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Targeting DNA base excision repair: a new strategy for personalised cancer therapy

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Introduction

Advanced cancer is a leading cause of death in the developed world.[1] Chemotherapy and ionising radiation are the two main treatment modalities currently available to improve outcomes in patients with disseminated malignancy. The cytotoxicity of many of these agents is directly related to their propensity to induce genomic DNA damage. However, the ability of cancer cells to recognise this damage and initiate DNA repair is an important mechanism for therapeutic resistance that negatively impacts upon treatment efficacy. DNA repair constituents may be useful as biomarkers to predict tumour response to treatment and improve outcome prognostication. Pharmacological inhibition of DNA repair pathways has the potential to enhance cytotoxicity of a diverse range of anticancer agents and overcome treatment resistance. The use of inhibitors of DNA damage pathways also seems to provide an exciting opportunity to target the genetic differences that exist between normal and tumour tissue.

DNA repair in cancer

Genomic DNA is at continuous risk of damage from spontaneous base lesions, metabolic by-products, and exogenous sources such as ultraviolet light, ionising radiation and chemical agents. This damage can result in non-canonical base pairing at replication, leading to the propagation of potentially mutagenic lesions. Mammalian cells have highly conserved DNA damage sensor mechanisms that result in several possible cellular responses to potentially carcinogenic insults, including damage tolerance, apoptosis and initiation of DNA repair.

The range of potential DNA lesions and adducts is broad, and hence a number of DNA repair pathways have evolved (Table 1). So critical are these repair pathways that mutations within constituent genes are associated with several cancer predisposition syndromes, such as hereditary non-polyposis carcinoma coli (HNPCC) caused by a mismatch repair defect, or *BRCA*-mutated breast and ovarian cancer syndromes, which are underpinned by deficient double-strand break repair. Polymorphisms in DNA repair genes have been identified and may confer suboptimal DNA repair capacity, influencing cancer susceptibility. Sporadic mutations in repair genes have also been implicated in early carcinogenesis by influencing the rate of acquisition of further mutations and so increasing the risk of malignant transformation – the 'mutator phenotype' hypothesis.

Table 1. Major DNA repair pathways in eukaryotes.

Repair pathway	Target lesion
Direct repair	• Reversal of specific base lesions (eg pyrimidine dimers and guanine methylation)
Base excision repair (BER)	• Base lesions induced by oxidation, alkylation and ring saturation
Nucleotide excision repair (NER)	• Bulky, helix-distorting base adducts
Mismatch repair (MMR)	• Mispaired bases introduced during replication
Non-homologous end joining (NHEJ)	• Non-replication-associated double-strand breaks (DSBs)
Homologous recombination (HR)	• Replication-associated DSBs

Targeting base excision repair for therapy

Base excision repair (BER) is responsible for detection and repair of damage caused by a number of mechanisms, including alkylation, oxidation, ring saturation, single-strand breaks and base deamination. Although complex, with at least two sub-pathways, BER generally proceeds via recognition and removal of a damaged base by a DNA glycosylase to form an abasic site intermediate, cleavage of the phosphodiester backbone 5' to the abasic site by apurinic/apyrimidinic endonuclease 1 (APE1), removal of the 5' sugar fragment, incorporation of the correct base by a DNA polymerase, and sealing of the strand break by a DNA ligase (Fig 1).

Failure by BER to repair an abasic site results in persistence of a single-strand break (SSB) at the site of damage. When encountered by the replication machinery during the S-phase, persistent SSBs cause replication fork collapse, with subsequent conversion of the SSB to a double-strand break (DSB). If DSBs occur at high frequency, the cell will deem the damage to be irreparable and apoptosis will be induced – forming the mechanistic basis of many DNA-damaging agents. It has been hypothesised that inhibiting critical enzymes within DNA repair pathways will

