COMMENTARY

The discovery of a new family of mammalian enzymes for repair of oxidatively damaged DNA, and its physiological implications

Tapas KHazra, Tadahide Izumi, Y.Wah Kow1 and Sankar Mitra2

Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555 and 1Division of Cancer Biology, Emory University, Atlanta, GA 30335, USA

2To whom correspondence should be addressed
Email: samitra@utmb.edu

Oxidatively damaged bases in the genome are likely to be responsible for mutations leading to sporadic carcinogenesis. Two structurally similar DNA glycosylases, NTH1 and OGG1, which are able to excise most of these damaged bases, were identified previously in mammalian cells. A distinct family, consisting of two human DNA glycosylases orthologous to enzymes in Escherichia coli, has recently been characterized; they have overlapping substrate ranges with NTH1 and OGG1. The presence of multiple enzymes with potential back-up functions underscores the importance of removing both endogenously and exogenously generated oxidatively damaged bases from the genome, and may explain why no cancer or other disease phenotype has so far been linked to the deficiency of a single DNA glycosylase.

Reactive oxygen species (ROS) are produced as by-products of respiration, and also due to the inflammatory response and the metabolism of xenobiotics. ROS are genotoxic, and generate strand breaks and a plethora of damaged bases in DNA, which can be mutagenic or induce cell death (1,2). It was estimated that human cells experience at least 10 000 oxidative damage events per cell per day (3). Genomic mutations and other chromosomal changes caused by these lesions are believed to be involved in the etiology of sporadic cancer as well as other diseases, and of aging. All of these types of damage except DNA double-strand breaks are repaired via the base excision repair (BER) pathway. The critical step in the repair of damaged bases via this pathway is their excision from the DNA by a class of enzymes named DNA glycosylases; the repair is completed in several subsequent steps. These glycosylases have broad substrate specificity, and their activities are conserved from bacteria to humans (4). The major glycosylases specific for oxidatively damaged bases have an intrinsic abasic (AP) lyase activity, in which an amino group of the active site residue forms a transient Schiff base bond at the abasic site, generating a 3’ phospho α,β-unsaturated aldehyde or 3’ phosphate, respectively (5,6).

Until recently, only two mammalian DNA glycosylases specific for oxidized bases had been characterized, the Escherichia coli endonuclease III (Nth) homolog NTH1, and 8-oxoguanine-DNA glycosylase (OGG1); these share common structural motifs and reaction mechanisms with Nth, and carry out β-elimination using an internal Lys residue as the active site nucleophile (7,8). These enzymes belong to the superfamily of repair enzymes that utilize a common helix–hairpin–helix (H-h-H) DNA-binding domain (9). The Nth family is widespread in both eukaryotes and prokaryotes. NTH1 recognizes a wide spectrum of oxidation products of pyrimidines such as thymine glycol, 5-hydroxyuracil and 5-hydroxycytosine; OGG1 recognizes predominantly the oxidation products of purines, such as 8-oxoguanine and formamidopurimidines (Table I). However, E.coli has two other oxidized base-specific DNA glycosylases, namely, MutM, and its paralog Nei; these enzymes utilize N-terminal Pro as the active site nucleophile, and carry out β-elimination reactions (4,10). In contrast to the Nth superfamily, the MutM family of repair enzymes utilizes two DNA-binding motifs, the helix-two turns-helix (H-2T-H) and a zinc finger (11). Structural orthologs of Nei/MutM are absent in yeast and Drosophila, and were not identified in mammals.

In view of the mutagenic and toxic consequences of oxidative damage to bases in the DNA, and their pervasive nature, it is expected that deficiency in their repair should have significant pathophysiological effects. However, mouse mutants lacking either NTH1 or OGG1 have no obvious phenotype, in spite of accumulation of mutagenic and toxic base lesions in their genomes (12,13). This paradox could be explained at least partially by the subsequent discovery that, in cells lacking OGG1, 8-oxoguanine is repaired quite efficiently from the DNA sequences, which are transcribed. These results thus suggested a general phenomenon that additional DNA glycosylases are present in mammalian cells, specific for repair of transcribed genes, which, unlike repair of the bulk of the genome, is critical for proper cellular functioning and survival (14). The dichotomy in repair of the bulk of the genome versus repair of transcribed sequences was recognized first in the nucleotide excision repair process and named global genome repair (GGR) and transcription-coupled repair (TCR), respectively (15). Three orthologs of E.coli MutM/Nei have recently been identified in the human and mouse genomic databases (16,17). The proteins encoded by these genes, originally named NEHs and now called NEILs (17; Nei-like), have distinct sizes, and are located on different human and mouse chromosomes (17,18). Interestingly, only NEIL2 but not NEIL1 has a Zn finger motif (17; T.Hazra, unpublished). However, both recombinant NEIL1 and NEIL2 have robust activity for excising damaged bases, but show significant differences in their expression in human tissues and cells, and in substrate

