIL1-Asn$^{252}$ and NEIL1-Arg$^{136}$ have been discovered in humans [55]. NEIL1-Cys$^{82}$ and NEIL1-Asn$^{252}$ exhibited near wild type glycosylase activity for FapyAde and FapyGua. In contrast, NEIL1-Asp$^{83}$ completely lacked this activity and also failed to catalyze the wild type β,δ-elimination reaction, only exhibiting β-elimination. These findings suggest that individuals with *neil1* mutations may be at risk for disease development. Recent work pointed to a significant role of NEIL1 in the prevention of disease processes. Decreased levels of NEIL1 led to accumulation of oxidatively induced DNA damage and enhanced spontaneous mutations in human and Chinese hamster cells [133]. Downregulation of NEIL1 expression by siRNA sensitized mouse embryonic stem cells to killing effects of ionizing radiation [126]. Oxidative stress increased *neil1* mRNA levels in human carcinoma cells [134]. There is evidence for a correlation of mutations in the *neil1* gene with human gastric cancer [135]. In the absence of exogenous oxidative stress, *neil1*$^{-/-}$ mice exhibited increased levels of mitochondrial DNA damage and deletions, and symptoms of diseases associated with the metabolic syndrome, suggesting a significant role for NEIL1 in disease prevention [136]. In a subsequent study, *neil1*$^{-/-}$ mice have been shown to accumulate greater levels of FapyAde and FapyGua, but not 8-OH-Gua, in their livers, kidneys and brains than control animals [137]. Figure 5 illustrates the levels of FapyAde, FapyGua and 8-OH-Gua in livers and kidneys of control and *neil1*$^{-/-}$ mice. This finding is on a par with the substrate specificity of mouse NEIL1 and human NEIL1, which was previously observed *in vitro* using DNA containing multiple lesions, as discussed above. *Neil1*$^{-/-}$ mice developed pulmonary and hepatocellular tumors in the second half of their lives [137]. Additional knockout of the *nth1* gene in these animals significantly increased the tumor incidence, indicating an important role for NEIL1 and NTH1 in cancer prevention. Figure 6 illustrates histological sections of liver tumors, pre-malignant livers and lung tumors of *neil1*$^{-/-}$, *nth1*$^{-/-}$ and *neil1*$^{-/-}$/*nth1*$^{-/-}$ mice. The pulmonary tumors contained activating GGT→GAT transitions in codon 12 of *K-ras* of their DNA. This is in contrast to the GGT→GTT transversions of codon 12 in *K-ras* found in the pulmonary tumors of mice lacking OGG1 and MUTY [138]. The accumulation of FapyAde and FapyGua in *neil1*$^{-/-}$ mice strongly suggests a role for these compounds in carcinogenesis, which are mutagenic as discussed below.

An additional function of NEIL1 has recently been discovered. Significant accumulation of *R*-cdA and *S*-cdA has been observed in livers of *neil1*$^{-/-}$ mice, but not in *ogg1*$^{-/-}$ mice [139]. Figure 7A illustrates the levels of S-cdA in control, *neil1*$^{-/-}$ and *ogg1*$^{-/-}$ mice. Since *R*-cdA and *S*-cdA are repaired by NER, not by BER, as discussed above, this finding strongly suggests that NEIL1 plays a role in NER in addition to its role as a DNA glycosylase in BER. This work also showed the accumulation of FapyGua, but no accumulation of 8-OH-Gua, in livers of *neil1*$^{-/-}$ mice; however, *ogg1*$^{-/-}$ mice accumulated 8-OH-Gua along with FapyGua [Figure 7B,C]. These findings are on a par with those reported previously [137], and with the substrate specificity of NEIL1 [50,54,55,131]. Accumulation of 8-OH-Gua and FapyGua in livers of *ogg1*$^{-/-}$ mice is in agreement with the substrate specificity of OGG1 [58]. Mechanism of action of NEIL1 in NER is not known. In this context, Cockayne syndrome B protein has been shown to stimulate the repair