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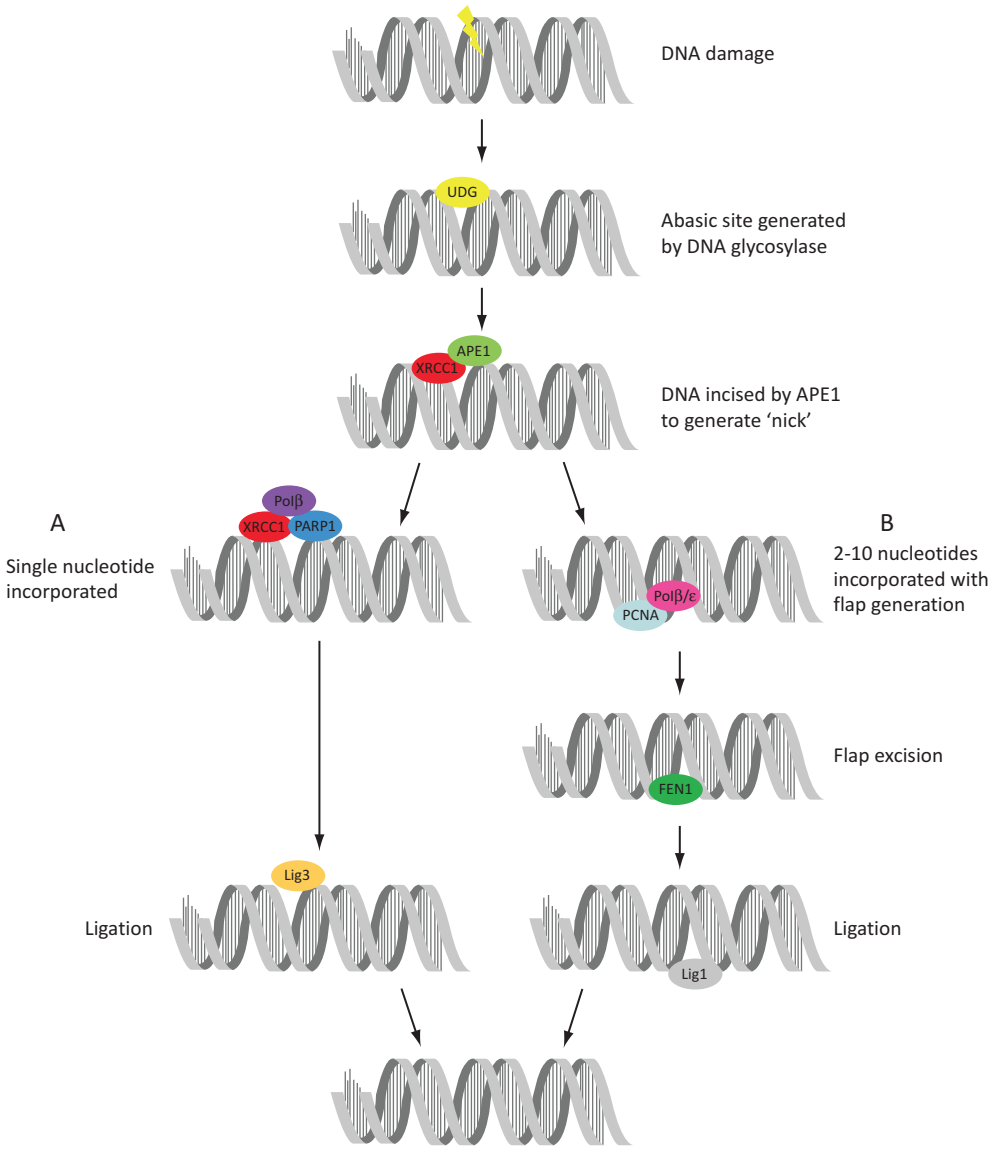

Fig 1. Base excision repair. APE 1 = apurinic/apyrimidinic endonuclease; Fen1 = flap endonuclease 1; Lig = ligase; PARP = poly (adenosine diphosphate (ADP) ribose) polymerase; PCNA = proliferating cell nuclear antigen; Pol = polymerase (DNA-directed); UDG = uracil-DNA glycosylase; XRCC1 = X-ray repair complementing defective repair in Chinese hamster cells 1.

increase the volume of damage caused by such chemotherapy drugs, hence increasing their efficacy.

