Review Article

Targeting Base Excision Repair to Improve Cancer Therapies

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Key Words: DNA repair, cancer therapy, radiosensitization, oxaliplatin.

Abstract

Most commonly used cancer therapies, particularly ionizing radiation and certain classes of cytotoxic chemotherapies, cause cell death by damaging DNA. Base excision repair (BER) is the major system responsible for the removal of corrupt DNA bases and repair of DNA single strand breaks generated spontaneously and induced by exogenous DNA damaging factors such as certain cancer therapies. In this review, the physico-chemical properties of the proteins involved in BER are discussed with particular emphasis on molecular mechanisms coordinating repair processes. The aim of this review is to apply extensive knowledge that currently exists regarding the biochemical mechanisms involved in human BER to the molecular biology of current therapies for cancer. It is anticipated that the application of this knowledge will translate into the development of novel effective therapies for improving existing treatments such as radiation therapy and oxaliplatin chemotherapy.

Contents

1. Intoduction

Base excision repair (BER) is the major system responsible for the removal of corrupt DNA bases and repair of DNA single strand breaks generated spontaneously and induced by exogenous DNA damaging factors, including ionizing radiation and alkylating agents that are frequently used in cancer therapy (reviewed in (Dianov et al., 2001; Fortini and Dogliotti, 2007; Lindahl, 1993; Lindahl and Wood, 1999; Weinfeld, 2001). Excision repair of damaged bases is initiated by specific DNA glycosylases that recognize and remove the damaged base by hydrolyzing the glycosylic bond linking the base residue to the DNA sugar phosphate backbone (Fig. 1). The abasic site (AP site) is further recognized by AP endonuclease 1 (APE1) that introduces a DNA single strand break (SSB) by cleaving the phosphodiester bond 5′ to the AP site generating a SSB containing a 5′-sugar phosphate. At this point, BER and SSB repair mechanisms are very similar. Poly(ADP-ribose)polymerase (PARP-1) temporarily binds to both SSBs induced by DNA damaging agents and repair intermediates arising during BER. Although the exact role of PARP-1 binding to the SSBs is unclear, it has been proposed that PARP-1 binding protects strand breaks from degradation by cellular nucleases where the amount of damage outstrips the repair capacity of the cell (Parsons et al., 2005a; Satoh and Lindahl, 1992). Following NAD^+-dependent poly(ADP-ribosyl)ation of PARP-1, automodified PARP-1 then dissociates from DNA. If enough BER enzymes are present after release of PARP-1, repair proceeds by virtue of end-processors that removing moieties blocking DNA repair synthesis and DNA ligation. DNA polymerase β, polynucleotide kinase, APE1 and aprataxin are the major players involved in processing of modified 3′ and 5′ -ends. After restoring conventional 3′ -hydroxyl and 5′-phosphate strand break ends, repair is accomplished by DNA polymerase β (Pol β) and the DNA ligase IIIα-XRCC1 heterodimer. Pol β adds one nucleotide to the 3′-OH end of the strand break, thus filling the one nucleotide gap created during repair, while DNA ligase seals the DNA ends thus completing the repair process (Fig. 1).

The minor BER pathway, also known as long-patch repair (Frosina et al., 1996), involves flap endonuclease 1 (FEN1), proliferating cell nuclear antigen (PCNA),

replication factor C (RFC), DNA polymerase δ/ ε and DNA ligase I in addition to DNA glycosylase and AP endonuclease (Klungland and Lindahl, 1997b; Pascucci et al., 1999) . Long-patch BER acts to repair AP sites at which the sugar residue has been modified in some way, e.g. oxidised or reduced (Klungland and Lindahl, 1997b; Matsumoto and Kim, 1995b). The AP lyase activity of Pol β is inactive at such AP sites, therefore to initiate this pathway, DNA polymerase β first adds one nucleotide to the 3' end of the nick and than DNA polymerase δ or ε add a few more nucleotides to generate part of a single-stranded flap structure containing a 5' sugar phosphate (Podlutsky et al., 2001a). This flap is recognised and excised by FEN1, which tracks down the length of the flap to cleave at the single-strand/double-strand junction, and the DNA is finally ligated by DNA ligase I; in this reaction, FEN1 activity is stimulated by PCNA (Klungland and Lindahl, 1997a). Alternatively, long-patch repair can also be catalysed by Pol δ plus PCNA. Long-patch repair events result in a 2-5 nucleotide repair patch (Klungland and Lindahl, 1997b). Since long-patch repair appears to be a relatively minor contributor to all BER activity (reviewed in Sung and Demple 2006), this essay will focus on short-patch repair pathway in mammalian cells.

2. Base excision repair proteins

2.1 DNA glycosylases

DNA glycosylases initiate the BER process by catalyzing hydrolysis of the C1'-N glycosylic bond to the damaged base, removing the base and leaving behind an abasic (AP) site. The ubiquity and wide utility of the glycosylase family reflects the essential role of the BER process in protecting genome stability. Eleven DNA glycosylases have so far been implicated in mammalian BER, and their various specificities are summarized in Table 1. Two mechanistic classes of DNA glycosylases are recognized:

mono-functional (i.e. glycosylase activity only) such as UDG, and bi-functional (i.e. glycosylase and β-lyase activities) such as human 7,8-dihydro-8-oxoguanine-DNA glycosylase (hOGG1). Mono-functional DNA glycosylases create AP sites through cleavage of the C1'-N-glycosylic bond using an activated water molecule as an active site nucleophile (McCullough et al., 1999). Additionally, bi-functional glycosylases have an associated AP lyase activity which can further process the AP site by incising the DNA backbone 3' to the AP site.

2.2 AP Endonucleases

Removal of damaged bases from DNA by DNA glycosylases leads to the formation of AP sites. A critical step in the BER pathway involves the cleavage of baseless sites in DNA by an AP endonuclease. The major AP endonuclease in mammalian cells is APE1, also known as Ref1 or HAP1, an enzyme that is homologous to bacterial exonuclease III (Demple et al., 1991; Robson and Hickson, 1991). APE1 is a multifunctional protein that is not only responsible for repair of AP sites, but it also functions as a reduction-oxidation (redox) factor maintaining transcription factors in an active reduced state; it has also been shown to be closely linked with cellular apoptosis (Reviewed in (Evans et al., 2000; Wilson and Barsky, 2001)). The functional importance of APE1 is underscored by the findings that mice nullizygous for the APE1 gene are embryonic lethal (Xanthoudakis et al., 1996) and that downregulation of APE1 levels in human cells using RNAi leads to accumulation of AP sites, halted cell proliferation and triggering of apoptosis (Fung and Demple, 2005).

