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Overview of Base Excision Repair Biochemistry

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COPING WITH ENDOGENOUS DNA DAMAGE

Many forms of endogenous DNA damage are generated spontaneously, in some cases at high frequency (Table 1). Base modifications are perhaps the most common type of endogenous DNA damage, accounting for thousands of lesions per mammalian genome per day (1). Altogether, frequent DNA alterations encompass alkylative and oxidative base products, abasic sites, strand breaks, and misincorporated nucleotides (Table 1). Each of these lesions can alter the base-pairing property of the genetic material, and thus, can be mutagenic and potentially carcinogenic. For instance, abasic sites, which lack the instructional information of the base moiety, are non-coding lesions and impact the local structure of DNA, destabilizing the duplex thermodynamically relative to the corresponding undamaged parent DNA duplex (2). Hydrolytic deamination of C or m⁵C generates either a U:G or T:G mismatch, respectively, which has the potential to be mutagenic if not repaired before replication. Since DNA of all aerobic organisms is also continually exposed to reactive oxygen species (ROS) produced by normal cellular metabolism, purine nucleotides frequently undergo oxidation of the ring atoms. This chemical modification can lead to the formation of guanine derivatives, namely 8-oxo-7,8-dihydroguanine (8-oxoG), which can induce G to T transversions, a common somatic mutation found in human cancers (3, 4). The 8-oxoG lesion is also prone to further oxidation under physiological conditions to yield several mutagenic products, such as cyanuric acid, oxaluric acid, and oxazolone (5–7). Thus, endogenous DNA damage must be corrected efficiently by DNA repair mechanisms in order to maintain genome integrity and a low rate of mutation frequency, since these outcomes are highly relevant to disease initiation and progression in humans.

The base excision repair (BER) pathway likely evolved to cope with the high level of spontaneous decay products that are formed in DNA, as well as those damages created upon reactions with natural endogenous chemicals, most notably ROS. BER predominantly deals with non-bulky small nucleobase lesions, excising and replacing incorrect (*e.g.* uracil) or damaged (*e.g.* 3-methyladenine, 8-oxoG) bases derived from deamination, alkylation or oxidation. As will be touched upon in more detail below, there are several variations in the BER theme, yet each pathway shares a set of common elements and generally invokes the five following steps:

1. Recognition and removal of an incorrect or damaged substrate base by a DNA glycosylase to create an abasic site intermediate
2. Abasic site incision by an apurinic/apyrimidinic (AP) endonuclease or AP lyase
3. Removal of the remaining sugar fragment by a lyase or phosphodiesterase
4. Gap filling by a DNA polymerase

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5. Sealing of the nick by a DNA ligase

We next describe the major biochemical steps of the BER pathways (Figure 1).

ENZYMATIC STEPS OF BER

Base Removal

The first enzymatic step in BER is typically excision of a substrate base from duplex DNA by a DNA glycosylase, enzymes that catalyze the cleavage of the *N*-glycosidic bond between the substrate base and the 2'-deoxyribose creating an abasic site (8–11). There are several DNA glycosylases (Table 2), and all possess binding pockets that selectively accommodate a set of target base modifications (8–11). To locate and facilitate recognition, DNA glycosylases appear to gently pinch the DNA while scanning, ultimately bending DNA at the position of the damaged base to create a widened and flattened minor groove. This localized DNA distortion promotes the damaged base to flip out of the double helix and enter the binding site of the enzyme for surveying and protein-substrate complex formation. This event, known as 'base flipping', is an integral step in the enzymatic process of all DNA glycosylases.

DNA glycosylases are frequently classified as either mono-functional or bi-functional based on their catalytic mechanism and ability to execute AP lyase strand cleavage activity (12, 13). The mono-functional glycosylases use a water molecule as a nucleophile to attack the aromatic carbon of the damaged base to promote base release, generating an abasic site product that is identical to that formed upon spontaneous DNA depurination or depyrimidination. This abasic site is a substrate for an AP endonuclease (see next section). The bi-functional glycosylases use an active site amine moiety as a nucleophile to excise the damaged base, and generate a covalent Schiff base protein-DNA intermediate during the catalytic process. This class of DNA glycosylases not only exhibits base excision activity, but also harbors the capacity to incise at the abasic site to create a single-strand break (SSB) with a non-conventional 3'-terminus. Cleavage of the phosphodiester backbone of DNA by a bi-functional glycosylase occurs within the phosphodiester linkage 3' to the AP site either by β -elimination or by two consecutive elimination steps (β,δ -elimination). Either of these incision events creates a DNA nick with a 3'-blocking residue, a phosphor- α,β -unsaturated aldehyde (PUA) or phosphate (PO_4), respectively, which requires further processing by a specific enzyme to provide a suitable substrate for a DNA polymerase (see section entitled "*Termini Clean-up*"). Mammals have evolved a panel of DNA glycosylases that target specific base modifications in DNA, although they often share overlapping specificity (Table 2).