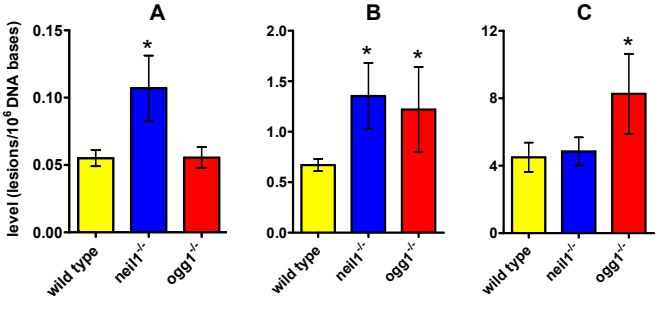


**Figure 7:** Levels of *S*-cdA (A), FapyGua (B) and 8-OH-Gua (C) in livers of control, *neil1*$^{-/-}$ and *ogg1*$^{-/-}$ mice. Stars denote statistical significance ($p$<0.05). The uncertainties are standard deviations (from ref. [139]).

of FapyAde and FapyGua by NEIL1 and play a role in NER of *S*-cdA [131,140]. This indicates that NEIL1 may interact with other proteins of the NER complex. Future studies may elucidate the mechanism of action of NEIL1 in NER.

Defective expression of DNA repair genes may affect DNA repair status of tumors and thus cause therapy resistance, and affect the outcome of cancer and survival of patients [141-143]. DNA repair may be increased in malignant tumors as recent evidence suggests. Nonsmall-cell lung cancer has been shown to exhibit resistance to chemotherapy, which is associated with elevated DNA repair in tumors [141,143]. Greater overexpression of *ogg1* mRNA for the excision of 8-OH-Gua has been observed in a number of lung cancer cell lines when compared to control lung cell lines [144]. Cancerous colon tissues exhibited lower levels of ethano-DNA adducts than non-cancerous tissues of colorectal cancer patients, indicating enhanced DNA repair in cancerous tissues [145,146]. During evolution of cancer, additional mutations resulting in an increase in genetic instability may provide cancerous cells with survival advantage. In contrast, increased mutations may also cause cell death late in tumor evolution. However, tumors that overexpress DNA repair genes may be favored by natural selection to become capable of surviving. With time, tumors may develop greater DNA repair capacity than non-cancerous tissues. Increased DNA repair in cancerous tissues may cause resistance to therapeutic agents and thus affect patient survival. These facts suggest that DNA repair capacity of tumors would be an essential factor to be determined prior to treatment. Thus far, accumulated evidence points to DNA repair proteins as important predictive, early detection, prognostic and therapeutic factors in cancer [95]. In this context, DNA repair pathways are promising drug targets for cancer treatment. DNA repair inhibitors are being developed to reduce DNA repair in cancerous tissues and thus increase the efficacy of therapy [95,96]. Selective development of DNA repair inhibitors for combination therapy or as single agents for monotherapy targeting both BER and NER pathways will be of outmost importance for future advances in cancer therapy.

## Biological effects of oxidatively induced DNA damage

Among the oxidatively induced DNA lesions, 8-OH-Gua has been investigated extensively for more than 25 years, perhaps at the expense of other lesions that may be equally important. 8-OH-Gua has been found to be strongly mutagenic and mispair with Ade leading to G→T transversions [147-150], which are the second most common somatic mutations found in human cancers, with 14.6% of all mutations in the tumor suppressor gene *TP53* following C→T transition mutations [44.2%] [151]. However, this does not mean that all G→T mutations result from 8-OH-Gua. Other Gua lesions may lead to this type of mutations such as FapyGua, another major oxidatively induced product of guanine. Albeit to a lesser extent, 8-OH-Ade is premutagenic and induces A→G transitions and A→C transversions in mammalian cells [152-154]. The other major purine lesions FapyAde and FapyGua also posses mutagenic properties. Early studies suggested that these lesions were lethal rather than mutagenic. This was based on the inference with lethal lesions *N7*-Me-FapyAde and *N7*-Me-FapyGua, which are alkylation products of Ade and Gua, respectively, [155-159], unlike FapyAde and FapyGua, the formation mechanism of which was discussed above. Recently, base-pairing properties and biological effects of FapyAde and FapyGua have definitively been established using synthetic oligodeoxynucleotides containing these compounds at a defined position. FapyAde pairs with Ade leading to A→T transversions and may be more mutagenic than 8-OH-Ade [160,161]. Similarly, Ade is misincorporated opposite FapyGua, resulting in G→T transversions [162]. This is the same mutation caused by 8-OH-Gua, as discussed above. FapyGua has been found to be 25-35% more mutagenic than 8-OH-Gua in simian kidney cells (COS-7) [161], although it is weakly mutagenic in *E. coli*, when bypassed in different sequence contexts [163]. These findings clearly proved, in contrast to the common assumption in the literature, that the well known G→T mutations may not be exclusively due to 8-OH-Gua as a result of oxidatively induced damage to DNA, and that the biological effects of FapyAde and FapyGua completely differ from those of *N7*-Me-FapyAde and *N7*-Me-FapyGua. 2-Hydroxyadenine, which is a minor product of Ade [164], is mutagenic in prokaryotic and eukaryotic cells, and pairs with Cyt leading to A→G transitions [165]. Its pairing with Ade has also been reported [166].