APE1 is the major human AP endonuclease that comprises more than 95% of cellular AP site incision activity (Chen et al., 1991). APE1 cleaves phosphodiester bonds hydrolytically in a Mg^{2+}-dependent manner, leaving a 3'-hydroxyl group and a 5'-deoxyribose phosphate (dRP) group flanking the nucleotide gap. In addition to AP endonuclease activity, APE1 has 3'-diesterase and phosphatase activity, although its phosphatase activity is approximately 100-fold lower than its AP endonuclease activity (Chen et al., 1991; Wilson and Barsky, 2001) and is most probably of limited physiological relevance (Wiederhold et al., 2004). Notably, it has recently been demonstrated that APE1 is the major activity in human cells responsible for removal of

3'-phosphoglycolate or 8-oxoguanine that occur at SSBs created by ionizing irradiation and oxidative agents (Parsons et al., 2004; Parsons et al., 2005b). Although APE1 expression is ubiquitous, it exhibits a complex and heterogeneous staining pattern that differs among tissue types and even differs between neighboring cells (Rivkees and Kelley, 1994; Wilson et al., 1996). As expected, APE1 is found in the nucleus of cells, in a fashion which corresponds to its repair functions. In some cell types, however, APE1 is also found in the cytoplasm and mitochondria (Tell et al., 2001), which may correspond either to its role as a DNA repair protein in the mitochondria (Sawyer and Van Houten, 1999) or, through its redox activity in maintaining newly synthesized transcription factors in a reduced state during transportation to the nucleus (Evans et al., 2000; Kakolyris, 1998).

Mammals also possess a second protein (APE2) with sequence homology to exonuclease III, but this protein exhibits comparatively weak AP site-specific and 3'-nuclease activities (Hadi et al., 2002).

2.3 Poly(ADP-ribose)polymerase

The enzyme poly(ADP-ribose) polymerase (PARP-1) has been strongly implicated in BER although its precise role is not yet fully understood. Poly(ADP)-ribose polymerase-1 (PARP-1) has a very high affinity for strand breaks and it is generally though to bind incised AP sites before any other repair proteins (Parsons et al., 2005a). The binding of strand breaks allosterically activates PARP-1's catalytic activity, modifying nuclear proteins with polymers of ADP-ribose that it synthesises from NAD^+ (see (Ame et al., 2004; Herceg and Wang, 2001; Smith, 2001) for review). One of the major targets of poly(ADP-ribosyl)ation is PARP-1 itself and the resulting accumulation of negative charge on the enzyme reduces its affinity for DNA via electrostatic repulsion. As a result, single strand break repair exhibits a dependence on NAD^+ due to the activity of PARP-1 (Satoh and Lindahl, 1992; Satoh et al., 1993). Similarly, the repair of damaged DNA bases via BER also exhibits a dependence on NAD^+ (Allinson et al., 2003). Inhibition of PARP-1 catalysis in cultured cells, by the use of either chemical inhibitors or expression of the catalytically inactive PARP-1 DNA binding domain, results in inhibition of poly(ADP-ribosyl)ation and a concomitant retardation of strand break repair

(Aoufouchi et al., 1999; Molinete et al., 1993; Perkins et al., 2001). Interestingly, PARP-1 null mice are hypersensitive to alkylating agents and ionizing radiation (Ménessier-de Murcia et al., 1997; Wang et al., 1997), and immortalised cells from these animals manifest characteristic features of genomic instability, including delayed rejoining of strand breaks, mixed ploidy and elevated frequencies of SCE and micronuclei formation upon treatment with DNA-damaging agents (Ménessier-de Murcia et al., 1997; Trucco et al., 1998; Wang et al., 1997).

It was recently demonstrated that *in vitro* repair reactions carried out in the absence of PARP-1 result in increased nucleolytic degradation of base excision repair intermediates and it has been suggested that PARP-1 functions to protect strand breaks and repair intermediates from degradation or recombination where the amount of damage outstrips the repair capacity of the cell (Parsons et al., 2005a). In support of this hypothesis, it has recently been reported that the genomes of PARP-1 null mice show an increased incidence of deletion mutations upon DNA damage (Shibata et al., 2005).

2.4 Polynucleotide Kinase

Human PNK is an important end-processor since it is the principal enzyme involved in the restoration of 5′-phosphate and 3′-hydroxyl ends at SSBs. Accordingly, human PNK has been cloned and characterized as the major DNA 5′-kinase and 3′-phosphatase that is able to phosphorylate the 5'-end of SSBs and removes blocking phosphate lesions from the 3'-end (Jilani et al., 1999; Karimi-Busheri et al., 1999). In human cells, the involvement of PNK in the repair of oxidative strand breaks has been revealed by its interaction with components of the BER pathway, namely X-ray cross complementing protein 1 (XRCC1), DNA ligase IIIα and Pol β (Whitehouse et al., 2001).

Recently, an important role for PNK in SSB repair was revealed by down-regulation of human PNK by RNAi silencing: A reduction in the levels of endogenous PNK enhanced the spontaneous mutation frequency and caused an elevated sensitivity of cells to genotoxic agents (Rasouli-Nia et al., 2004).

2.5 Aprataxin

Aprataxin is a member of the HIT domain superfamily of nucleotide hydrolases/transferases (Brenner, 2002; Kijas et al., 2006). Mutations in the aprataxin gene give rise to the neurological disorder ataxia oculomotor apraxia (AOA1) that is similar to SCAN1 pathology (Date et al., 2004; Moreira et al., 2001). Aprataxin has been found to interact with XRCC1 (Caldecott, 2003; Date et al., 2004) and cells deficient in aprataxin are defective in DNA SSB repair (Clements et al., 2004; Gueven et al., 2004).

Recently, aprataxin has been found to be involved in the resolution of abortive DNA ligation intermediates by catalyzing the release of adenylate groups covalently linked to 5′-phosphate termini at single strand nicks and gaps in DNA. Based on these data, it was proposed that such adenylated 5'-ends are formed as a result of abortive DNA ligation during attempted ligation of SSBs containing "damaged" unligatable DNA ends (Ahel et al., 2006).