Uracil is one of the most frequent lesions found in DNA, arising either by spontaneous hydrolytic deamination of cytosine (1, 14) or by misincorporation of uracil during replication (15). Uracil preferentially pairs with adenine during replication, so deamination of cytosine can lead to C:G to T:A transition mutations. Uracil is generally detected and removed by a member of the uracil DNA glycosylase (UDG) superfamily (16, 17). UDG superfamily proteins are mono-functional DNA glycosylases and four different family members have been identified in humans: uracil DNA *N*-glycosylase (UNG), thymine DNA glycosylase (TDG), single-strand-selective mono-functional uracil-DNA glycosylase 1 (SMUG1), and methyl-CpG-binding domain 4 (MBD4). UDG family members can catalyze the removal of uracil from single-stranded DNA, as well as from double-stranded DNA when positioned opposite G, A, or a CpG sequence. UNG is one of the most well characterized UDG superfamily members, and specifically recognizes and excises uracil via the formation of a precise hydrogen bonding network between active site residues and the uracil base itself (18). Specifically, Leu272 of UNG intercalates into the DNA duplex and

replaces the flipped-out uracil within the base stack (19). Backbone amide nitrogen atoms from Gln144, Asp145, and Phe158 donate hydrogen bonds to uracil, and the side chains of Asn204 and His268 interact with the excised base. These interactions allow for uracil-specific recognition by UNG and protein-DNA complex formation. Tyr147 blocks entrance of thymine, because the methyl group of the base would clash with the amino acid side chain. The side chain of Asn204 forms hydrogen bonds with the O4 and N3 positions of uracil to exclude binding of cytosine. UNG has a remarkably high turnover number, capable of catalyzing the removal of 1000 uracil residues from DNA every minute (20). Like other mono-functional DNA glycosylases, the mechanism for uracil excision by UNG involves a nucleophilic attack by an activated water or a hydroxyl ion, and leaves behind an abasic site product (21).

Natural intracellular compounds, environmental chemicals, and certain types of chemotherapeutic agents can modify DNA bases through the addition of a methyl or alkyl group (alkylation). For instance, the biological methyl group donor S-adenosylmethionine (SAM) can react with DNA to produce alkylated bases, such as 3-methyladenine (3-MeA), at a rate of several hundred per day per human genome (Table 1). Since it blocks replication, 3-MeA is the major cytotoxic alkylative base damage. In humans, only one DNA glycosylase has been identified that is dedicated to catalyzing the removal of alkylated base lesions, *i.e.* the mono-functional alkyladenine/methylpurine DNA glycosylase (AAG/MPG) (22, 23). In addition to excising 3-MeA, this glycosylase can release base modifications such as 7-methyladenine (7-MeA), 3-methylguanine (3-MeG), 7-methylguanine (7-MeG), N⁶-methyladenine (m⁶A), and 1,N⁶-ethenoadenine (24–26). AAG/MPG specifically binds to DNA containing a target damaged base by inserting a β -hairpin loop into the minor groove of DNA (27). The flipped-out substrate base is then stacked between Tyr127, His136 and Tyr159 of AAG/MPG, while Tyr162 intercalates into the vacated gap between the 3' and 5' bases. AAG/MPG appears to use an acidic amino acid to protonate neutral bases, which are then excised upon activation of a water nucleophile using a second basic amino acid residue (27).

