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EVIDENCE FOR BASE EXCISION REPAIR PROCESSING OF DNA INTERSTRAND CROSSLINKS

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Abstract

Many bifunctional alkylating agents and anticancer drugs exert their cytotoxicity by producing cross links between the two complementary strands of DNA, termed interstrand crosslinks (ICLs). This blocks the strand separating processes during DNA replication and transcription, which can lead to cell cycle arrest and apoptosis. Cells use multiple DNA repair systems to eliminate the ICLs. Concerted action of repair proteins involved in Nucleotide Excision Repair and Homologous Recombination pathways are suggested to play a key role in the ICL repair. However, recent studies indicate a possible role for Base Excision Repair (BER) in mediating the cytotoxicity of ICL inducing agents in mammalian cells. Elucidating the mechanism of BER mediated modulation of ICL repair would help in understanding the recognition and removal of ICLs and aid in the development of potential therapeutic agents. In this review, the influence of BER proteins on ICL DNA repair and the possible mechanisms of action are discussed.

Keywords

AP endonuclease; Base Excision Repair; BCNU; Cisplatin; DNA glycosylase; Interstrand crosslinks; Mitomycin C; Nitrogen mustard; Oxaliplatin; Psoralen; DNA Polymerase beta

Introduction

Cellular DNA is under constant threat from endogenous sources such as reactive oxygen species (ROS) and exogenous sources such as environmental oxidants, alkylating agents and anticancer drugs. The most common DNA lesions are base modifications such as alkylation, oxidation, loss of bases and single strand breaks. Complex and more toxic lesions include crosslinks and double strand breaks [1, 2]. Cells are endowed with the inherent capacity to respond to and eliminate these DNA lesions. The lesions are typically recognized and removed by various DNA repair pathways [3]. The base excision repair (BER) pathway as its name suggests is mainly involved in the excision of damaged bases from the DNA. It is

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Conflict of interest
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considered as the predominant repair system in the protection of cells against a broad range of small base lesions resulting from oxidation, alkylation and deamination [4]. The BER pathway is a highly conserved, multistep process which requires the concerted action of several proteins [5]. It has been estimated that cells encounter ~10000 damaged bases per day, most of which are removed by BER [6–8].

The initiation of BER occurs by the action of DNA glycosylases which recognize alterations to the DNA bases and remove the altered bases by hydrolyzing the N-glycosidic bond. Once the damaged base is removed by a glycosylase, the resulting sugar-phosphate backbone without the base is called an apurinic/apyrimidinic (AP) site [9, 10]. AP endonuclease1 (APE1) cleaves the phosphate backbone resulting in a nick with a 3′ hydroxyl group and 5′ deoxyribose phosphate (dRP) residue. The oxidation/reduction state of this 5′ deoxyribose is a crucial factor in determining the subsequent downstream processing. If the dRP is not oxidized/reduced, this will lead to the activation of the short-patch BER pathway with the recruitment of DNA Polymerase β (Pol β). The dRP is cleaved by the lyase activity of Pol β and the one nucleotide gap is also filled by Pol β. The final nick is subsequently ligated by the DNA ligase III and XRCC1 complex [10]. If there is any change in the oxidative state of the dRP residue, this leads to the inhibition of the lyase activity of Pol β and activation of other polymerase activity resulting in strand displacement which leads to a 2–10 nucleotide flap intermediate, which is cleaved by FEN1 and joined by DNA Ligase-I [11]. The latter process is termed long-patch repair and requires the action of PCNA [10]. In addition to the oxidized state of the dRP residue, lesion specificity, protein-protein interaction and cell cycle status can also influence the specific choice of BER sub-pathways [12, 13]. The nucleotide incision pathway (NIR) is suggested to be the backup of the BER pathway where Ape1 incises the damaged DNA independent of glycosylase cleavage [14].

Recent studies indicate that BER proteins have broad substrate specificity and they interact with each other to catalyze the repair of DNA lesions [15, 16]. However, in the context of drug therapy, effective BER can render cells resistant to alkylating agents by repairing the DNA adducts that would otherwise be cytotoxic [17, 18]. For example, BER repairs the DNA lesions induced by alkylating agents such as methyl methane sulphonate (MMS) and temozolomide and over expression of BER proteins enhance resistance to these drugs [19, 20]. Therefore, several attempts have been made to target the BER proteins to increase cell sensitivity to alkylating agents [21, 22]. Generation of knock-out mice and identification of small molecule inhibitors of BER proteins have proven to be useful tools to dissect the mechanisms of drug resistance. Several small molecule inhibitors of APE1, Pol β and PARP were tested extensively for their ability to enhance the cytotoxicity of alkylating anticancer agents and some of them have been successful in clinical trials [23–28]. BER proteins interact with proteins from other DNA repair pathways and this cross-talk/co-ordination has implications for combination therapy targeting two DNA repair pathways simultaneously [29, 30].