2.6 BER DNA Polymerases

Excision of a damaged base and subsequent processing of the resulting SSB results in an intermediate containing a 3'-hydroxyl, suitable for priming strand resynthesis by a polymerase. The majority of BER proceeds through the so-called "short-patch" sub-pathway in which a single nucleotide is removed and replaced. In this pathway, DNA resynthesis is carried out by Pol β, a member of the X family of polymerases (Dianov et al., 1992; Sobol et al., 1996; Wiebauer and Jiricny, 1990). Like other X family polymerases, Pol β is a compact (39 kDa) single subunit protein. It contains two domains connected by a hinge region: a C-terminal 31 kDa polymerase domain (Kumar et al., 1990) and an N-terminal 8 kDa dRP lyase domain (Matsumoto and Kim, 1995a), both of these activities are required for short-patch BER (Podlutsky et al., 2001b). The dRP lyase activity of Pol β is required for processing the SSB created by APE1 since it contains 5'-dRP residue blocking DNA ligation. Pol β first removes the dRP residue through catalysis of a β-elimination reaction (Matsumoto and Kim, 1995a) and then fills created one nucleotide gap. The two activities of Pol β are likely to be tightly coordinated since co-complementation of Pol β null cell extracts with both dRP lyase and polymerase deficient mutants of Pol β is unable to restore single nucleotide patch repair to these extracts (Podlutsky et al., 2001b). Pol β lacks any intrinsic proof-reading activity

yet carries out DNA synthesis of moderately high fidelity compared to that of the so-called error-prone polymerases. The error frequency of short patch base excision repair by mammalian cell extracts has been calculated to be of the order of 5 to 10 x 10^{-4}, with one nucleotide deletions predominating (Bennett et al., 2001; Sanderson and Mosbaugh, 1998; Zhang and Dianov, 2005).

Mice nullizygous for the Pol β gene are non-viable and die early in embryogenesis (Gu et al., 1994). Embryonic fibroblasts derived from these mice are hypersensitive to the methylating agent, MMS (Sobol et al., 1996). As might be expected, under normal circumstances Pol β is detectable in all tissues. It is generally found at low levels, except for testicular tissue in which meiosis is an important physiological process (Hirose et al., 1989). Pol β is expressed constitutively and without demonstrable cell-cycle dependence (Zmudzka et al., 1988). Levels of Pol β are most probably kept low in undamaged cells due to the mis-insertion frequency that is inherent to the activity of Pol β when one compares it to the replicative polymerases.

2.7 DNA ligases

Two DNA ligases have been implicated in BER: DNA ligase I and DNA ligase III (Tomkinson and Levin, 1997). DNA ligase III is the major DNA ligase involved in single-nucleotide patch BER. DNA ligase III exists in the nucleus as two isoforms, denoted DNA ligase IIIα (103 kDa) and DNA ligase IIIβ (96 kDa), which arise through differential splicing of RNA transcripts (Mackey et al., 1997). DNA ligase IIIα is ubiquitously expressed in human tissues, whereas DNA ligase IIIβ is found only in testicular tissue and is believed to be involved in homologous recombination during meiosis (Tomkinson and Mackey, 1998). The two isoforms share many features in common and differ only at their C-termini, with the C-terminal 77 amino acids of DNA ligase IIIα being replaced with 17 different amino acids in DNA ligase IIIβ (Tomkinson et al., 2001).

DNA ligase IIIα was originally purified in a complex with XRCC1 (Caldecott et al., 1994; Caldecott et al., 1995). This interaction has been characterized *in vitro* and is mediated through the BRCT domain located at the C-terminus of DNA ligase IIIα (Nash

et al., 1997). XRCC1 null cell lines have 4-6 fold reduced levels and activity of DNA ligase IIIα, indicating that the XRCC1 protein is important for maintaining cellular level of DNA ligase IIIα (Caldecott et al., 1995). In support of this finding, it has been shown that the activity of DNA ligase III can be restored in mutant XRCC1 cells (EM9) by transfection of the XRCC1 gene product (Ljungquist et al., 1994)). Since XRCC1 null cells are characteristically deficient in short-patch BER, it has been suggested that the XRCC1-DNA ligase IIIα complex plays an important role in this sub-pathway (Cappelli et al., 1997).

DNA ligase I (102 kDa) is conserved in all eukaryotes, with orthologs being found in organisms from yeast to mammals (Tomkinson and Mackey, 1998). It plays an essential role in DNA replication (Waga and Stillman, 1998). Human cell lines containing a partially inactive DNA ligase I mutant exhibit hypersensitivity to DNA-damaging agents such as ionizing radiation and alkylating agents (Barnes et al., 1992; Prigent et al., 1994; Webster et al., 1992), suggesting a role for DNA ligase I in BER. However, a mouse model with an identical mutation was defective in DNA replication but not in DNA repair (Harrison et al., 2002). Despite this controversy, there is abundant evidence to suggest the involvement of DNA ligase I in the long-patch pathway of BER (DeMott et al., 1998; Dianov et al., 1999; Levin et al., 2000).

2.8 XRCC1

Originally identified as a result of its absence from a mutant Chinese hamster ovary cell line (EM9), which showed hypersensitivity to both alkylating agents and ionizing radiation (Thompson et al., 1990), XRCC1 has been increasingly implicated as a key player in the BER process. Cells deficient in XRCC1 show many of the hallmarks of defective BER, including hypersensitivity to ionizing radiation and alkylating agents, delayed single strand break rejoining and increased rates of sister chromatid exchange (SCE) and induced mutations (reviewed in (Thompson and West, 2000)). Extracts from such cells show a partial defect in BER efficiency when compared to wild type cells (Cappelli et al., 1997; Sleeth et al., 2004). The importance of the *XRCC1* gene for cellular functioning is underlined by the fact that its targeted knockout results in

embryonic lethality (Tebbs et al., 1999). Although it has no known enzymatic function of its own, XRCC1 interacts with a number of enzymes involved in BER, including DNA ligase IIIα (Caldecott et al., 1994; Nash et al., 1997), Pol β (Caldecott et al., 1996; Kubota et al., 1996), PARP-1 (Caldecott et al., 1996; Masson et al., 1998), APE1 (Vidal et al., 2001) and PCNA (Fan et al., 2004).

XRCC1 is a 69.5 kDa multi-domain protein with an N-terminal DNA binding domain and two BRCT motifs. The first BRCT motif, BRCT I, is the site of interaction with PARP-1 (Masson et al., 1998), while the C-terminal BRCT motif (BRCT II) is responsible for the interaction with DNA ligase IIIα (Nash et al., 1997). Mutations in the BRCT domains of XRCC1 seriously compromise single-strand break repair (SSBR) *in vivo*, but with differing cell cycle dependencies. The BRCT II domain seems to be required only for SSBR during the G_0/G_1 phase of the cell cycle with SSBR being unaffected in S phase (Taylor et al., 2000). Disruption of this domain affects the survival of quiescent cells only: cycling cells with a mutated BRCT II domain show similar repair efficiencies and survival to those with normal XRCC1 (Moore et al., 2000). Conversely, the BRCT I domain is required for SSBR in both G_1 and S/G_2 (Taylor et al., 2002) and disruption of this domain compromises cells' ability to reinitiate DNA synthesis following exposure to an alkylating agent (Kubota and Horiuchi, 2003).