The major oxidatively induced product of Cyt is Cyt glycol, which yields 5-OH-Cyt by dehydration, Ura glycol by deamination and 5-OH-Ura by deamination and dehydration [13]. All these compounds may simultaneously exist in DNA [167]. Ura glycol and 5-OH-Ura pair with Ade and efficiently induce C→T transitions [5,168-170]. This is the most frequently observed mutation resulting from oxidatively induced DNA damage [171,172]. 5-OH-Cyt is less mutagenic than Ura glycol and 5-OH-Ura and leads to C→T transitions as well as C→G transversions[169,172,173]. Tandem double CC→TT mutations have also been observed due to oxidatively induced DNA damage; however, the identity of the Cyt product(s) leading to these mutations is not known [174]. Thy glycol, which is the most extensively studied pyrimidine lesion, pairs with Ade and is poorly mutagenic [5,175,176]. However, when inserted into a bypass sequence, a low level of pairing with Gua also occurs giving rise to T→C transitions [177]. Thy glycol is a strong block to most DNA polymerases *in vitro*, and thus a strongly lethal lesion *in vivo* [5,175,176,178-180]. Other pyrimidine lesions listed in Figure 1 are either weakly mutagenic or lethal lesions [1,5].

Biological consequences of 8,5′-cyclopurine-2′-deoxynucleosides have been investigated in the past decade. *S*-cdA is a strong block to DNA polymerases and reduces transcription by blocking transcription binding factor, reducing gene expression [71,72,181,182]. Furthermore, *S*-cdA causes transcriptional mutagenesis [183], which occurs when prokaryotic RNA polymerases bypass non-bulky DNA lesions such as modified DNA bases [184]. Mutant transcripts resulting from bypass of *S*-cdA by RNA polymerase II *in vivo* have been characterized [183]. Bypass of *S*-cdA by RNA polymerase II misincorporates an adenosine opposite to the next nucleotide 5'- to *S*-cdA and causes multiple nucleotide deletions [183]. *S*-cdA may give rise to neuronal death in a number of diseases such as NER-defective xeroderma pigmentosum by blocking neuronal gene expression [185]. A more recent work showed that *S*-cdG is a strong block to replication and a highly mutagenic lesion leading to G→A transitions with G→T transversions to a lesser extent, and is inefficiently repaired in *E. coli* [186]. Elevated levels of 8,5′-cyclopurine-2′-deoxynucleosides in genomic DNA *in vivo* in a number of diseases including cancer suggest that these lesions may play a role in carcinogenesis and other disease processes [25,38,187-190].