Interstrand crosslinks (ICLs)

DNA interstrand crosslinks are formed between both strands of DNA and these covalent links are highly toxic to cells [31, 32]. It has been shown that it takes only a single ICL to kill repair-deficient bacteria and yeast, and about 40 ICLs to kill repair-deficient mammalian cells [33, 34]. The ICLs form an absolute block to metabolic processes such as DNA replication and transcription, trigger cell cycle arrest and apoptosis, ultimately resulting in cell death [35]. In addition, ICLs are shown to cause mutations and genomic instability [36, 37]. Certain endogenous and environmental agents form DNA ICLs and the most important class of ICLs are chemotherapeutic agents such as nitrogen mustards (eg. melphalan),

nitrosureas (eg. BCNU), platinum agents (cisplatin, carboplatin, oxaliplatin, transplatin etc), mitomycin C and psoralen [38].

With continued exposure, cells develop strategies to eliminate these ICLs in order to survive [39]. However, enhanced repair of ICLs induced by chemotherapeutic agents in tumor cells is detrimental to the efficacy of the treatment [40–42]. Therefore, it is clinically important to elucidate the mechanism of elimination of the ICLs in order to develop strategies to overcome drug resistance. Because of its complexity, the repair of ICLs requires the concerted action of multiple DNA repair pathways [43]. It has been shown that nucleotide excision repair (NER) and homologous recombination (HR) as well as Fanconi Anemia (FA) proteins are involved in the repair of ICLs [44–47]. Translesion synthesis (TLS) can also occur across the ICLs where TLS polymerases bypass the processed (unhooked) ICL intermediates and the low fidelity of these lesion bypass polymerases increases mutations at the ICL site [36, 48]. The ICL repair events are shown to be both replication-dependent [49] and replication-independent [50]. The replication-dependent ICL repair occurs in S or G2 phase of the cell cycle [51, 52]. ICL repair is initiated by DNA replication fork collapse which activates signaling pathways for cell cycle arrest, to repair the DNA lesion [53]. When the damage is not repaired, the apoptotic signaling pathways are triggered to kill the cell [54]. Evidences also suggest that ICL repair occurs outside of S phase and does not require replication of DNA substrates [55, 56].

Several studies have shown that cells defective in DNA repair pathways such as NER and FA are hypersensitive to crosslinking agents, indicating the role of these pathways in the processing of ICLs [57, 58]. A recent model of ICL repair suggests that Mus81-Eme1 endonuclease cleaves 3′ of the ICL lesion on one strand and ERCC1-XPF cleaves 5′ of the lesion unhooking the crosslink [59, 60]. This can be repaired in a recombination-dependent manner using the undamaged sister chromatid [37, 61, 62]. When the undamaged template is not available (since ICLs affect both strands of DNA), translesion synthesis past the crosslink can play a role in the repair process. This recombination-independent repair is error-prone and mutagenic, and mainly occurs in non-dividing cells and in the G1 phase of dividing cells [63–65].

The ICLs distort the DNA double helix and distortion levels affect the recognition and repair of the ICLs. Each cross-linking agent forms different ICL DNA structures and therefore can influence the repair of these lesions [50]. For example, nitrogen mustard ICLs reside in the DNA major grove and do not affect hydrogen bonding of G-C, but ICLs induced by nitrosureas affect this base pair bonding [31, 66]. Psoralen ICLs create significant distortions to the DNA double helix, whereas mitomycin C ICLs are relatively non-distorting [67, 68]. Cisplatin ICLs bend and unwind the DNA significantly, where the cytosines adjacent to cross-linked guanines are flipped extrahelical and are exposed to the cellular environment [69–71]. Cisplatin analogues, oxaliplatin and transplatin also forms ICLs, but without extrahelical flipping of the bases [72, 73]. These differences between the crosslinks formed by ICL inducing agents have a significant impact on the way these adducts are recognized and repaired [74]. NER has been shown to be involved in the elimination of bulky DNA lesions. Studies by us and others have suggested that BER can play a role in the processing of bulky and structure distorting DNA lesions such as ICLs [75–77]. This review describes the possible role of the major BER proteins in the processing of ICLs *in vitro* and *in vivo*. Table 1 summarizes the list of BER proteins and their cytotoxic response to the ICL inducing agents.