Nucleic acid bases are susceptible to numerous modifications by a wide variety of chemical agents. Most notably, several types of ROS, such as singlet oxygen (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), are generated as byproducts of normal oxidative metabolism or by ionizing radiation exposure (*e.g.* X-rays and γ -rays). ROS can induce oxidative modification of DNA bases, producing lesions such as the ring-opened formamido-pyrimidine derivatives of guanine and adenine (faPyG and faPyA), 8-oxoG, formyluracil (fU), dihydroxyuracil (DHU), and thymine glycol (Tg) (28–30). There are six glycosylases in humans that repair mainly oxidized DNA lesions: MutY homolog (MYH), 8-oxoguanine glycosylase 1 (OGG1), endonuclease three homolog 1 (NTH1), and Nei endonuclease VIII-like 1, 2, and 3 (NEIL1, NEIL2, and NEIL3). MYH catalyzes the excision of adenine opposite guanine or 8-oxoG, the latter of which arises upon erroneous replication of DNA containing 8-oxoG, and has a controversial or weak AP lyase activity (31–36). NTH1 and OGG1 are bi-functional DNA glycosylases specific for oxidized bases such as Tg and 8-oxoG, respectively, and exhibit β -elimination AP lyase activity (36, 37). OGG1 cleaves the *N*-glycosidic bond using a conserved active site lysine residue that initiates nucleophilic attack at C1 of the target nucleotide and forms a covalent protein-DNA intermediate. The subsequent general base-catalyzed abstraction of the C2 hydrogen then promotes β -elimination of the DNA strand 3' to the AP site. The NEIL glycosylase family can efficiently remove oxidized pyrimidines, faPyA, faPyG, hydroxyuracil, and urea from DNA (8, 36, 38–40). BER initiated by NEIL1 or NEIL2 (Figure 1) typically involves β , δ -elimination cleavage at the AP site to produce a one base gap with a 3'- PO_4 , which is not an effective substrate for DNA polymerases or the AP endonuclease in higher eukaryotes (41).

In mammals, this problem is solved through the activity of polynucleotide kinase/phosphatase (PNKP, see section '*Termini Clean-up*').

AP Site Incision

After base removal by a DNA glycosylase, an AP endonuclease incises the DNA backbone immediately 5' to the abasic site to generate a strand break with a priming 3'-OH group and a non-conventional 5'-deoxyribose phosphate (dRP). The major AP endonuclease in mammals is APE1, which accounts for more than 95% of the total cellular AP site incision activity (42, 43). APE1 executes an acid-base catalytic reaction to incise the phosphodiester bond (P-O) of DNA, and this hydrolytic reaction mechanism is promoted by the metal ion Mg^{2+} (44). Mg^{2+} is thought to stabilize the developing charge on the phosphate oxygen atoms, strategically positioning them for the subsequent nucleophilic attack by an active site-generated hydroxyl radical. There are several amino acid residues that are required for full APE1 nuclease activity. For instance, the catalytic reaction of APE1 is mediated through a triad structure involving Asp283-His309-H₂O (44). Asp283 and His309 are active site residues that form a hydrogen bond that has been postulated to allow the histidine residue to function as the general base in the reaction, accepting a proton from water to generate the reactive hydroxyl nucleophile (45, 46). Glu96 and Asp210 are also critical for the catalytic reaction of APE1. Indeed, double mutation of these residues results in a variant protein that tightly binds to abasic DNA, while abolishing AP site incision activity (47). Arg177 can penetrate the major groove of the AP site containing DNA and donate a hydrogen bond to the AP site 3'-PO₄ (46), and as a result, the interaction of Arg177 slows APE1 dissociation from the cleaved DNA intermediate. Consistently, an Arg177Ala substitution enhances the AP endonuclease activity of the mutant protein relative to the wild type APE1 protein (~25%). This observation prompted the conclusion that human BER enzymes have evolved for the effectiveness of the pathway, *i.e.* remaining bound to the product to facilitate "hand-off" to the next protein, in a mechanism referred to as "passing-the-baton" (46, 48).

There are several additional DNA processing functions of APE1 besides its primary AP endonuclease activity. For instance, APE1 is capable of catalyzing the removal of 3'-blocking ends generated by bi-functional DNA glycosylases, normal aerobic metabolism, ionizing radiation, and some anticancer drugs (see section '*Termini Clean-up*'). In addition, a 3' to 5' exonuclease activity of APE1 may serve as an autonomous proofreading function to remove mismatched or damaged nucleotides during the synthesis step of BER (49, 50). The mammalian APE1 protein has also acquired a unique N-terminal extension that appears to have imparted a function distinct from its roles in direct DNA processing, *i.e.* its so-called Ref-1 (redox effector factor 1) activity. In particular, the N-terminus of APE1 regulates the DNA binding potential of several important transcription factors, including activator protein 1 (AP-1), hypoxia-inducible factor 1 α (HIF-1 α), nuclear factor κ B (NF- κ B), cAMP response element-binding factor (CREB), and p53, by providing an enriched reducing environment for transcription factor activation (51). However, the molecular mechanism(s) of the redox regulation by APE1 is still unclear.