The first BER protein to be identified as a potential therapeutic target was poly (adenosine diphosphate (ADP) ribose) polymerase 1 (PARP1), which plays an important role in SSB repair (SSBR), a subpathway related to BER. Binding of PARP1 at the sites of SSBs stabilises the DNA ends and recruits downstream repair proteins. Modified expression of PARP1 in cancer cell lines and tumour xenografts enhances sensitivity to DNA-damaging agents. This has led to the development of small molecule inhibitors, which exhibit synergism of cytotoxicity in combination with chemotherapy agents and ionising radiation, and also function as single agents in certain DNA repair-deficient tumour cell lines such as *BRCA*-deficient breast and ovarian cancers (see 'Synthetic lethality as a treatment strategy' below). A number of PARP inhibitors have shown promise in early clinical trials, although more recent results have been disappointing. In January 2011, it was reported that iniparib had failed to progress through phase 3 evaluation in combination with gemcitabine and paclitaxel in metastatic 'triple negative' (negative for oestrogen, progesterone and HER2 receptors) breast cancer after failing to meet primary survival endpoints, although subgroup analysis did indicate a survival benefit when the combination was used as second- or third-line treatment. Furthermore, olaparib was withdrawn from planned phase 3 evaluation after interim analysis of phase 2 data indicated that it was unlikely to offer an overall survival benefit in the treatment of serous ovarian cancer.

Despite these failures, interest in targeting the BER pathway remains strong. The body of preclinical data is robust, supporting the rationale of targeting DNA repair. Iniparib has recently been found to be a weak inhibitor of PARP1, which likely exerts its effects via an alternative mechanism, so other compounds may offer improved efficacy. In addition, the patient population of the iniparib trial had a high level of genetic heterogeneity that may have influenced outcomes, and reassessment of patient selection criteria using improved biomarker analysis may help maximise outcomes in future trials.[2] Furthermore, alternative BER targets may offer improved specificity and efficacy given that PARP1 is both multifunctional, with further roles in gene transcription and chromatin modulation, and belongs to a family of about 20 related PARP proteins, many of which have not been functionally characterised.

Apurinic/apyrimidinic endonuclease as a new BER target

Apurinic/apyrimidinic (AP) endonuclease (APE1) is a critical BER protein of 35 kDa encoded by a 2.6 kb gene located on