Glycosylases

In BER, specific DNA glycosylases recognize corresponding damaged bases and cleave the N-glycosidic bond between abnormal bases and deoxyribose, leaving either an abasic site or a DNA single-strand break [78]. Several DNA glycosylases are identified in humans, which bind specifically to the modified base initiating the BER pathway. Cell survival assays display differential effects of glycosylases on the sensitivity of ICL inducing agents. 3-alkyladenine-DNA glycosylase (AAG), also called 3-methyladenine-DNA glycosylase (MPG) excises 3 methyladenine from DNA and it is the only alkyl specific DNA glycosylase present in mammalian cells to date. Studies show that *Aag* null mouse ES cells are sensitive to 1,3-bis(2-chloroethyl)-1-nitrosurea (BCNU), mitomycin C (MMC) and psoralen, but not to nitrogen mustards [79–81]. It was shown that AAG protects against BCNU- and MMC- induced apoptosis and chromosomal damage possibly through initiating the repair of the DNA adducts formed by these agents or oxidative DNA damage [80]. This indicates that a variety of damaged bases can be substrates for AAG. However, AAG has no incision activity on MMC and psoralen mono adducts or ICLs [80, 81], suggesting a non-enzymatic, structural role for AAG [75]. This may also be due to enhanced repair of these adducts by DNA repair pathways such as NER. Even though, bacterial and mammalian (human and rat) AAG excised mustard adducts [82], the absence of AAG in mouse did not show mustard hypersensitivity [80]. These observations collectively indicate species specificity and agent/drug specificity in AAG initiated BER. Further studies are warranted to explore the possibility of species specificity. Decreased and delayed formation of psoralen ICL-induced DNA double strand breaks in the absence of AAG indicates its possible role in ICL repair. However, the exact mechanism is not understood. It was suggested that AAG does not prevent conversion of psoralen mono adducts into ICLs. Instead, it might enhance ICL repair by binding to an ICL intermediate either in the early or later stages of ICL DNA processing [81]. The ICL inducing agents also form mono adducts and intrastrand adducts, thus making it difficult to infer the types of adducts repaired by AAG. Interestingly, while ES cells are hypersensitive to BCNU and MMC, bone marrow cells showed equal sensitivities [83] and neuronal cells showed low level resistance to nitrogen mustard [84] in the absence of AAG. In human cervical cancer cells, down regulation of AAG resulted in BCNU hypersensitivity [85]. It is important to note that AAG is over expressed in cervical neoplasia while neurons exhibit relatively lower levels of AAG. This indicates that lineage and cell type specificity, and expression levels of AAG can also influence crosslink sensitivity and the initiation of BER.

Neil1 is the human homologue of the *E.coli* oxidized base-specific DNA glycosylase/endonuclease VIII (Nei) and acts on oxidized, saturated, and ring-fragmented bases. Depletion of Neil1 in HeLa cells results in hypersensitivity to psoralen [76]. A recent study suggested that hypersensitivity of FA cells to ICL could be partly due to Neil1 deficiency and over expression of Neil1 in these cells enhanced resistance to ICL agents [86]. In mammalian cells, uracil residues in DNA are rapidly removed by uracil-DNA glycosylase (UNG). The expression of UNG is deregulated in cancer and it has been shown that cisplatin treatment up regulates UNG expression and activity in lung cancer cell lines [87]. We have shown that loss of UNG results in a cisplatin resistant phenotype and enhanced cisplatin ICL repair in UNG null mouse embryonic fibroblasts [77]. Knockdown of human NTH1, the bifunctional DNA glycosylase/apurinic/apyrimidinic lyase enhanced sensitivity to cisplatin, but had no effect on MMC cytotoxicity. Cisplatin, but not MMC induces oxidative stress resulting in the generation of oxidized bases which are substrates for NTH1[88]. Ape1 was shown to incise the abasic sites generated by the removal of uracil by UNG from cisplatin ICL DNA substrates [77]. It would be interesting to determine if bifunctional glycosylases with AP lyase activity could process cisplatin ICLs without the requirement of Ape1 activity. The glycosylase substrates if left unrepaired are less cytotoxic to cells, however,

enhanced levels of glycosylases result in the accumulation of more toxic abasic sites and single-strand breaks which overwhelm the cells. Over expression of OGG1 (8 oxoguanine DNA glycosylase) in mitochondria increased the sensitivity of cancer cells to cisplatin treatment [89]. Extensive mitochondrial damage, increased production of intracellular free radicals and enhanced apoptosis contributed to the observed hypersensitivity. However, sensitivity of MMC, cisplatin or nitrogen mustards was not affected by MPG over expression [90, 91].