Abbreviations: DHU, 5,6 dihydro uracil; Fapy, formamidopurimidine; 5-OHC, 5-hydroxycytosine, 5-OHU, 5-hydroxyuracil; 8-oxoG, 7,8 dihydro 8-oxoguanine; ROS, reactive oxygen species; TCR, transcription-coupled repair; TG, thymine glycol.
Table I. Substrate specificity of human oxidatively damaged base-specific DNA glycosylases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Preferred lesions</th>
<th>Comments</th>
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<tbody>
<tr>
<td>NTH1</td>
<td>5-OHU, 5-OHC, DGU and Fapy G</td>
<td>Probably the major glycosylase that recognizes oxidized pyrimidines in most tissues. Possibly involved in both genomic and transcription-coupled repair.</td>
</tr>
<tr>
<td>OGG1</td>
<td>8-OxoG, Fapy G; prefers lesion opposite C</td>
<td>Probably the major repair enzyme that recognizes oxidized. Primarily purines in most tissues involved in genomic repair.</td>
</tr>
<tr>
<td>NEIL1</td>
<td>5-OHU, 5-OHC, DGU, TG, DHT, 8-oxoG, Fapy G and Fapy A</td>
<td>Expression level tissue-specific. Highly expressed during S phase. Specific for cytosine-derived lesions.</td>
</tr>
<tr>
<td>NEIL2</td>
<td>5-OHU and 5-OHC</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DHU, 5,6 dihydro uracil; Fapy, formamidinopyrimidine; 5-OHC, 5-hydroxycytosine; 5-OHU, 5-hydroxyuracil; TG, thymine glycol; 8-oxoG, 7,8 dihydro 8-oxoguanine.

Preference. For example, the highest level of NEIL1 mRNA was observed in the liver, with low expression in muscle and testis. In contrast, NEIL2 expression was the highest in the testis and muscle, and lower in other organs. More interestingly, NEIL1 expression in primary human fibroblasts is strongly dependent on the S-phase, whereas NEIL2 expression appears to be independent of the cell cycle (17,18). Both enzymes prefer ROS-derived lesions of pyrimidines, although, like its E.coli counterpart Nei, NEIL1 does excise 8-oxoguanine from oligonucleotides. Surprisingly, NEIL2 appears to have a rather limited substrate range, preferring only cytosine-derived lesions, with 5-hydroxyuracil as the preferred substrate (18). The substrate preferences of the cloned oxidized base-specific DNA glycosylases are summarized in Table I.