Termini Clean-up

In addition to the DNA strand break intermediates generated by either bi-functional glycosylases or the AP endonuclease, ROS or direct action of physical agents such as ionizing radiation can create non-conventional 5'- or 3'- termini (*i.e.* 5'- or 3'-blocking ends). Terminal blocking groups include 5'-dRP, 5'-OH, 3'-PO₄, 3'-phosphoglycolate (3'-PG), and 3'-PUA. Such termini are refractory to DNA polymerase repair synthesis or nick ligation, and thus, must be 'cleaned' for the final steps of repair. In mammals, specialized proteins have evolved to remove these non-conventional ends (52). For instance, in humans, 5'-dRP removal is primarily carried out by DNA polymerase β (Pol β), which has two

distinct enzymatic activities: a polymerase activity and a dRP lyase activity (53, 54). Using its intrinsic dRP excision activity, Pol β can remove the blocking moiety to create the required 5'-PO $_4$ for DNA ligase nick sealing. DNA polymerase λ (Pol λ) and DNA polymerase ι (Pol ι) also harbor 5'-dRPase activity capable of removing 5'-dRP lesions following APE1 incision (55, 56). APE1, besides being the predominant mammalian AP endonuclease, exhibits 3'-phosphodiesterase activity that can remove 3'-PG residues to create 3'-OH priming ends needed for DNA repair synthesis (57–59). APE1 also removes 3'-PO $_4$ groups, yet via a relatively weak 3'-phosphatase activity. In most cases, PNKP is the primary enzyme for removing 3'-PO $_4$ blocking groups. PNKP is a bi-functional DNA repair enzyme, fusing both a DNA kinase and phosphatase domain, and is responsible for preparing nicked DNA for ligation (60). In particular, the 3'-phosphatase activity hydrolyzes a 3'-PO $_4$ to generate a 3'-OH end, and the 5'-kinase activity phosphorylates a 5'-OH end to generate a 5'-PO $_4$.

Repair Synthesis

To replace the excised nucleotide, organisms utilize DNA polymerases to execute repair synthesis. A DNA polymerase requires a 3'-OH terminus on the primer strand, and ideally an unmodified template to incorporate the correct complementary deoxynucleotide(s) (53). Many DNA polymerases also have 3' to 5' exonuclease (proofreading) activity that removes non-complementary or altered nucleotides immediately after phosphodiester bond formation and before the addition of another nucleotide (53). Pol β is the main human DNA polymerase that operates on short nucleotide gaps, such as those that arise during short-patch BER (SP-BER) (54). Pol λ in certain circumstances can compensate for the absence of Pol β (61, 62). However, Pol λ is less efficient at binding to nicked DNA, even though this enzyme can incorporate a nucleotide into a one-nucleotide gap. The Pol β SP-BER pathway typically involves the incorporation of only a single nucleotide (Figure 1, left).

In addition to SP-BER, the long-patch BER (LP-BER) pathway incorporates multiple nucleotides ranging from 2 to 12 during the repair synthesis step (9, 63–65). During LP-BER, DNA synthesis is thought to be mediated by Pol δ and Pol ϵ in conjunction with the accessory 'clamp' protein, proliferating cell nuclear antigen (PCNA). These polymerases perform 'strand displacement synthesis,' where the downstream 5' DNA end is 'displaced' to form a flap intermediate. The displaced strand is then removed by a structure-specific nuclease, primarily flap endonuclease 1 (FEN1), to create a ligatable substrate. FEN1 shows a strong preference for a 'long' 5' flap adjacent to a 1-nt 3' flap in mammals (66, 67). Pol β can also carry out strand-displacement synthesis, and therefore can potentially participate in either SP- or LP-BER (54).