For decades, it was assumed that glycosylases excise only spontaneously formed, oxidized, and alkylated bases. However, recent studies indicate that ICLs formed between the two strands of DNA can also be substrates for the BER pathway. Couve-Privat and Couve et al have shown that Neil1 excises cross-linked thymine bases of the unhooked psoralen ICLs *in vitro* and this acts as a substrate for further BER processing. We have demonstrated that cisplatin ICLs with a flanking uracil base can be a substrate for the BER machinery [77]. UNG removed the uracil bases and the resulting AP site was cleaved by APE1. In addition, we have shown that depletion of UNG enhanced cisplatin ICL repair consistent with BER playing a positive role in mediating cisplatin cytotoxicity. Collectively, these studies suggest a possible role for DNA glycosylases in the processing of ICL DNA substrates as an alternate to the classic ICL-DNA repair pathways. The ICL substrate specificity and the capacity of other DNA repair systems to compensate for the loss or deregulation of BER can influence the cytotoxicity of an ICL inducing agent. Figure 1 depicts possible models for BER processing of cisplatin ICL and psoralen ICLs initiated by UNG and Neil1, respectively.

Endonucleases

Ape1/Ref1 performs complex functions in cells through redox dependent and independent mechanisms. It acts as a transcriptional coactivator, regulates apoptosis, proliferation, differentiation, and production of ROS. In the BER pathway, it acts as an endonuclease that cleaves the abasic sites generated by the action of DNA glycosylases [92, 93]. Over expression of Ape1 has been observed in cancer cells and tumor samples which serves as a diagnostic and prognostic marker and also correlates with clinical outcome of chemotherapy [94–99]. High level expression of Ape1 has been shown to be associated with cisplatin resistance in head and neck and lung cancer [98, 100] but not in ovarian cancer [101]. Accordingly, down regulation of Ape1 enhanced cisplatin sensitivity in lung adenocarcinoma cells [100] and over expression protected melanoma cells from cisplatin-induced apoptosis [102]. This anti-apoptotic effect of Ape1 is suggested to be mediated by redox activation of cell survival signals as cisplatin induced apoptosis is mediated by ROS production. Targeting Ape1 also enhanced sensitivity to BCNU, MMC and psoralen in cancer cells and accumulation of unrepaired AP sites in the absence of Ape1 is likely responsible for the observed hypersensitivity [76, 95, 103]. However, expression of a dominant-negative form of Ape1 sensitized CHO cells to BCNU, but not to melphalan, cisplatin and MMC [104, 105]. It is not clear whether agent specificity or species specificity plays a role in Ape1 reactivity. Madhusudan et al have shown that CRT0044876, an inhibitor of Ape1 did not enhance cellular sensitivity to nitrogen mustard [25]. Methoxyamine, a small molecule inhibitor which binds to AP sites and inhibits Ape1 cleavage potentiated the cytotoxicity of BCNU and MMC due to accumulation of persistent AP sites and possible generation of DSBs [77, 106]. However, in our recent study, methoxyamine enhanced cisplatin resistance through enhanced repair of cisplatin ICLs in mouse embryonic fibroblasts and human cancer cells [77].

Ape1 is predominantly expressed in the nucleus and its over expression has been shown to result from increased cytoplasmic localization. Moore et al suggested that translocation of

nuclear enzyme into cytoplasm could affect the nuclear DNA repair capacity which implies that sub-cellular compartmentalization contributes to the functions of Ape1 [97, 107–109]. It appears that both cytoplasmic and nuclear functions of Ape1 are essential as repair of AP sites also occur in the mitochondria. Consistent with this, studies indicate that both redox and DNA repair functions of Ape1are involved in mediating cytotoxicity to DNA damaging agents. It was suggested that independent of glycosylses, the NIR pathway can incise DNA lesions as a backup to the NER pathway [110]. Our recent study has shown that Ape1 can incise adjacent to a cisplatin ICL only if it contains an abasic site, which suggests that the glycosylase activity is required to create an abasic site flanking the cisplatin ICL for Ape1 cleavage [77]. Couve et al have shown that Ape1 together with Neil1 initiate an alternative repair pathway for bulky psoralen adducts [111]. These studies suggest a possible role for Ape1 in ICL DNA processing which is likely dependent on the specific ICL agent.