The nick-binding N-terminal domain of XRCC1 interacts with the palm-thumb domain of Pol β allowing it to form a tertiary complex with Pol β and nicked DNA, in which both proteins almost completely surround the nicked site (Gryk et al., 2002; Marintchev et al., 1999; Marintchev et al., 2000). The biological importance of the XRCC1-Pol β interaction was recently confirmed when a mutant of XRCC1 incapable of interaction with Pol β was unable to fully reverse the hydrogen peroxide hypersensitivity of an XRCC1 deficient cell line and was less efficient at performing the ligation step in reconstituted repair reactions (Dianova et al., 2004).

3. Links with Cancer

As discussed above, BER is a highly coordinated cellular biochemical system in which protein-DNA and protein-protein interactions play essential roles. It is likely that there

exists a fine balance between the concentrations of the enzymes involved in the BER pathway (Glassner et al., 1998). Significant under-expression of a BER enzyme may result in attenuation of repair efficiency as the dynamics of the pathway would alter and the under-expressed enzyme may then become rate-limiting. The consequences for a cell may depend on the abundance of backup repair proteins (see below). If the repair capacity of BER is reduced, base damage may accumulate in DNA resulting in genomic instability or apoptosis. Alternatively, and probably more commonly in cancer, over-expression of a BER enzyme may have effects in the cell such as genomic instability or increased translesion synthesis (see below). In this section, the effects of imbalances in DNA repair activities will be reviewed with particular reference to their potential contributions to carcinogenesis.

3.1 DNA Glycosylases

DNA glycosylases which recognise DNA lesions and initiate BER have overlapping functions, which permit backup mechanisms to exist if the function of one DNA glycosylase is lost. Compatible with this statement, mouse knockout models have been constructed for AAG (Engelward et al., 1997; Hang et al., 1997), OGG1 (Klungland et al., 1999; Minowa et al., 2000), UNG2 (Nilsen et al., 2000), NTH1 (Elder and Dianov, 2002; Ocampo et al., 2002; Takao et al., 2002), MBD4 (Millar et al., 2002) and MYH DNA glycosylases (Xie et al., 2004), which have proven invaluable in furthering understanding of BER *in vivo*. Perhaps surprisingly, only in the OGG1 knockout mice has there been shown to be significant accumulation of the oxidised base, 8-oxo-guanine; an observation made in tissues of older animals compared to wild-type mice (Klungland et al., 1999; Minowa et al., 2000).

The predisposition of OGG1 -/- mice to develop cancer is demonstrated by the incidence of lung cancer in mice aged 18 months (Sakumi et al., 2003). The fact that mice do not develop cancer until old age is likely to represent some degree of protection conferred by the existence of backup pathways such as NEIL1 and NEIL2 DNA glycosylases. Despite this protection, OGG1 -/- mice do have accelerated rates of mutations in response to mutagens such as cigarette smoke and premature senescence of cells, the latter being particularly apparent in mitochondrial DNA (de Souza-Pinto et al.,

2001). Mitochondrial DNA is more susceptible to the damaging effects of reactive oxygen species since it lacks the relative protection of histones. Compared to the predisposition to develop lung cancer in old age in OGG1 -/- mice, a much earlier predisposition to both lung cancer and ovarian cancer is observed in mice which possess knockouts for both OGG1 and MYH (Xie et al., 2004). In this study, G to T transversions were observed in the *K-ras* gene of affected animals, a mutation that has been detected in several human cancers (Friday and Adjei, 2005).

Results regarding potential links between genetic polymorphisms of BER genes and cancer risk have been inconsistent and definite conclusions cannot be drawn. In a useful meta-analysis performed by Hung and colleagues using 3,253 cases and 3,371 controls from seven studies and focusing on OGG1, APE1 and XRCC1, increased lung cancer risk was found in subjects carrying the *OGG1 Cys/Cys* genotype (odds ratio (OR) = 1.24, 95% confidence interval (CI): 1.01, 1.53) (Hung et al., 2005). They also found a protective effect of the *XRCC1 194Trp* allele for tobacco-related cancers (OR = 0.86, 95% CI: 0.77, 0.95) using 4,895 cases and 5,977 controls from 16 studies. The *XRCC1 399Gln/399Gln* genotype was associated with increased risk of tobacco-related cancers among light smokers (OR = 1.38, 95% CI: 0.99, 1.94) but decreased risk among heavy smokers (OR = 0.71, 95% CI: 0.51, 0.99), suggesting effect modification by tobacco smoking. Some of these data are supported by experimental evidence that certain isoforms of these gene products exhibit decreased enzymatic activity or lower mutagen sensitivity in animal models (reviewed in (Hecht, 1999)).

The first demonstration of a link between an inherited disorder involving BER and human carcinogenesis is the monofunctional DNA glycosylase, hMYH, which removes A that is misincorporated opposite 8-oxoguanine, hence preventing formation of G:C to T:A transversions. In a seminal study, the autosomal recessive syndrome of adenomatous polyposis coli (APC), which confers a 100% lifetime risk of colorectal cancer unless the organ is surgically removed, was linked with biallelic mutations in hMYH (Jones et al., 2002). This finding was considered surprising since preclinical studies of the E.coli homologue, MutY, in E.coli and mouse knockout models had not suggested that a defect in this BER protein alone should be strongly linked with carcinogenesis (Moriya and Grollman, 1993; Xie et al., 2004). The clinical finding of

biallelic mutation has been confirmed by subsequent preclinical and clinical studies (Nielsen et al., 2005; Sieber et al., 2004; Tenesa et al., 2006), resulting in the description of a new disease, MYH-associated polyposis (MAP). MAP is an autosomal recessive disease characterized by multiple colorectal adenomas and biallelic mutation of MYH. It is of particular interest to pediatricians and physicians who see colorectal cancer in young patients who do not meet the criteria for the APC-inherited disease, familial adenomatous polyposis (FAP), or the mismatch repair disease, hereditary non-polyposis colorectal cancer (HNPCC). More recently, a study of 928 colorectal cancer cases and 845 healthy controls from Scotland, supported by a meta- analysis from published data on the association between mutations at MYH and colorectal cancer risk, has suggested that a small but significant mono- allelic effect may also exist (Farrington et al., 2005). Recent data suggest phenotypic similarities between colorectal cancers characterized by mismatch repair defects and those characterized by biallelic mutation of MYH (Aaltonen et al., 2007; Peterlongo et al., 2006). Other studies are emerging which associate mutations in hMYH with other human cancers such as gastric cancer (Zhang et al., 2006).

3.2 DNA Polymerases

Whereas DNA glycosylases show "functional redundancy" due to the existence of backup enzymes with overlapping activities, BER enzymes downstream of the glycosylase step play key roles in coordinating BER and are essential in embryonic development. Absence of APE1 (Xanthoudakis et al., 1996), Pol β (Gu et al., 1994), XRCC1 (Tebbs et al., 1999) and DNA ligase III (Puebla-Osorio et al., 2006) is embryonic lethal. In the case of APE1, for example, under-expression leads to apoptosis of cells essential to neurological development (Izumi et al., 2005).