The choice between the SP- and LP-BER sub-pathway appears to be influenced, at least in part, by the relative ATP concentration after the dRP removal step (68). For example, if the ATP concentration is high, the pathway reaction is likely to proceed immediately to ligation by DNA ligase III α (LIGIII α). Conversely, LP-BER will occur more frequently at low ATP concentration, where ligation is less favored. Other factors may also influence the preference between SP- and LP-BER, such as the type of initiating lesion, protein-protein interactions, the cell cycle stage, or whether the cell is terminally differentiated or actively dividing (64). Most notably, some lesions, such as C1'-oxidized abasic lesion 2-deoxyribonolactone, are resistant to Pol β lyase activity and therefore must be processed by LP-BER (69). The atypical involvement of error-prone translesion DNA polymerases, such as Pol η , Pol ι , and Pol κ , during the BER synthesis step can increase the frequency of misincorporation on undamaged DNA (70). Should these polymerases be recruited to the site of a BER lesion, they form a dead-end Schiff base complex with the dRP moiety in DNA, preventing correct nucleotide insertion by the polymerase and creating a non-conventional 5'-terminal block, which requires processing (52).

The so-called SSB repair (SSBR) pathway, now considered more of a specialized sub-pathway of BER, invokes many of the same proteins as BER, including APE1, Pol β , LIGIII α , along with the nick sensor poly(ADP-ribose)polymerase 1 (PARP1) and the scaffold protein X-ray cross-complementation group 1 (XRCC1) (71, 72). PARP1 and XRCC1 are needed to stabilize DNA strand breaks until repair can take place to prepare ends for gap-filling synthesis and nick ligation. In this case, activation of PARP1 by poly-(ADP)ribosylation helps facilitate the recruitment of XRCC1 protein. PARP1 also promotes the repair of 8-oxoG in Pol β -deficient cells by LP-BER (73).

Nick Sealing

The final step of any DNA repair pathway is to seal the lingering DNA strand break or nick. The responsibility of DNA ligases is to generate a covalent phosphodiester bond between the 3'-OH end of the upstream nucleotide and the 5'-PO $_4$ end of the downstream nucleotide (74). DNA ligases utilize the energy of phosphoanhydride hydrolysis to catalyze phosphodiester bond formation, in either an ATP-dependent or NAD $^{+}$ -dependent manner. BER is specifically completed by DNA ligase I (LIGI) or the LIGIII α /XRCC1 complex, both of which join two correctly processed ends to restore the original, unmodified DNA state (74). LIGI, which is an ATP-dependent enzyme, is the main DNA ligase that participates in chromosomal replication and LP-BER (Figure 1, right). LIGI encircles DNA and interacts with the minor groove to ensure compatible Watson-Crick base pairs. LIGIII α is also an ATP-dependent enzyme, but is primarily involved in the SP-BER pathway (Figure 1, left). LIGIII α requires XRCC1, a non-enzymatic scaffold protein, for protein stability and full activity (75, 76).

ROLES OF BER PROTEINS IN DNA-DAMAGING AGENT RESISTANCE

In this section of the review, we will highlight recent studies that indicate a role for BER proteins in resistance to DNA-damaging agents, with a particular focus on DNA-interactive drugs used in anticancer therapy. Cancer cells can continuously divide and each division requires *i*) replication of the DNA (S-phase) and *ii*) the transcription and translation of the many genes needed for continued growth. Therefore, chemicals that interfere with the mechanisms that regulate replicative capacity have the potential to inhibit cell division and eventually delay the proliferation and spread of cancer cells. For this reason, many chemotherapeutic agents (*e.g.* alkylators, cross-linking agents, intercalating agents, topoisomerase inhibitors, DNA cleaving agents, and certain antimetabolites) along with radiotherapy used to eradicate or manage neoplastic disease induce the formation of complex DNA lesions/intermediates that ultimately block multiplication of rapidly dividing cells, such as cancer cells, and activate cell death responses (77, 78).

As described above, BER removes a wide spectrum of DNA lesions, such as those caused by alkylators (*e.g.* 7-MeG, 3-MeA, and AP sites), as well as DNA modifications caused by ionizing radiation and radiomimetic drugs like bleomycin (*e.g.* 3'-PG or -PO $_4$ containing strand breaks). As will be described next, recent evidence has implicated BER capacity as an important factor in determining the therapeutic response to clinical DNA-damaging agents (Table 3). Thus, many current efforts are focused on either strategic down-regulation of DNA repair (*e.g.* inhibition) or creating a targeted imbalance in the repair pathway as a mechanism for cancer cell sensitization during treatment paradigms involving chemotherapeutic agents.