FEN1, a structure-specific endonuclease is involved in the long patch BER pathway that removes 5′ overhanging flaps and in the processing of 5′ ends of Okazaki fragments in lagging strand DNA synthesis. FEN1 has also been shown to facilitate HR by DSB processing [112]. Absence of FEN1 enhanced sensitivity to cisplatin, MMC and nitrogen mustard indicating a possible role for long patch BER in ICL processing, inhibition of DNA replication processing resulting in the inhibition of ICL repair or possibly involvement of FEN1 in HR processing of ICLs [113–115]. Over expression of FEN1 is observed in several cancers [116–119] and down regulation of FEN1 enhanced cisplatin sensitivity and apoptosis in glioma cells [113]. Mutations in FEN1 is associated with cancer and mouse embryonic fibroblasts harboring a E160D mutation identified in human cancer shows deficiency in nuclease activities and undergoes MMC-induced apoptosis [114]. Additionally, expression of nuclease deficient FEN1 enhanced cisplatin sensitivity compared to wt FEN1 suggesting the requirement of the nuclease activity to elicit resistance to cisplatin [120]. However, loss of FEN1 in chicken DT40 cells did not affect cisplatin sensitivity [43]. Yeast Rad27 mutant (homologue of human FEN1) did not affect DSB formation following nitrogen mustard treatment which shows that Rad27 is not involved in the repair of ICL-induced DSBs [115]. FEN1 is localized to nuclear repair foci in response to DNA damage. Cisplatin and MMC treatment results in recruitment of FEN1 to sites of arrested replication foci [120, 121]. FEN1 co-localizes with WRN at arrested replication forks following MMC treatment [122]. Cisplatin induced FEN1 localization is dependent on the BRCA1/RAD51 pathway as evident by the decreased FEN1 and Rad51 foci in BRCA1-deficient cells. This could be associated with a role of FEN1 in HR complexes following ICL treatment. ERCC1 and XPG are recruited to repair foci in FEN1 nuclease deficient cells indicating that NER could compensate for the FEN1 deficiency [120]. In addition to templates with a primer having an unannealed 5′-tail or flap structure, FEN1 also cleaves flaps containing small covalent adducts [123]. Even though FEN1 could cleave cisplatin GG-intrastrand adduct substrates [124], cisplatin ICLs prevented FEN1 cleavage [123]. This could be due to the structural distortion induced by the cisplatin ICL, since it has been shown that DNA secondary structures inhibit flap processing. These observations raise the possibility that hypersensitivity of ICL inducing agents during the loss of FEN1 could be due to a role in DNA replication dependent ICL processing as ICLs are known to stall replication forks, due to its role in flap processing in long-patch BER or possibly due to a role for FEN1 in HR ICL processing.

Polymerases

Polymerase β(Pol β) belongs to the X family DNA polymerases and is well characterized for a role in DNA repair [125]. Studies have established the role of Pol β in both short-patch and long-patch BER [126]. Pol β has dRP lyase activity which is shown to be a rate-limiting step in the BER pathway [127]. This gap filling polymerase has been identified as being

error prone which is evident by increased mutagenesis when it is over expressed. Over-expression of Pol β leads to bifunctional DNA damage tolerance and facilitates the error-prone translesion synthesis past the DNA lesions that otherwise would block DNA replication and kill the cells. This affects the genomic stability of the cells and influences tumorigenesis [128]. We recently demonstrated additional base incorporation at cisplatin ICL sites that resulted from strand displacement synthesis by Pol β [77]. We also showed that Pol β had a low fidelity at the site flanking the cisplatin ICL even in the presence of correct nucleotides.