While we are currently characterizing the enzymatic activity of the third candidate glycosylase (NEIL3), the results obtained so far on NEIL1 and NEIL2 highlight several interesting aspects of the repair process for oxidized bases: (i) Redundancy of base excision enzymes. Redundancy seems to be quite common in the repair of base lesions via the BER pathway. Unlike the repair of bulky adducts via the nucleotide excision repair pathway, which involves the same enzymes for distinct lesions, multiple enzymes have evolved which specifically recognize, and excise oxidized bases, presumably because of their abundance and spontaneous formation. Even for repair of U, one of the most abundant DNA base lesions generated due to deamination of C or misincorporation from the deoxy-nucleotide pool, multiple enzymes including UNG, TDG and SMUG1, are present in mammalian cells (19). (ii) Presumptive role of OGG1 and NTH1 in repair of the bulk genome. The bulk of the nuclear genome contains transcriptionally inactive sequences, and hence could tolerate the accumulation of damaged bases without any significant pathological consequence, as long as the transcribed sequences are repaired efficiently. Thus, the deficiency of OGG1 or NTH1 may not induce an obvious phenotype if these enzymes are primarily involved in GGR while other glycosylases initiate TCR. The phenomenon of TCR for oxidized bases has already been documented in cellular studies (20–22). However, it is possible that under certain conditions OGG1 and NTH1 could be involved in TCR as well. On the other hand, the NEILs could be involved in TCR and not GGR, particularly in the presence of OGG1 or NTH1. (iii) Base excision coupled to DNA replication. Either incorporation of mutagenic damaged bases or misincorporation of normal bases opposite unrepaird base lesions warrants repair in concert with DNA replication in order to maintain genetic fidelity. NEIL1, with its S-phase specific activation, may be involved in such replication-associated repair. NEIL1 avidly excises both Fapy A and Fapy G (ROS-induced ring-opened products of the purines in DNA), and is the only mammalian enzyme identified so far with this activity (17). The Fapy, generated at a comparable level as 8-oxoG, are highly mutagenic, and could block replication (23,24). The specificity of NEIL2 for ROS-induced products of C namely, 5-OHU, 5-OHC and possibly uracil glycol, could be rationalized by the fact that these lesions are also highly abundant, and invariably mutagenic because of their preference to pair with A (25). In fact, ROS-induced GC→AT mutations, which are generated at a high frequency, could be explained by misreplication of C-induced lesions in DNA. (iv) Tissue specificity and compensatory expression of BER enzymes. It is interesting to note that OGG1 is poorly expressed in brain tissues while NEIL1 is highly expressed. Similarly, NEIL1 and OGG1 are poorly expressed and NEIL2 highly expressed in testes and skeletal muscles (17,18). It is therefore probable that any phenotype associated with the lack of these oxidative repair enzymes will be highly tissue-specific. However, these enzymes could also serve as pinch hitters by stepping in as back-up enzymes in the event of deficiency of the primary glycosylases. (v) Existence of yet undiscovered DNA glycosylases. Identification of the new family of DNA glycosylases was a direct dividend of the Human Genome Project. Whereas the activity of NEIL3 has yet to be demonstrated, it is highly probable that other mammalian DNA glycosylases are left to be discovered, but are unlikely to be identified or cloned on the basis of their activity in cell extracts. For example, we had characterized a second 8-oxoG-DNA glycosylase (OGG2) from HeLa cell extract, which, based on size and β lyase activity, is unlikely to be identical to one of the NEILs (26); however, we have not been able to clone its cDNA because the enzyme was inactivated during extensive purification (T.Hazra et al., unpublished experiment). In fact, nearly all of the human DNA glycosylases were cloned, not on the basis of their amino acid sequence of purified enzymes, but on the basis of functional complementation or sequence homology with E.coli or yeast homologs. Two distinct thymine glycol excision activities have recently been separated from the liver extract of NTH1-null mice (13). Neither of these enzymes appears to be identical to NEIL1 or NEIL2 (27). Sequence homology searches of genomic databases could identify genes encoding these and other undiscovered glycosylases. Elucidating the functions of these DNA glycosylases will necessitate production of mutant mice lacking these enzymes, and examination of their phenotypes. It is possible that in some cases the pathophysiological changes will be observed only after inactivation of multiple genes. However, some enzymes (like NEIL1, which appears to be linked to DNA replication), may be critical for cellular survival, and hence
its deficiency should cause a strong phenotype. This possibility is supported by the observation that the loss of the long arm of chromosome 15, in which NEIL1 is located, is observed in many types of cancer (16). In general, the redundancy of excision enzymes, particularly for oxidatively damaged bases, may have a teleological basis in ensuring protection of the genome from the most insidious genotoxic agent, namely, free radicals. In fact, lesion bypass DNA polymerases, e.g. DNA polymerase <i>eta</i>, which incorporates C opposite 8-oxoG may provide the last line of defence against mutations induced by 8-oxoG (28). In any event, the multiplicity of DNA glycosylases with overlapping substrate ranges could explain why, unlike the etiologic linkage of cancer to deoxyribonucleotide excision repair processes, where there is no redundancy, deficiency of the base excision enzymes examined so far has not been linked to susceptibility to cancer or other pathophysiological states.

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