DNA glycosylases

Several studies clearly indicate that DNA glycosylase repair status has the ability to influence anticancer agent resistance. For example, mouse embryonic stem (ES) cell lines

depleted via RNA interference (RNAi) for NEIL1 are hypersensitive to low levels of γ -irradiation (38). Recent studies have also shown that the overexpression of SMUG1 prevents 5-fluorouracil (5-FU)-mediated cell killing by removing the base analog from DNA (79). The OGG1 DNA glycosylase can protect hematopoietic cells from N,N',N"-triethylenethiophosphoramidate (ThioTEPA)-induced DNA damage (80). *Aag/mpg* null mouse ES cells exhibit a pronounced hypersensitivity to carmustine (*a.k.a.* bis-chloronitrosourea or BCNU) and mitomycin C (MMC) (81). Similarly, small interfering RNA (siRNA) knockdown of AAG/MPG in the human cervical carcinoma HeLa cell line increases sensitivity to clinical alkylators including temozolomide (TMZ) and carmustine (82).

It is noteworthy that creating an imbalance in the enzymatic steps of BER can also have a profound effect on the biological consequences of endogenous (and presumably induced) DNA damage. For instance, overexpression of AAG/MPG in human breast cancer cells results in increased sensitivity to TMZ, suggesting transient AAG/MPG overexpression as a potential therapeutic approach for increasing cellular sensitivity to clinical alkylators (83). This increased alkylation-induced cytotoxicity of AAG/MPG-overexpressing cells apparently stems from the conversion of non-toxic 7-MeG adducts to toxic repair intermediates, such as abasic sites or SSBs, by the DNA glycosylase (84).

APE1

There is evidence that suppression of APE1 activity by various strategies increases cellular sensitivity to DNA-interactive therapeutics. For instance, a reduction in APE1 protein in human osteosarcoma cells increases sensitivity to ThioTEPA, etoposide, and ionizing radiation (85). Antisense suppression of APE1 also increases the cytotoxicity of TMZ and carmustine in *mgmt*^{-/-} SNB19 human glioma cells (86, 87), and it appears that the AP endonuclease activity of APE1 contributes to the alkylating-agent resistance in these cells (86, 87). A later study using 52 medulloblastomas and 10 primitive neuroectodermal tumors supports that the APE1 endonuclease activity confers resistance to the combined treatment of radiation with alkylating and/or platinating agents (87). In the same study, AP site incision activity of APE1 was found to be predictive of outcome following adjuvant therapy in patients with medulloblastomas and primitive neuroectodermal tumors. Lam *et al.* found that over-production of a nuclease-competent APE1 protein improved cellular resistance to antimetabolite compounds β -L-dioxolane-cytidine (L-OddC, BCH-4556, Troxacitabine) and β -L-2',3'-dideoxy-2',3'-didehydro-5-fluorocytidine (L-Fd4C) (88). Additionally, down-regulation of APE1 via short hairpin RNA (shRNA) sensitized human colorectal cancer cells by approximately 2-fold to these nucleoside analogs, indicating a contribution of APE1 to regulating responsiveness to certain antimetabolites (88). The expression of a dominant-negative form of APE1 (a double mutant of Glu96 and Asp210, termed ED) enhances sensitivity to carmustine, TMZ, and 5-FU in Chinese hamster ovary (CHO) cells and/or the human non-small cell lung cancer cell H1299 (47, 89). The combination of these results indicates that APE1 is a rationale target for improving treatment paradigms for certain human cancers.

Methoxyamine (MX) is a small molecule inhibitor of the BER process that reacts with the C1' position of the aldehyde group of an "open-ring" abasic site to form an alkoxyamine adduct in DNA (90). Addition of MX to the deoxyribose inhibits alkaline rupture of the adjacent phosphodiester bond and prevents efficient cleavage by APE1 at the modified abasic damage, making the compound an indirect inhibitor of the repair endonuclease (91, 92). This stabilization of the AP site creates a cytotoxic intermediate that is not efficiently removed. Treatment with MX, and consequent inhibition of APE1 activity, leads to increased DNA SSBs, double-strand breaks (DSBs), and cytotoxicity in colon cancer cells when combined with alkylating agents, such as TMZ (93, 94).