Using matched normal and tumor samples from different tissues, Srivastava et al have shown that approximately one third of tumor samples over express Pol β [129]. However, down regulation of this enzyme was observed in some tumors [130]. Pol β gene sequencing in different cancer cells revealed different types of mutations [131]. Sweasy et al studied the transformation activity of Pol β variants which when expressed in mouse cells resulted in cellular transformation [132–134]. Studies have shown that targeting Pol β modulates sensitivity of cisplatin, oxaliplatin, MMC and melphalan [77, 135–138]. Pol β deficiency resulted in MMC hypersensitivity and enhanced apoptosis [137]. However, evidence for the effect of down regulation of Pol β in modulating cisplatin and oxaliplatin chemosensitivity is conflicting. Yang et al have shown that knock down or down regulation of Pol β in colon cancer cells and MEFs enhanced oxaliplatin sensitivity and this was attributed to the delayed repair of oxaliplatin intrastrand adducts as well as ICLs, and increased apoptosis [136]. In contrast to this, down regulation or loss of Pol β in breast cancer cells and MEFs did not affect oxaliplatin sensitivity as well as oxaliplatin ICL repair in our study [77]. Similarly, studies show that cisplatin cytotoxicity when targeting Pol β in MEFs as well as cancer cells resulted in a) hypersensitivity, b) resistance and c) no effect [130, 135, 139–141]. A Pol β inhibitor conferred resistance to cisplatin in MEFs, but not in ovarian cancer cells [142]. In our study, we have shown that down regulation of Pol β plays a positive role in cisplatin cytotoxicity and its deficiency resulted in a resistant phenotype through enhanced repair of cisplatin-ICLs [77]. The discrepancy in these observations of drug cytotoxicity upon deficiency of Pol β is not clear. However, mutations in the Pol β gene, basal level expression of Pol β and overall DNA repair capacity could influence a cells response to the crosslinking agents. We have shown that BER machinery processes the flanking DNA at the cisplatin ICL site resulting in the non-productive repair of the ICL, therefore competing with the productive ICL DNA repair mechanisms. We have also shown that Pol β has low fidelity at the cisplatin ICL site [77] and Pol β mediated misincorporation of nucleotides could generate mismatched bases initiating the mismatch repair (MMR) apparatus (Manuscript in preparation).

Polymerase δ and ε, components of the replication complex are important replication polymerases involved in the synthesis process in both NER and long-patch BER. These polymerases can also bypass across different types of DNA lesions indicating a possible role in translesion replication [143, 144]. Aphidicolin, which selectively binds to the replication polymerases and inhibits DNA synthesis, has been used in cancer chemotherapy. *In vitro* and *in vivo* studies have shown that aphidicolin potentiates cisplatin cytotoxicity in tumor samples and cancer cells [145–148]. The modulatory effect of aphidocolin was significantly higher in cisplatin-resistant cells implying that inhibition of these polymerases can overcome platinum resistance. Aphidicolin also increased BCNU and melphalan activity in cancer cells [138, 149]. Zhang et al have shown that these polymerases are involved in psoralen ICL processing, at least in the late stages [150]. Aphidicolin mediated inhibition of DNA synthesis, replication stress and cell cycle arrest are potential mechanisms for cytoxicity of the crosslinking agents. Since these replication polymerases are components of several DNA repair pathways including NER, MMR, HR as well as long-patch BER, the mechanism that aphidicolin utilizes to exert its ICL cytotoxic enhancement is unclear.

Other BER proteins

BER proteins including XRCC1 and PARP are also involved in processing ICLs. XRCC1 is involved in the repair of single strand breaks (SSB) generated during BER and acts as a scaffold, connecting other BER proteins such as PARP, Pol β and DNA ligase III [151]. Polymorphisms in the XRCC1 gene have been associated with increased risk of developing certain cancers and also used as a prognostic marker in platinum-treated lung and gastric cancer patients [151–153]. Down regulation of XRCC1 enhanced cellular sensitivity to cisplatin and mitomycin C [154, 155]. PARP plays a key role in the repair of single-stranded breaks (SSBs) via BER/SSB repair pathways apart from other cellular functions. Cells with PTEN and BRCA mutations were found to be sensitive to PARP inhibitors and some of the PARP inhibitors are in clinical use for the treatment of melanoma, breast, ovarian and colorectal cancers [27, 28]. The role of these proteins in ICL repair is further strengthened by the findings of Zhu and Lippard where XRCC1, DNA ligase III and PARP1 bind to cisplatin ICLs *in vitro* [156].