Of the BER enzymes downstream of the glycosylase step, Pol β has been the most extensively studied in relation to carcinogenesis. Pol β null mice display a tissue-specific reduction of 1-nt frameshift mutations as well as increased apoptosis compared to wild type embryos (Sweasy et al., 2005). Studies have shown that under-expression of Pol β increases the rate of chromosomal aberrations by reducing BER activity (Dalal et al., 2005; Matsuzaki et al., 1996). The accumulation of such DNA damages ultimately results in genomic mutagenesis or apoptosis. Using expression arrays as a screening tool,

Albertella and colleagues observed reduced levels of Pol β mRNA and protein in approximately one fifth of all tumours sampled, particularly breast and colon cancers (Albertella et al., 2005b). Furthermore, they found that Pol β under-expression correlates with under-expression of other DNA polymerases, providing potential links with other cellular pathways.

Pol β over-expression appears to be more common in cancer than under-expression. Early observations indicated that Pol β is overexpressed in some cancer cells grown in vitro (Bergoglio et al., 2001; Canitrot et al., 1999; Srivastava et al., 1999; Tan et al., 2005). Indeed, overexpression of Pol β by 2 to 4-fold in mammalian cells can increase mutation rate (Canitrot et al., 1998) and promote genomic instability. Albertella and colleagues used expression arrays to systematically study the expression patterns of DNA polymerases (Pol β, λ, δ, ε) in samples of cancer tissue compared to normal tissue (Albertella et al., 2005b). They found that Pol β was overexpressed at the mRNA level and the protein level in approximately one third of tumours sampled, particularly samples of cancer taken form the uterus, ovary, prostate and stomach. Pol λ was also overexpressed in a range of tumours, but to a lesser extent than Pol β. The long-patch BER DNA polymerases, Pol δ and ε, were not commonly overexpressed in this study (Albertella et al., 2005b).

Studies comparing mouse embryonic fibroblasts (MEF) cells that are wild-type or null for Pol β have yielded interesting results regarding the potential role of Pol β in the response to ionizing radiation or chemotherapy with alkylating agents. The Pol β-deficient MEFs exhibited hypersensitivity to DNA alkylating agents (Poltoratsky et al., 2005). Surprisingly, this hypersensitivity was lost when only the N-terminal dRp lyase domain of Pol β was provided, suggesting greater functional redundancy for BER polymerase activities than for dRp excision activities.

3.3 PARP-1

As discussed above, poly(ADP-ribose) polymerase-1 (PARP-1) is one of the most abundant proteins in the nucleus of normal cells. In response to DNA damage, PARP1 undergoes self-ADP-ribosylation and is known to interact with XRCC1, DNA ligase III and Pol β. PARP-1 cooperatively interacts with PARP2 in DNA repair processes.

Consequently, double deficient models have been studied to elucidate the role of PARP1 in DNA repair and carcinogenesis. This approach revealed that PARP-1 null mice are hypersensitive to alkylating agents and ionizing radiation (Menissier-de Murcia et al., 1997; Wang et al., 1997). Several research teams have studied radiation-induced DNA alterations and carcinogenesis in PARP-1-deficient mice. Unfortunately, results have not been consistent across studies and definite conclusions cannot be drawn. For example, PARP1-deficient mice with a p53-knockout background or treated with oral carcinogens yielded diverse results ranging from an enhancement of the frequency of induced tumours to a reduction in carcinogenesis (Beneke and Moroy, 2001; Conde et al., 2001).

Studies with PARP inhibitors have yielded further discrepant results. Such discrepancy between knockout models and pharmacological inhibition may not be surprising since studies using PARP-1-deficient experimental systems do not distinguish between findings that are related to the physical absence of the enzyme (its 'scaffold' function) and those owing to the lack of catalytic activity (its 'enzymatic' function). Generally speaking, although its specific functions are not known, PARP-1 seems to have more important roles in DNA repair under stress conditions (such as radiation, carcinogen exposure or exposure to certain antitumour drugs) than in normal (non-stressed) cells (Eberhart, 2003). PARP-1 does not appear to have an active role in apoptosis (Jagtap and Szabo, 2005).

Since PARP-1 can be activated up to 100-fold by DNA strand breaks, changes in the expression or abundance of PARP-1 are not considered of primary regulatory relevance. Nevertheless, several recent reports have shown up- or down-regulation of PARP1 in pathophysiological conditions, including its up-regulation in chronic heart failure and in inflammatory conditions such as arthritis and colitis (Hong et al., 2004; Szabo and Dawson, 1998). Similarly, up-regulation of PARP-1 expression/activity has been observed in several cancers. Elevated PARP-1 activity has been shown in cell lines, including those derived from small cell lung cancer and androgen-sensitive prostate cancer (reviewed in (Jagtap and Szabo, 2005)). It has also been demonstrated in tissue samples obtained from patients with colorectal cancer, malignant melanoma and hepatocellular carcinoma (Iizuka et al., 2006; Nosho et al., 2006; Staibano et al., 2005).

PARP-1 mRNA levels have been found to be increased in diffuse large B-cell lymphoma samples, compared to normal or reactive lymph node samples (Menegazzi et al., 1999).

4. Targeting BER to improve cancer therapy

Most commonly used cancer therapies, particularly ionizing radiation and certain classes of cytotoxic chemotherapies, cause cell death by damaging DNA. The therapeutic index refers to the amount of tumor cell kill relative to normal tissue damage. Molecularly targeted agents have been developed over the past decade to try to improve the therapeutic index in the treatment of cancer. The development of resistance to cytotoxic therapy is a major clinical problem. It is extremely common in the clinic to observe the development of resistance to chemotherapy that was initially effective. In this section of the review, the potential for targeting BER will be discussed with a view to improving the therapeutic index of existing cancer therapies and reducing the development of resistance to therapy.

4.1 Targeting APE1

Several preclinical studies using antisense and RNA interference have shown that downregulation of BER enzymes sensitizes cells to various anticancer agents. In particular, down-regulation of APE1 sensitizes cells to alkylating agents and ionizing radiation (Tell et al., 2005).