Pol β

Consistent with a prominent role for Pol β in BER, studies have found that mouse embryonic fibroblasts (MEFs) deficient in Pol β are more susceptible to cell killing by the alkylator TMZ than isogenic wild-type cells or AAG/MPG-deficient cells, presumably due to an accumulation of BER intermediates (95, 96). Consistently, knockdown of Pol β in mouse fibroblasts via RNAi increases TMZ-induced cell death, particularly upon long drug exposure (*i.e.* from 2 to 48 hr) (96). Artificial AAG/MPG overexpression exacerbates the cytotoxicity of TMZ in Pol β -deficient human breast cancer cells, supporting a contribution of BER intermediates in the cytotoxicity of this anticancer drug (97).

DNA ligases

As seen with the other core BER proteins (98), mice homozygous for LIGIII disruption show early embryonic lethality, indicating a requirement for animal development (99). HeLa cells stably suppressed for LIGIII via RNAi grow normally, suggesting that this protein is not required for cell viability in the absence of exogenous stressors (100). LIGI-deficient mouse cells are only slightly more susceptible to UV cell killing, and are indistinguishable from control cells for ionizing radiation sensitivity (101). The fibroblast cell strain 46BR, derived from a patient displaying symptoms of immunodeficiency, stunted growth and sun sensitivity, was shown to be hypersensitive to the lethal effects of a variety of laboratory alkylators, including the monofunctional methylating agents methyl methanesulfonate (MMS), N-methyl-N-nitrosourea (MNU), and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (102). It was later discovered that two missense mutations in the different alleles of *LIG1* give rise to poorly active protein forms, and presumably, the deficiencies associated with the 46BR cells, most notably, the impaired ligation activity (103). 46BR cells, while displaying a defect in re-ligation of DNA SSBs, exhibit normal repair of γ -radiation-induced DNA DSBs (104).

Besides being implicated in alkylating agent resistance, very little is known about the role of DNA ligases in mediating anticancer agent survival. Recently, Chen *et al.* identified novel small molecule inhibitors that block the second and the third steps of the ligation reaction in which the enzyme interacts with nicked DNA (105). These inhibitors also exhibit different specificities for human DNA ligases I, III, and IV *in vitro*. The selected ligase inhibitors increase the cytotoxicity of human breast cancer cells to DNA damaging agents, such as MMS and ionizing radiation, in comparison to normal human breast cells.

XRCC1 and PARP1

Mammalian cells deficient in XRCC1 exhibit a clear defect in SSBR kinetics, likely stemming from inefficient DNA termini “clean-up” and nick ligation. XRCC1 mutant CHO cells display a pronounced hypersensitivity to laboratory alkylating agents as well as the topoisomerase I inhibitor camptothecin (106). In addition, human cells lacking XRCC1 via siRNA strategies show increased sensitivity to MMS, carmustine, hydrogen peroxide, and ionizing radiation (107–109). There is some evidence that the XRCC1 protein amount may correlate with the degree of camptothecin resistance (110). Specifically, the *XRCC1* gene was amplified in camptothecin-resistant cell lines, and XRCC1 appears to play a role in the processing of camptothecin-induced topoisomerase I-DNA covalent intermediates. A more exhaustive look at the effects of XRCC1 deficiency on cellular responsiveness to anticancer therapeutics is warranted.

DNA damage caused by alkylators, topoisomerase I inhibitors (*e.g.* irinotecan), antimetabolites (*e.g.* gemcitabine), and ionizing radiation can increase protein expression and the enzymatic activity of PARP1 (its so-called poly ADP-ribosylation activity) in normal and malignant human cell lines (111–114). In addition, elevated PARP1 levels are

found in a number of human cancers (113, 114). Thus, inhibition of PARP1, resulting in suppression of the BER/SSBR pathway, has been a focus of preclinical and clinical development in an effort to sensitize human cancers to chemotherapy and radiotherapy (111–114).

Antisense depletion of PARP1 leads to a significant reduction in the initial rate of SSBR following MMS treatment, although the extent of repair is ultimately similar to control cells (115). Moreover, there is an excellent correlation between the potency of the compounds as PARP1 inhibitors and their effect on cell survival and DNA repair (112, 116). It was demonstrated early on, using the PARP1 inhibitor 3-aminobenzamide, that inactivation of PARP1 function results in impaired rejoining of strand breaks induced by alkylating compounds (117, 118). More recent studies employing a collection of PARP1 inhibitors [*i.e.* 3-aminobenzamide, benzamide, 3,4-dihydro-5-methoxyisoquinolin-1(2H)-one (PD 128763) and 8-hydroxy-2-methylquinazolin-4(3H)-one (NU1025)] found that such compounds acted in a concentration-dependent manner to potentiate the cytotoxicity and increase DNA strand break levels in cells treated with the monofunctional alkylator TMZ. Furthermore, DNA-PK and PARP1 inhibitors individually or in combination act as potent radiosensitizers and show potential as tools for anticancer therapeutic intervention (119).