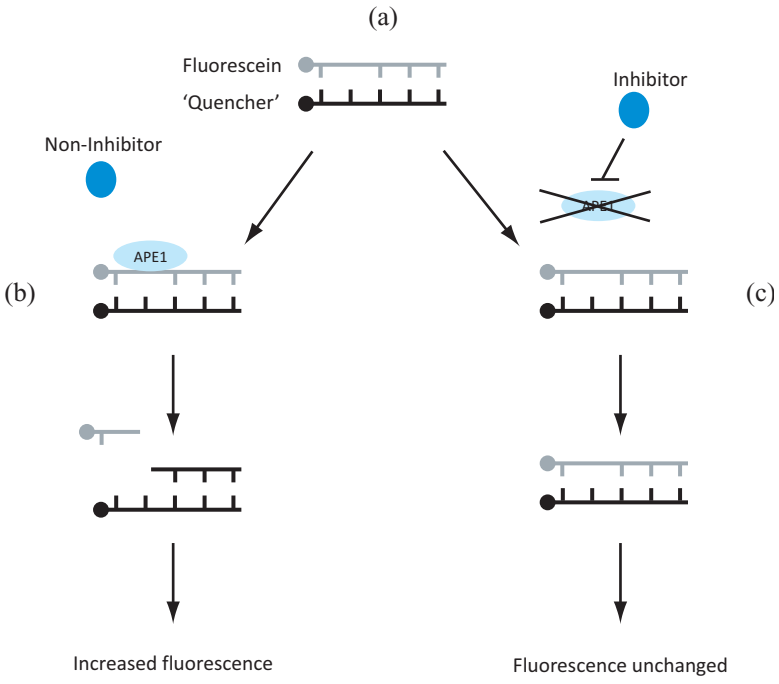

Fig 2. Fluorescence-based assay to identify potential small molecular inhibitors of apurinic/apyrimidinic endonuclease (APE1). (a) The assay substrate is a short DNA duplex containing a fluorescein molecule. While remaining in close proximity to the complementary strand, fluorescence emission is absorbed by the 'quenching' Dabcyl molecule. (b) An abasic site is present adjacent to the fluorescein molecule. Uninhibited APE1 cleaves the DNA backbone at this site, allowing dissociation of the fluorescein molecule. This is associated with a quantifiable increase in fluorescence. (c) Inhibition of APE1 prevents DNA cleavage and fluorescence remains quenched.

chromosome 14q11.2-12. It is a multifunctional protein, accounting for 95% of all endonuclease activity in human cells and also possessing structurally distinct roles in redox regulation of transcription factors, acetylation-mediated gene regulation and RNA quality control.[3]

APE1 has been implicated as a prognostic biomarker in cancer. Upregulation is a common feature of many advanced malignancies, with higher levels associated with more aggressive phenotypic features and poor survival outcomes. This upregulation may be the result of the hypoxic and acidic tumour microenvironment found in advanced disease, which necessitates efficient repair of increased rates of DNA damage induced by the associated high levels of intracellular reactive oxygen species. Overexpression of APE1 may also have a role in treatment response. The efficacy of ionising radiation and many chemotherapeutic agents is dependent on the ability to induce overwhelmingly cytotoxic levels of DNA damage within malignant cells. Efficient repair of this damage therefore impacts negatively upon treatment response. Immunohistochemical analysis of tumour specimens of various origins indicates that high intrinsic expression of APE1 is commonly associated with a poor response to treatment. This has been confirmed *in vitro*, where engineered overexpression of APE1 in cancer cell lines is associated with resistance to DNA-damaging agents compared with wildtype control cells. Furthermore, treatment-induced APE1 overexpression may play a role in the development of drug resistance. In lung cancer cells, cisplatin is able to induce overexpression of APE1 in a dose-dependent manner, resulting in a reduced cytotoxic response on recurrent exposure.[4]

Based on these prognostic implications of overexpression, APE1 has emerged as a possible target for therapeutic inhibition. Preclinical evidence supports this approach. *In-vitro* downregulation using antisense or small inhibitory RNA (siRNA) strategies induces abasic site accumulation, S-phase arrest and apoptosis in several cancer cell lines. Furthermore, APE1 depletion in cell and mouse models potentiates the effect of various DNA-damaging agents, including hydrogen peroxide, the alkylating agent methane methylsulphonate (MMS), and anticancer therapies such as temozolomide, cisplatin and ionising radiation.

A number of groups have reported the identification of small molecule inhibitors of APE1. A commonly used strategy employs a high-throughput fluorescence-based assay of a physical library of several thousand compounds (Fig 2).[5] An alternative approach has utilised knowledge of the crystal structure of the APE1 active site to strategically design inhibitor templates that are applied in a virtual screen of much larger compound libraries.[6] Using these strategies, a number of potential inhibitors have been identified and are currently undergoing preclinical evaluation.

Two compounds with APE1 inhibitory activity have been evaluated in phase 1 clinical trials. Lucanthone is a topoisomerase II inhibitor that may also inhibit APE1 by binding at the active site and subsequent protein cleavage. In common with APE1 gene knockdown and small molecule protein inhibition, lucanthone is able to potentiate the cytotoxic effect of alkylating agents in human cancer cell lines. Clinically, it has been evaluated as a radiosensitiser and seems to accelerate the regression of brain metastases following whole brain radiotherapy. However, it is unclear whether this effect is mediated via APE1 blockade or its topoisomerase activity.[7]

Methoxyamine is an indirect inhibitor of APE1 that irreversibly binds to abasic DNA sites. In preclinical evaluation, it is able to potentiate temozolomide in cancer cell lines and tumour xenografts. It is under evaluation in phase 1 trials in combination with either pemetrexed or temozolomide.