Methoxyamine is a small molecule which binds to apurinic (AP) sites by reacting with aldehyde group of deoxyribose residue, thus preventing their processing by APE1. In the case of patients treated with cancer chemotherapeutic agents such as temozolomide (TMZ) or 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), AP sites are formed following the removal of the drug-induced methylated purines by the DNA glycosylase. Blocking the processing of such AP sites with agents such as methoxyamine leads to an accumulation of these potentially cytotoxic sites in DNA (Liu and Gerson, 2004). Methoxyamine has been shown to augment the cytotoxicity of a range of DNA damaging agents in cells grown in vitro and in xenograft studies (Liu et al., 2002; Taverna et al., 2003). Methoxyamine appears to potentiate cytotoxicity in both MMR-proficient and -deficient cells (Taverna et al., 2001). It also appears to bypass other potential resistance

factors such as the loss of p53 tumour suppressor function (Liu and Gerson, 2004). An example of these concepts is provided by a recent project in which the enhancement of TMZ cytotoxicity was studied by the addition of methoxyamine to three ovarian cancer cell lines, SKOV-3x, Ovcar-3, and IGROV-1, grown in vitro (Fishel et al., 2007). These experiments demonstrated the effective modulation of the cytotoxic activity of TMZ via methoxyamine. Transfection studies revealed that this effect was not dependent on p53 status but that it was influenced by N-methylpurine DNA glycosylase (MPG) overexpression (Fishel et al., 2007). Such findings lend support to the hypothesis that BER modulators may have a role in augmenting clinical responses to cytotoxic agents currently used in the clinic (see below). A Phase I clinical trial of methoxyamine in combination with TMZ is currently in progress in the USA.

With regard to sensitization of cells to radiotherapy, the halogenated thymidine analogue, 5-iodo-2'-deoxyuridine, has been shown to radiosensitize cancer cells in preclinical studies. Moreover, combining methoxyamine and 5-iodo-2'-deoxyuridine has been shown to potentiate this radiosensitization (Taverna et al., 2003).

A related strategy for targeting AP sites is to interfere with the function of the repair enzyme APE1 which processes the AP site. One reason why such targeting of the enzyme might be a more promising strategy than the use of methoxyamine or its derivatives is that APE1 is a multifunctional enzyme which has additional roles in BER and DNA strand break repair unrelated to AP site processing. For example, APE1 is the major 3'-phosphoglycolate diesterase in human cells; this activity may be important in protecting cells against the potentially carcinogenic effects of ionizing radiation (Parsons et al., 2004). APE1 also regulates the DNA binding activity of several key transcription factors involved in redox signaling (Tell et al., 2005). Several research groups have generated small molecule inhibitors of APE1. One such APE1 inhibitor, CRT0044876, inhibits the AP endonuclease, 3'-phosphodiesterase and 3'-phosphatase activities of APE1 at low micromolar concentrations (Madhusudan et al., 2005). At non-cytotoxic concentrations, CRT0044876 enhances the cytotoxicity of several DNA base-targeting compounds, including the DNA alkylating and methylating agent, TMZ. An alternative agent, lucanthone, inhibits the repair activity of the exonuclease III family members (APE1 and ExoIII), but not endonuclease IV AP endonucleases, nor

bifunctional glycosylase/lyases such as endonuclease VIII or formamidopyrimidine-DNA glycosylase (Fpg). The addition of lucanthone inhibits APE1 repair activity from cellular extracts and enhances the cell killing effect of the alkylating agents, methyl methanesulfonate (MMS) and TMZ (Luo and Kelley, 2004).

5.2 PARP-1 inhibitors

As discussed in section 2.3 above, PARP plays a role in BER that is not fully understood. Despite this incomplete knowledge base, PARP inhibitors have been used to suppress DNA repair and to promote apoptosis in cancer cells and PARP-1 inhibitors are in clinical trials in combination with certain anticancer agents (Table 2).

The combination with TMZ is the best contemporary example of this clinical development. Resistance to TMZ therapy frequently develops during the treatment of cancers such as glioblastoma multiforme and malignant melanoma and, on account of the lack of alternative effective therapies, such resistance represents a major clinical problem. The efficient repair of methyl adducts at the O^6 position of guanine is mediated by O^6-alkylguanine DNA alkyltransferase (AGT) or the mismatch-repair system. The combination of PARP-1 inhibitors with TMZ interferes with the repair of methylpurines. These agents do not usually contribute to the cytotoxicity that is induced by TMZ. When PARP-1 is inhibited, it remains bound to SSB and it hampers strand rejoining, which in turn leads to the generation of permanent single strand breaks that trigger the apoptotic process. Studies performed in vitro and in vivo have shown that combining PARP inhibitors with TMZ enhances the cytotoxic effects of TMZ (Liu et al., 1999; Tentori et al., 2003; Tentori et al., 2006). Indeed, such a combinatorial approach restores the sensitivity of mismatch repair-deficient tumours to TMZ (Liu et al., 1999).

PARP-1 inhibitors have also been shown to enhance the cytotoxicity of the camptothecin DNA topoisomerase I inhibitors, irinotecan and topotecan. These agents are currently used in the clinic to treat colorectal cancer, other gastrointestinal cancers, lung cancer and ovarian cancer. PARP-1 interacts with, and promotes the activity of, topoisomerase I. Poly (ADP-ribosyl)ated PARP1 and PARP2 counteract the action of the camptothecin compounds by facilitating the resealing of DNA-strand breaks (Park and Cheng, 2005). The combination of the anthracycline, doxorubicin, and the PARP-1

inhibitors appears particularly effective in p53-deficient breast cancer lines (Munoz-Gamez et al., 2005). In this context, PARP inhibition might be of clinical value, as it may also prevent the cardiotoxic side effects of doxorubicin which currently limit its cumulative dosing in patients. More recently, collaboration between PARP1 and DNA topoisomerase II-associated proteins has also been discovered (Lis and Kraus, 2006).

Another recently recognized use of PARP-1 inhibitors could be in the context of enhancing cancer-cell killing in cells deficient in homologous recombination. Two complementary studies demonstrate that cells that are deficient in BRCA1 or BRCA2 are acutely sensitive to killing by PARP-1 inhibitors in vitro and in vivo (Bryant et al., 2005; Farmer et al., 2005). Although the majority of sporadic breast cancer is not deficient in either of these genes, these findings offer significant hope for specific treatment in patients whose tumours are shown to be deficient in BRCA1 or BRCA2, particularly patients with inherited breast cancer. A clinical trial is due to open to recruitment in the UK to accumulate early clinical data in such patient groups treated with a PARP-1 inhibitor developed by KuDOS Pharmaceuticals (Cambridge, UK).

A further potential application of PARP inhibitors in cancer treatment might be related to the enhancement of the cytotoxic effects of radiotherapy. Various classes of PARP-1 inhibitors have been shown to exert radiosensitizing effects in vitro (Brock et al., 2004; Noel et al., 2006; Veuger et al., 2003). The in vivo efficacy of PARP inhibition for radiosensitization has been demonstrated in a preclinical study, which found that the intraperitoneal administration of the PARP-1 inhibitor AG14361 enhanced the sensitivity of colon carcinoma subcutaneous xenografts to radiation therapy (Calabrese et al., 2004).