Closing Thoughts

Resistance to chemotherapy and radiotherapy is believed to result in treatment failure for patients with metastatic cancer. Clearly, if drug resistance could be overcome, the impact on survival and quality of life would be significant. The collection of data, some of which has been reviewed in this article, currently indicates that BER plays a significant role in dictating the cellular response to various chemotherapeutic agents and radiotherapy. Indeed, activation of BER enzymes can lead to DNA-damaging agent resistance. Furthermore, BER inhibitors appear to improve the efficacy of current treatment strategies by promoting sensitization of cancerous cells to relevant clinical DNA-interactive drugs. The additional reviews of this Special Issue highlight current research aimed at inhibiting specific targets in DNA repair.

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Figure 1.
Human BER pathways

Table 1

Endogenous DNA lesions in human cells

Lesion	Mode of formation	Number of residues generated daily per human genome	Genome steady state level in normal, repair-proficient cells
Uracil	Cytosine deamination	400	~1
Thymine (opposite guanine)	5-Methylcytosine deamination	30	10–20
Hypoxanthine	Adenine deamination	10	~1
8-oxo-7,8-dihydroguanine	Guanine oxidation	~1000	~1
faPy	Guanine oxidation	~200	~5
Thymine glycol and similar oxidized pyrimidines	Pyrimidine oxidation	~500	~5
Etheno C	Lipid peroxidation of cytosine	~200	~5
Etheno A	Lipid peroxidation of adenine	~200	~5
3-Methyladenine	SAM methylation of adenine	~600	~5
7-Methylguanine	SAM methylation of guanine	4000	3000
O ⁶ -Methylguanine	Genomic alkylation by endogenous nitrosamines	~200	~1
Abasic site	Hydrolytic depurination	9000	~5

* Adapted from Trends in Genetics, 1999;15:93–4

Table 2

Human DNA glycosylases and primary substrates

Damaged base type	Acronym	Full name	AP lyase activity	Substrates
Deaminated base	UNG	Uracil DNA N-glycosylase	No	U, U:G, U:A, 5-FU
	TDG	Thymine DNA glycosylase	No	U:G, EthenoC:G, T:G
	SMUG1	Single-strand-selective monofunctional uracil-DNA glycosylase 1	No	U, U:A, U:G
	MBD4	Methyl-CpG-binding domain 4	No	U or T in U/TpG:5-meCpG
Alkylated base	AAG/MPG	Alkyladenine DNA glycosylase/ Methyl purine DNA glycosylase	No	3-MeA, 7-MeA, 3-MeG, 7-MeG, Etheno A, m ⁶ A
Oxidized base	MYH	MutY homolog	Yes (β)/No	A:G, A:8-oxoG
	OGG1	8-oxo-guanine glycosylase 1	Yes(β)	8-oxoG:C, faPyG
	NTH1	Endonuclease three homolog 1	Yes(β)	Tg, Cg, faPyG, DHU, 5-ohU, 5-ohC
	NEIL1	Neiendonuclease VIII-like 1	Yes (β, δ)	Tg, 5-ohU, 5-ohC, faPyA/G, Urea, 8-oxoG
	NEIL2	Neiendonuclease VIII-like 2	Yes (β, δ)	Overlap with NTH1 and NEIL1
	NEIL3	Neiendonuclease VIII-like 3	Yes (β, δ)	Oxidized purines, faPyG, faPyA

Table 3

BER protein and clinical DNA-damaging agent resistance

BER proteins	DNA damaging agents
AAG/MPG	TMZ, Carmustine, MMC
SMUG1	5-FU
OGG1	ThioTEPA
NEIL1	IR
APE1	ThioTEPA, Carmustine, Etoposide, TMZ, L-Oddc, IR, 5-FU
Pol β	TMZ
XRCC1	Carmustine, IR
PARP1	IR