Synthetic lethality as a treatment strategy

Using BER inhibitors in combination with DNA-damaging agents may improve treatment efficacy and overcome drug resistance. However, indiscriminate downregulation of BER in tumour and normal cells may increase drug toxicity and limit the available dose range. One possible approach might involve targeting the damaging agents – for example, by utilising

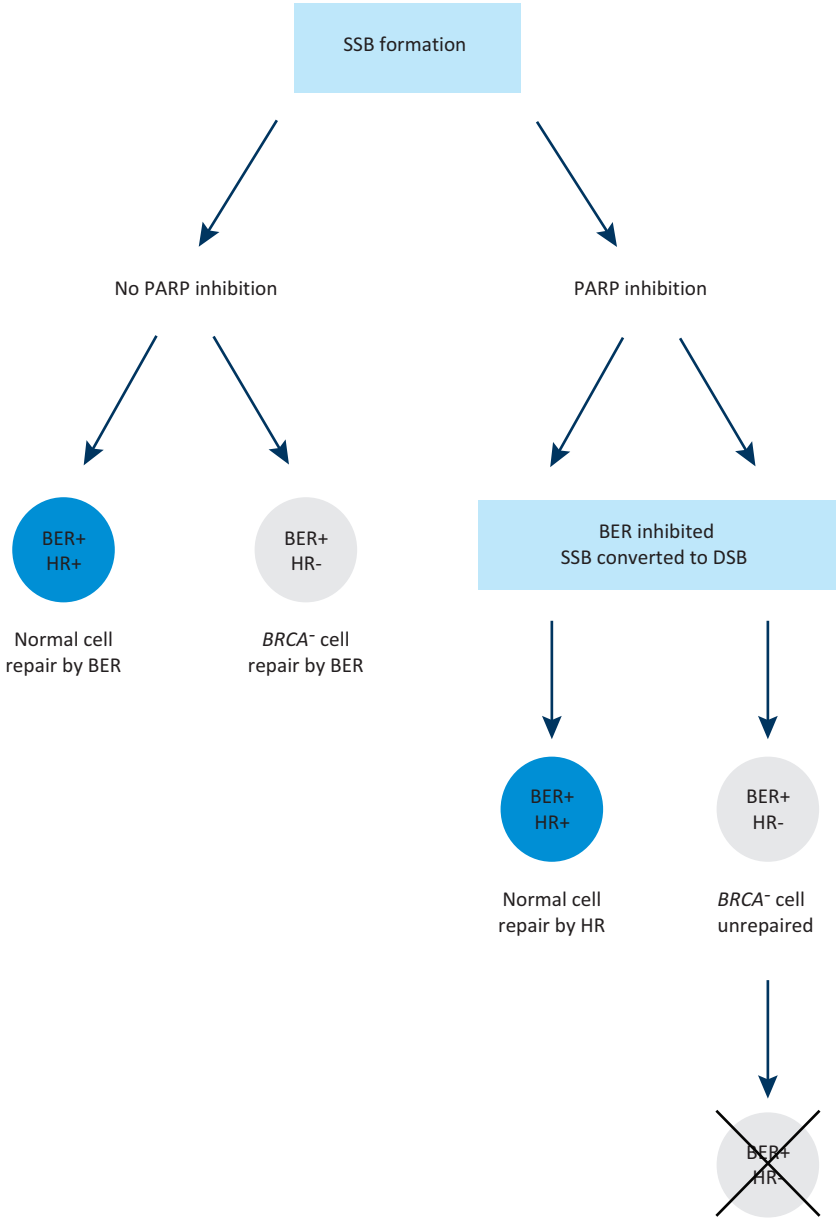

Fig 3. Synthetic lethality in *BRCA* deficiency. BER = base excision repair; DSB = double-strand break; HR = homologous recombination; PARP = poly (adenosine diphosphate (ADP) ribose) polymerase; SSB = single-strand break.

ionising radiation to limit DNA damage in normal cells. Alternatively, limiting the use of BER inhibitors to patients with histological evidence of BER protein overexpression may also reduce the toxicity in normal cells.