## Oxidatively induced DNA lesions and DNA repair proteins as biomarkers

The findings reviewed above suggest that DNA lesions and DNA repair proteins may serve as potential biomarkers for cancer risk assessment and monitoring of the therapy. Noninvasive biomarkers such as those found in human urine have been extensively investigated. DNA lesions excreted into urine may be used as tumor markers for diagnosis, early detection and therapy monitoring, as well as in

population-based studies such as epidemiological investigations. In terms of oxidatively induced DNA damage, early studies suggested two oxidatively modified DNA nucleosides, 8-hydroxy-2'-deoxyguanosine (8-OH-dG) and 2'-deoxythymidine glycol as suitable biological markers [191,192]. Since then, 8-OH-dG and its free base 8-OH-Gua in urine have been the mostly investigated lesions as biomarkers, albeit with some significant controversy in terms of the measurement by different analytical techniques between laboratories (reviewed in [193]). The source of these lesions in urine has also been debated for some time. In general, the possible sources of oxidatively induced DNA lesions have been considered to be diet, cell death/turnover and DNA repair [194]. Some recent work provided strong evidence that diet does not significantly contribute to the urinary levels of 8-OH-dG and 8-OH-Gua [195]. This may also be true for cell death [196,197]. BER is a likely source for the presence of 8-OH-Gua in urine [198], since this lesion is efficiently removed from DNA by OGG1 as was discussed above. However, this repair pathway cannot remove the nucleoside 8-OH-dG. It is not clear how DNA repair would remove this lesion. NER may excise 8-OH-dG and 2'-deoxythymidine glycol within an oligodeoxynucleotide as was discussed above. However, no oligodeoxynucleotides containing 8-OH-dG have been identified in human urine [196]. Further processing of such oligodeoxynucleotides by unknown mechanisms may yield 8-OH-dG. The nucleotide pool may also be a major source of 8-OH-dG in urine [199,200]. Whatever the source of 8-OH-dG or 8-OH-Gua is in human urine, these lesions appear to be potential biomarkers, especially for DNA repair activity in tumors. Other modified DNA bases such as 5-OH-Ura, 8-OH-Ade and FapyGua have also been identified in urine as potential biomarkers [201,202]; however, these lesions have thus far not received as much attention as 8-OH-dG or 8-OH-Gua. A more recent work discovered the presence of *R*-cdA and *S*-cdA as free nucleosides in human urine [203]. These lesions are subject to NER rather than BER (see above); therefore, their source in urine is likely to be NER. This suggests that *R*-cdA and *S*-cdA may serve as alternative well-suited biomarkers for cancer.

As was discussed above, DNA repair capacity is a risk factor for carcinogenesis. DNA repair pathways are promising drug targets and DNA repair inhibitors are being developed to inhibit the activity of DNA repair proteins in tumors to enhance the efficacy of cancer therapy [95,96]. All the accumulated evidence points to DNA repair proteins as early detection, prognostic and therapeutic biomarkers in cancer. For this purpose, the accurate measurement of these proteins in normal and cancerous human tissues will be of outmost importance. As a first step in this direction to positively identify and accurately quantify DNA repair proteins, we recently developed methodologies using mass spectrometric techniques with isotopically labeled analogues of these proteins as internal standards [204,205]. More efforts will have to be spent to assess DNA repair proteins as suitable biomarkers in cancer. In view of the important role of DNA repair in protection of the genomic stability, there is no doubt that these proteins will become powerful biomarkers not only for cancer, but also for other diseases.

## Conclusions

Evidence accumulated over many years points to an important role of oxidatively induced DNA damage in the etiology of cancer. This type of damage occurs in living organisms endogenously due to oxygen metabolism and by a variety of exogenous sources. The existence of various repair mechanisms and repair proteins *in vivo* for the repair of oxidatively induced DNA lesions is a strong testament to the detrimental biological effects caused by these lesions in living organisms. Many of the lesions are strongly mutagenic leading to transition and transversion mutations that are commonly found in cancer. They also accumulate significantly in cancerous tissues when compared to normal tissues, possibly contributing to genomic instability and metastatic potential. Recent evidence suggests an increase of DNA repair in some types of tumors, which may cause resistance to therapy. There is evidence that defective DNA repair, and mutations and polymorphisms in DNA repair genes contribute to carcinogenesis. Oxidatively induced DNA lesions and DNA repair enzymes are potential cancer biomarkers.

DNA repair enzymes are emerging as important early detection, prognostic and therapeutic factors in cancer. DNA repair inhibitors are being developed to increase the efficacy of cancer therapy. More research in the field of oxidatively induced genomic DNA damage and its repair may lead to development of cancer biomarkers, DNA repair inhibitors and enhancement of our capabilities to understand and fight cancer.

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