Conclusion

ICLs covalently link the two strands of DNA and block the denaturing cellular processes that occur during DNA replication and transcription. ICLs are cytotoxic DNA lesions that are formed by a variety of anticancer drugs such as cisplatin, mitomycin C, psoralen, nitrosureas and nitrogen mustard derivatives. These crosslinking agents distort the DNA double helix, each in a unique manner. The repair of ICLs is still not completely understood in eukaryotes and the participation of different DNA repair pathways in ICL DNA repair is important. Recent evidence indicates that the BER pathway may play a novel role in the repair and processing of ICLs. Several mechanisms have been proposed on the role of BER in mediating ICL repair or processing. Accumulating evidence indicates that a variety of DNA lesions including ICLs can serve as substrates for the BER machinery. However, differential cellular sensitivity towards the ICL inducing agents suggests that the type of lesions formed determines the BER processing event and the effect is not general, but agent specific. The ICLs formed by each agent bend and distort the DNA in a unique manner leading to differential protein recognition and the distortion levels have a significant impact in the way these adducts are recognized and repaired. Each ICL structure therefore, has the potential to be processed differently and these unique physical structures contribute to the initiation of BER. The ICL inducing agents also form monoadducts and intrastrand adducts, which could also be substrates of BER. Some types of mono adducts can convert to ICLs and by repairing monoadducts, BER could prevent ICL formation and thereby, modulate the cellular responses to these agents. However, *in vitro* studies demonstrating differential recognition and processing of monoadducts by different glycosylases implies the existence of an alternate mechanism. In many cases, BER processing also depends on cell and tissue specificity as well as normal vs cancer cells based on the relative levels of basal protein expression and activity. Additionally, inherent DNA repair capacity also affects cellular responses to ICLs as it was suggested that BER may be able to compensate for the loss of other DNA repair pathways. BER could facilitate the repair of ICLs by interacting with other DNA repair pathways or compete with them to inhibit or delay the repair processes. BER mediated ICL processing could be productive as well as non-productive depending on the specific cross linking agents. BER can also act as an alternative or back-up pathway to NER in ICL repair as seen with psoralen. Collectively, it can be concluded that BER proteins process each ICL differently, mostly based on the distinct DNA structural distortions generated by the ICL inducing agents.

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Highlights

- BER pathway is mainly involved in the excision of damaged bases from the DNA
- BER is also involved in the processing of bulky lesions and interstrand crosslinks
- BER proteins process ICLs, mostly based on the distinct DNA structural distortions