Several small molecule PARP-1 inhibitors have been synthesized, listed in Table 3. Most of the PARP targeting inhibitors have structural similarity to the natural substrate, oxidized nicotinamide adenine dinucleotide (NAD+), and therefore act as competitive inhibitors. The effects in preclinical models appear similar for most of the agents studied. For example, in preclinical models, NU1025 enhances the cytotoxicity of ionizing radiation, TMZ and topoisomerase inhibitors (Bowman et al., 2001; Delaney et al., 2000). Similar effects have been demonstrated with NU1064, NU1085 and AG14361 (Agouron Pharmaceuticals, Inc, La Jolla, CA, USA), GPI15427 (Guilford Pharmaceutical

Inc., Baltimore, MD, USA) and CEP-6800 (Cephalon, Inc., West Chester, PA, USA) (Madhusudan and Middleton, 2005).

One general concern relating to systemic treatment with PARP-1 inhibitors is the impairment of DNA repair in normal cells, which might render them more susceptible to the toxic effects of chemotherapy or radiotherapy. Such an effect would not improve the therapeutic index and would not represent a significant advance in improving existing treatments. Although TMZ works primarily against actively proliferating cells, the combination of TMZ and PARP-1 inhibitors can be toxic to resting cells (Tentori et al., 2001). Although no significant toxicities have been reported so far, clinical trials must be conducted to carefully monitor early effects on normal tissues, particularly gut epithelium and bone marrow. It should also be noted that, analagous to the effects of radiation on certain normal tissues, covert toxicity to differentiated resting cells may not become apparent until months or years have passed since treatment with PARP-1 inhibitors.

5.3 Resistance to platinum agents

Cisplatin and carboplatin are amongst the chemotherapeutic agents most commonly used to treat solid cancers in humans, particularly in chemo-radiation strategies in which chemotherapy and radiation therapy are administered concomitantly. Platinum resistance, either intrinsic or acquired during cyclical treatment with the agent, is a major clinical problem since additional agents that can be added to therapy in order to circumvent tumour resistance do not currently exist. Biochemical studies have demonstrated that cells can become resistant to cisplatin by various cellular mechanisms, such as a reduction in the uptake of the drug, increased detoxification via interaction of the drug with intracellular thiols, and alterations in the ability of the cell to recognise and process cisplatin–DNA adducts. One current paradigm postulates that the DNA adducts produced by the platinum drugs are recognised by a detector, the detector activates a series of damage-responsive signal transduction pathways, and this in turn triggers apoptosis (Chaney et al., 2005). Failure of any one component of this sequential process may result in drug resistance.

Oxaliplatin is a third generation platinum compound that differs from cisplatin and carboplatin in that it has a broader spectrum of antitumour activity. Like cisplatin, it is employed as a radiosensitiser in chemo-radiation therapeutic strategies (Sharma et al., 2007). Although cisplatin and oxaliplatin form the same types of adducts at the same sites on the DNA (approximately 60–65% intrastrand GG, 25–30% intrastrand AG, 5–10% intrastrand GNG, and 1–3% interstrand GG diadducts), biochemical studies have suggested that oxaliplatin adducts are recognised and processed differently from those produced by the earlier generation platinum-containing drugs (Chaney et al., 2005; Eastman, 1987). Whereas nucleotide excision repair and recombination repair do not discriminate between the repair of cisplatin and oxaliplatin adduct, the following repair processes appear to discriminate between cisplatin-DNA adducts and oxaliplatin-DNA adducts: specific damage-recognition proteins and the translesion DNA polymerases (Chaney et al., 2005; Wu et al., 2004).

In particular, there is accumulating evidence for the concept that tumour resistance may be mediated by enhanced tolerance to adducts in DNA. Translesion DNA synthesis is a pathway of post-replication repair that allows replication of Pt–damaged DNA without removal of the adduct. Several mammalian DNA polymerases are capable of bypassing adducts engendered by oxaliplatin, which would normally arrest cellular replication if bypass does not occur (Havener et al., 2003; Vaisman and Chaney, 2000). Upregulation of levels of or enhanced activity of such enzymes would be expected to permit survival of cancer cells under conditions of "adduct load" that would normally be lethal.

A series of studies have demonstrated that human DNA polymerases β, γ, and η can bypass oxaliplatin (as well as cisplatin) adducts in human DNA. Both p53 and DNA mismatch repair proteins appear to play a role in modulating the extent to which bypass replication occurs (Avkin et al., 2006). The efficiency of bypass, measured by the primer extension assay, developed by Chaney and colleagues, is: human pol η > human pol μ > human pol β $\gg$ human pol γ $\sim$ yeast pol ζ (Havener et al., 2003; Vaisman and Chaney, 2000). Whereas Pol μ bypasses Pt–DNA adducts in a mostly error-prone manner and catalyzes a high percentage of frame-shifts in the vicinity of the adduct, the

other translesion polymerases predominantly catalyze misinsertions opposite Pt–DNA adducts (Havener et al., 2003; Vaisman and Chaney, 2000). Of the human DNA polymerases tested to date, both pol η and pol β bypass oxaliplatin–GG adducts with higher efficiency than cisplatin–GG adducts (Bassett et al., 2003; Havener et al., 2003). The efficiency and fidelity of bypass by these translesion polymerases is likely to affect the mutagenicity of the adducts. The potential mutagenicity of translesion synthesis is of note since it may be an important factor in genomic instability and clonal evolution of cancers during treatment with oxaliplatin-based combination chemotherapy.

Recent in vitro studies have suggested that targeting DNA polymerases involved in translesion synthesis past cisplatin and oxaliplatin adducts may overcome intrinsic or acquired tumour resistance to chemotherapy. Certain investigators have demonstrated a general requirement for pol η expression in providing tolerance to oxaliplatin, cisplatin and carboplatin using xeroderma pigmentosum variant (XPV) cell lines (polymerase η-null) grown in vitro and transfection techniques (Albertella et al., 2005a). In certain cell types, the absence of pol η expression resulted in a reduced ability to overcome cisplatin-induced S phase arrest. Other researchers have compared normal human fibroblasts (NHF1) with XPV cells after treatment with cisplatin, as well as comparing XPV cells complemented for polymerase 71 expression with the isogenic cells carrying the empty expression vector (Bassett et al., 2004). Cytotoxicity and induced mutagenicity experiments were performed, demonstrating that equitoxic doses of cisplatin induced mutations in fibroblasts lacking polymerase η at frequencies 2- to 2.5-fold higher than in fibroblasts with either normal or high levels of polymerase η. Collectively, these findings in cells grown in vitro demonstrate the role of polymerase η in translesion synthesis and potential mutagenicity and therefore identify it as a target for intervention in order to inhibit the development of platinum resistance during cancer therapy.