An exciting alternative approach involves employing a synthetic lethality strategy, which has been employed in many early clinical trials of PARP inhibitors. Synthetic lethality exploits intergene relationships in which the loss of function of either of two related genes is non-lethal but loss of both causes cell death. This offers the potential to specifically target cancer cells through inhibition of a gene known to be in a synthetic lethal relationship with a mutated tumour suppressor gene.

Particular focus in the development of PARP inhibitors has been in the setting of *BRCA1*- and *BRCA2*-deficient breast and ovarian cancers. *BRCA1* and *BRCA2* have long been identified as tumour suppressors, being mutated in an inherited cancer predisposition syndrome that increases susceptibility to breast and ovarian tumours. Both gene products have a role in the homologous recombination (HR) DNA repair pathway, which repairs DSBs. Inhibitors of PARP can be used to specifically target HR-deficient cells by taking advantage of the synthetic lethality relationship that exists between HR and the PARP1 target pathway SSBR. Inhibition of PARP1 blocks SSBR, causing persistence of SSBs that results in replication fork collapse with subsequent conversion to DSBs. In normal cells (including heterozygosity at a *BRCA* allele, which is associated with wildtype HR efficiency), these DSBs are repaired via the HR pathway. In *BRCA*-deficient tumour cells, however, loss of effective HR leads to DSB persistence, with subsequent cell cycle arrest and apoptosis (Fig 3). Preclinical evaluation has consistently demonstrated synthetic lethality in *BRCA*-deficient cell lines and tumour xenografts when treated with specific inhibitors of PARP1.[8,9] A number of phase 2 trials of a single-agent PARP inhibitor in *BRCA*-deficient tumours have demonstrated favourable efficacy and limited toxicity (reviewed in reference 10) although no phase 3 trials in this setting have yet been initiated.

New approaches to synthetic lethality

Beyond PARP1, BER targets are under exploration for use in a synthetic lethality approach. Confirmation that APE1 and DSB repair share a synthetic lethality relation is evidenced by the enhanced cytotoxicity of DSB repair inhibitors in APE1 knockout cell lines. Our laboratory has identified a number of specific and potent APE1 inhibitors that exhibit synthetic lethality in *BRCA2*-deficient Chinese hamster ovary cells.[6,11] An alternative approach in sporadic tumours might take advantage of cytotoxicity induced by APE1 inhibition in cell lines cultured in acidic environments. Tumour microenvironments are often acidic and have been associated with upregulation of BER proteins, including APE1. Conversely, other DNA repair mechanisms, including HR, are often downregulated under such conditions. Identification of tumours with BER upregulation and HR depletion may therefore offer an opportunity to exploit synthetic lethality through APE1 inhibition.[12]

Although PARP inhibitors show promise in *BRCA*-deficient tumours, these are a small subset of all malignancies. Alternative mechanisms of DSB repair downregulation are under investigation for a synthetic lethality relationship with BER. Recently, germline mutations in the HR protein Rad51D have been identified as conferring susceptibility to ovarian cancer and may offer a target for BER inhibitors in a small subset of women. Alternatively, '*BRCA*-ness' refers to a subset of breast cancers, including triple negative and 'basal phenotype' cancers, that possess molecular and histopathological similarity to *BRCA*-deficient tumours and that may successfully be targeted by BER inhibition. A number of exploratory biomarker studies are currently underway in conjunction with clinical trials involving DNA repair inhibitors to validate alternative synthetic lethality targets.

Conclusions

Chemotherapy and radiotherapy, the main treatment modalities available in the management of advanced cancer, commonly rely upon the overwhelming induction of DNA damage to exert a cytotoxic effect. A cancer cell's ability to resist this damage, via several DNA repair mechanisms, directly impacts upon the response to treatment. Modulation of DNA repair is an exciting strategy in cancer therapeutics and offers the possibility of improving the efficacy of existing DNA-damaging agents or overcoming previous treatment resistance. Furthermore, DNA repair inhibition may allow targeted cytotoxicity by exploiting the genetic differences between normal and tumour cells developing a 'synthetic lethality' strategy.

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