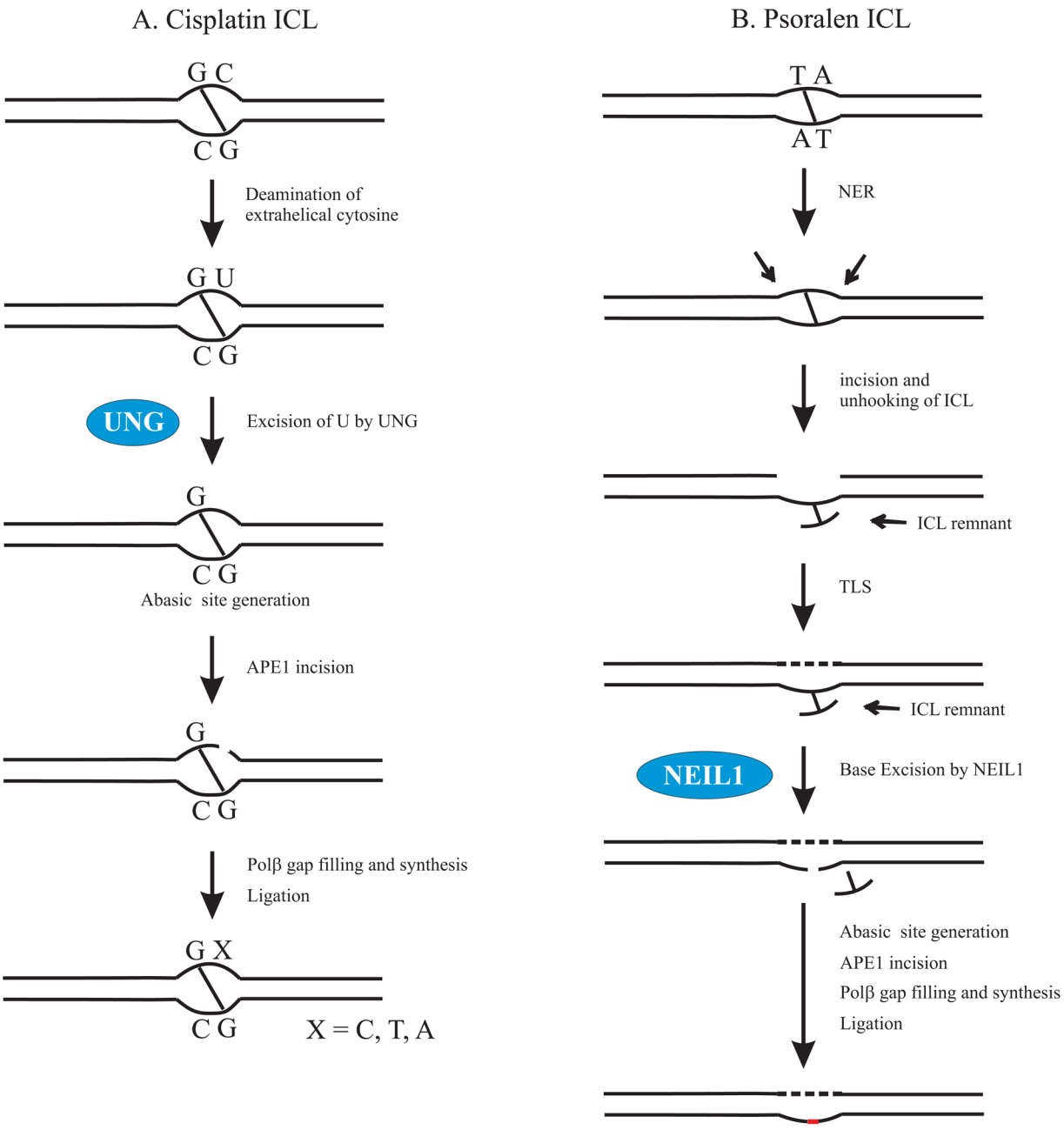

Figure 1.

Differential processing of ICLs by BER. **A**. BER processing of cisplatin ICL. The cytosines adjacent to the cisplatin ICL are extrahelical and flipped away from the double helix. The extrahelical cytosines are more susceptible to oxidative deamination and can convert to uracils. If cytosine deamination occurs, UNG initiates BER at the cisplatin ICL site by excising uracil from the DNA creating an abasic site. This is followed by Ape1 incision and Polβ mediated gap filling and synthesis. Polβ is shown to exhibit low fidelity at the cisplatin ICL site [77]. **B**. BER processing of psoralen ICL. NER may be involved in the initial recognition and incision of the psoralen ICLs by endonucleases. Translesion synthesis occurs across the unhooked ICLs where TLS polymerases bypass the ICL intermediate.

NEIL1 initiates BER by excising the unhooked ICL remnant swinging out of the helix. Ape1 and Polβ complete the downstream BER processing [111].

Table 1

BER proteins and their cytotoxic response to different ICL inducing agents.

BER protein	ICL agent	Cells	Cytotoxicity
AAG	BCNU	Mouse ES cells	Sensitive (79, 80)
		Mouse bone marrow cells	No effect (83)
		Cervical cancer cells (HeLa)	Sensitive (85)
	MMC	Mouse ES cells	Sensitive (79, 80)
	Psoralen	Mouse bone marrow cells	No effect (83)
		Mouse ES cells	Sensitive (81)
	Nitrogen mustard	Mouse ES cells	No effect (80)
		Mouse neuronal cells	Resistance (84)
NEIL1	Psoralen	Cervical cancer cells (HeLa)	Sensitive (76)
UNG	MMC	MEF	Sensitive (77)
	Cisplatin	MEF	Resistant (77)
NTH1	Cisplatin	Breast cancer cells (MCF7)	Sensitive (88)
	MMC	Breast cancer cells (MCF7)	No effect (88)
OGG1	Cisplatin	Hepatoma cells (HepG2)	Sensitive (89)
APE1	Cisplatin	Lung cancer cells (A549)	Sensitive (100)
		Melanoma cells (wm3211)	Sensitive (102)
		CHO	No effect (105)
	MMC	Cervical cancer cells (HeLa)	Sensitive (103)
		CHO cell	No effect (105)
	BCNU	Glioma (SNB19)	Sensitive (95)
		CHO cell	Sensitive (104, 105)
	Melphalan	CHO cell	No effect (104)
	Psoralen	Cervical cancer cells (HeLa)	Sensitive (76)
FEN1	Cisplatin	Glioblastoma (LN308)	Sensitive (113)
		Neuroblastoma (SK-N-MC)	Sensitive (120)
		Chicken DT40	No effect (43)
	MMC	MEF	Sensitive (114)
	Nitrogen mustard	*Saccharomyces cerevisiae*	Sensitive (115)
Polβ	Cisplatin	Cervical cancer cells (HeLa)	Sensitive (130)
		Ovarian cancer cells (SKOV-3)	Sensitive (130)
		Breast cancer cells (MDAMB231)	Resistant (77)
		MEF	Resistant (77, 139)
		NIH3T3	Sensitive (141)
	Oxaliplatin	MEF	No effect (136, 137, 140)
		Breast cancer cells (MDAMB231)	No effect (77)

BER protein	ICL agent	Cells	Cytotoxicity
		MEF	Sensitive (136)
	MMC	MEF	No effect (77)
	Melphalan	MEF	Sensitive (77, 137)
		MEF	No effect (137)
		NIH3T3	No effect (141)