Conclusions

Although extensive knowledge currently exists regarding the biochemical mechanisms involved in human BER, the cellular functions of specific BER pathways remains relatively unknown. For example, the effect of the cell cycle on the availability and activity of BER enzymes remains poorly understood. The ability of cancer cells to evolve or adapt subpathways of BER in order to survive potentially mutagenic insults, such as treatment with platinum agents, requires particular investigation. The discrepancy between the studies of E.coli *MutY* in preclinical models and the findings regarding its human homologue hMYH in patients with adenomatous polyposis coli, resulting in the description of a new disease entity, emphasizes the need for translational research, particularly from bedside back to bench, in order to discover the link between biochemical knowledge of BER and human carcinogenesis. Early clinical trials of agents targeting specific BER enzymes, such as PARP1, APE1 or DNA polymerases, represent a valuable opportunity to obtain tissues for the analysis of BER mechanisms in human tissues, which may ultimately result in the translation of biochemical knowledge into effective therapies for cancer.

Acknowledgement

RAS is supported by Cancer Research UK. GLD's research is supported by the UK Medical Research Council.

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Table 1: Mammalian DNA glycosylases

Glycosylase	Bifunctional?	Substrates	References
UNG2	No	U	(Krokan et al , 2001; Olsen et al, 1989)
SMUG1	No	U, OHMeU	(Boorstein et al., 2001; Haushalter et al., 1999)
TDG	No	**U**:G, **T**:G, ethenoC	(Neddermann et al., 1996; Wiebauer and Jiricny, 1990)
MBD4	No	**U**:G, **T**:G in CpG sites	(Hendrich et al., 1999; Petronzelli et al., 2000)
OGG1	Yes	**8oxoG**:C, FapyA, FapyG	(Radicella et al., 1997; Roldan-Arjona et al., 1997; Rosenquist et al., 1997)
MYH	No	**A**:8oxoG	(Slupska et al., 1996; Slupska et al., 1999)
NTH1	Yes	Oxidised and fragmented pyrimidines (e.g. Tg, DHU), Fapy	(Aspinwall et al, 1997; Ikeda et al, 1998)
AAG (MPG)	No	3-meA, 7-meG, ethenoA, ethenoG, hypoxanthine	(Chakravarti et al., 1991; O'Connor and Laval, 1991; Samson et al., 1991)
NEIL1	Yes	FapyA, FapyG, dihydrouracil, thymine glycol, 8-oxoG (low)	{Jaruga et al., 2004; Rosenquist et al., 2003)
NEIL2	Yes	Cytosine oxidation products (5-OHU, DHU)	(Dou et al., 2003)

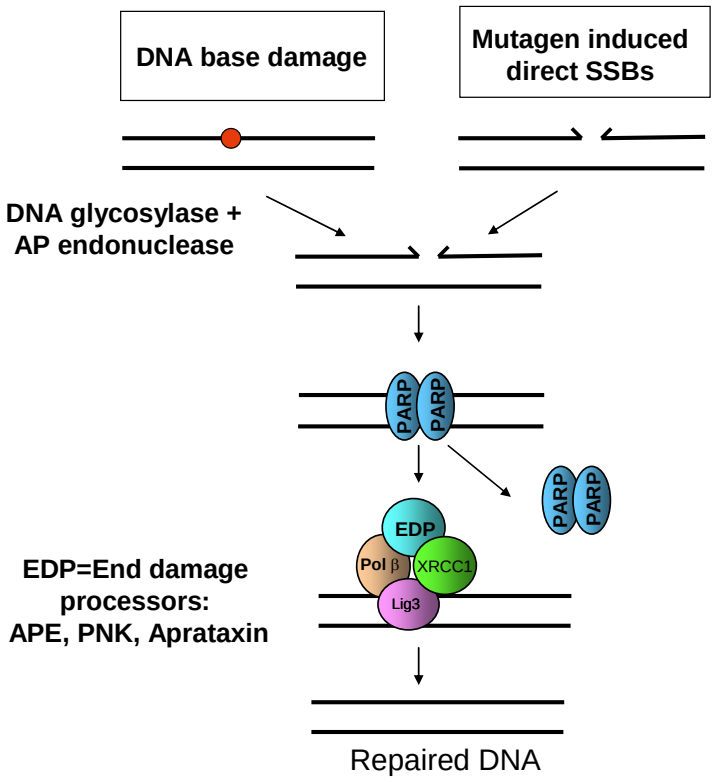

Figure 1. Model for base excision repair.

Damaged base processing is initiated by a specific DNA glycosylase, which removes the damaged base creating an abasic site (AP site). AP endonuclease (APE) then cleaves the phosphodiester bond 5' to the AP site containing 3'-OH and 5'-sugar phosphate ends. Both AP endonuclease induced and direct strand break are recognized by PARP-1 dimer. Following NAD^+-dependent poly(ADP-ribosyl)ation of PARP-1, automodified PARP-1 then dissociates from DNA. The release of automodified PARP-1 exposes SSB to the end damage processors (EDP), DNA polymerase β (Pol β) and XRCC1-DNA ligase IIIα that perform the remainder of the repair. After "cleaning" of the SSB ends (if required) Pol β than adds one nucleotide to the 3'-end and DNA ligase complete the repair by sealing DNA ends.

Table 2. PARP inhibitors currently in clinical trials being developed as potential treatments for cancer

Company [1]	PARP Inhibitor	Route of administration	Phase of Trial (country)	Subject Group
Pfizer (New York, USA)	AG-014699 + temozolomide antibody (C595)	Intravenous	Phase I (UK)	Patients with malignant melanoma
	AG-014699 + temozolomide	Intravenous	Phase II (UK)	Patients with malignant melanoma
KuDOS (Cambridge, UK)	KU 59436	Oral	Phase I (UK+Netherlands)	Patients with advanced solid tumors
Inotek (Beverly, MA, USA)	INO-1001	Intravenous	Phase IB (USA)	Patients with advanced solid tumors
	INO-1001 + temozolomide	Intravenous	Phase I (USA)	Pateints with glioblastoma multiforme
	INO-1001	Intravenous	Phase II (USA)	Acute coronary indications
Bipar Sciences (Brisbane, CA, USA)	BSI-201	Intravenous	Phase I (USA)	Patients with advanced solid tumors
MGI Pharma (Bloomington, MN, USA)	GPI 21016 + temozolomide	Oral	Phase I proposed (USA)	Patients with advanced solid tumors
Abbott Laboratories (Chicago, IL, USA)	ABT-888	Oral	Pilot study (USA)	Patients with advanced solid tumors and lymphoid malignancies

[1] Websites for source data: http://www.pfizer.com/pfizer/main.jsp; www.**kudos**pharma.co.uk/; http://www.inotekcorp.com/; www.**mgipharma**.com/; www.**abbott**.com/; www.biparsciences.com