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Review

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.

Contents

1. 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 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

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encounter ~10,000 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].

2. 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 (*e.g.* melphalan), nitrosureas (*e.g.* 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.

3. 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