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Differential role of base excision repair proteins in mediating cisplatin cytotoxicity

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ABSTRACT

Interstrand crosslinks (ICLs) are covalent lesions formed by cisplatin. The mechanism for the processing and removal of ICLs by DNA repair proteins involves nucleotide excision repair (NER), homologous recombination (HR) and fanconi anemia (FA) pathways. In this report, we monitored the processing of a flanking uracil adjacent to a cisplatin ICL by the proteins involved in the base excision repair (BER) pathway. Using a combination of extracts, purified proteins, inhibitors, functional assays and cell culture studies, we determined the specific BER proteins required for processing a DNA substrate with a uracil adjacent to a cisplatin ICL. Uracil DNA glycosylase (UNG) is the primary glycosylase responsible for the removal of uracils adjacent to cisplatin ICLs, whereas other uracil glycosylases can process uracils in the context of undamaged DNA. Repair of the uracil adjacent to cisplatin ICLs proceeds through the classical BER pathway, highlighting the importance of specific proteins in this redundant pathway. Removal of uracil is followed by the generation of an abasic site and subsequent cleavage by AP endonuclease 1 (APE1). Inhibition of either the repair or redox domain of APE1 gives rise to cisplatin resistance. Inhibition of the lyase domain of Polymerase β (Polβ) does not influence cisplatin cytotoxicity. In addition, lack of XRCC1 leads to increased DNA damage and results in increased cisplatin cytotoxicity. Our results indicate that BER activation at cisplatin ICLs influences crosslink repair and modulates cisplatin cytotoxicity via specific UNG, APE1 and Polβ polymerase functions.

1. Introduction

Cisplatin is a commonly used chemotherapeutic for the treatment of a wide variety of malignancies [1,2]. Many cancers, however, develop resistance to treatment which limits clinical success [3]. Cisplatin forms several types of lesions on the DNA, and the dynamics of the formation of these lesions, and their subsequent removal, is crucial for understanding the mechanisms underlying drug resistance. The majority of the lesions generated by cisplatin are intrastrand adducts, formed between adjoining purines on the same strand of DNA. Nucleotide excision repair (NER) is the primary pathway responsible for the removal of the intrastrand adducts formed by the drug. In addition, cisplatin also forms covalent interstrand crosslinks (ICLs) between guanines on opposite strands of DNA. However, it has been postulated that cells utilize multiple DNA repair pathways for the removal of cisplatin ICLs. The formation of ICLs blocks fundamental cellular processes like DNA replication and RNA transcription, influencing cell survival. It is important to note that repair of ICLs formed by cisplatin is likely different from the repair of the ICLs formed by other crosslinking agents like nitrogen mustards, psoralen and mitomycin C (MMC), and that the same mechanisms cannot be generalized for the repair of all ICLs. Unique structural distortions created by ICLs formed by various drugs influence the choice of specific repair pathways as well as the physiological rates of their removal. Crystallographic data and NMR solution structures of a cisplatin ICL have provided key insights on the degree of distortion created by a cisplatin ICL DNA lesion [4]. A cisplatin ICL produces significant distortion of the double helical DNA compared to other crosslinking agents. Due to the crosslink the DNA is unwound by 70–80° and bent towards the minor groove. This DNA distortion forces the cytosines adjacent to

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the crosslinked guanines from the double helix, exposing them to the nuclear environment.

We have recently demonstrated that these flipped out cytosines have a propensity to undergo preferential oxidative deamination, creating a uracil adjacent to the platinum crosslink [5]. By using purified proteins, we further showed that these newly formed uracils adjacent to the ICL are processed by proteins involved in the BER pathway. Purified uracil DNA glycosylase (UDG) was able remove the uracil base, the abasic site was cleaved by apurinic/apyrimidinic endonuclease 1 (APE1), and subsequently DNA polymerase β (Polβ) filled in the gap. However, there are four other DNA glycosylases present in human cells that have the ability to excise uracil from DNA [6]. Our studies have shown a deficiency in UNG, the primary uracil DNA glycosylase in humans, gives rise to cisplatin resistance [5]. In addition to UNG, single-strand selective mono-functional uracil DNA glycosylase (SMUG1) also has the ability to excise uracil from DNA. In some scenarios, other glycosylases such as methyl-CpG domain protein 4 (MBD4) and thymine DNA glycosylase (TDG), which are involved in the removal of thymine bases generated by deamination of 5-methylcytosine, may also participate in the removal of uracil.

After the removal of uracil by a glycosylase, an abasic site is formed and is recognized by APE1. APE1 is a multifunctional protein that has a wide variety of cellular functions in response to oxidative stress. APE1 facilitates DNA repair by cleaving the phosphodiester bond near the 5′ end of the apurinic/apyrimidinic (AP) site, which can be further processed by Polβ. APE1 contains both a redox domain responsible for transcriptional regulation of gene expression as well as the DNA repair domain required for AP site cleavage in the BER pathway. Studies show both repair and redox functions of APE1 are required for cell growth and survival [7]. Increased expression of APE1 in the A549 lung adenocarcinoma cell line has been associated with cisplatin resistance [8]. However, in the case of ovarian cancer, no significant difference was found between APE1 expression in platinum sensitive and platinum refractory cancers [9]. Targeting APE1 has been shown to mediate sensitivity to DNA damaging agents such as BCNU and MMC [10,11]. Accumulation of abasic sites and subsequent generation of DNA double strand breaks (DSBs) are likely responsible for this cellular sensitivity. On the other hand, previous studies in our lab have shown that pretreatment with methoxyamine (MX), which binds to abasic sites and blocks the access of APE1, enhanced repair of cisplatin ICLs and induced cisplatin resistance in human cancer cells [5]. Structural differences in chemotherapeutic agents could be the basis for the activation of different repair pathways. Cisplatin and MMC both form ICLs on DNA, however, the resulting structural changes are unique to each DNA damaging agent.

Polβ is a 39 kDa protein with a well characterized function in the BER pathway and contains two catalytically active units. The polymerase activity is located in the 31-kDa C-terminal polymerase domain, while the 8-kDa lyase domain (on the N-terminus) is responsible for the dRP lyase removal activity. Our lab has demonstrated that the polymerase domain of Polβ is required for cisplatin sensitivity [12]. DNA synthesis by Polβ near cisplatin ICLs is prone to errors, leading to the subsequent recruitment of mismatch repair (MMR) proteins. We have shown that this non-productive repair by BER and MMR proteins inhibits repair of cisplatin ICLs leading to increased cellular sensitivity [12]. Moreover, Polβ D256A variant deficient in polymerase activity was sensitive to cisplatin treatment, suggesting the requirement of Polβ mediated DNA synthesis for mediating cisplatin sensitivity. Misincorporation by Polβ gives rise to DNA mismatches near ICL sites, and we have previously shown that MMR protein binding is dependent on DNA synthesis by Polβ [12]. However, the role of dRP lyase activity of Polβ in this mechanism has not been studied, and it is unclear whether the proposed cisplatin ICL processing mechanism occurs via short patch (SP-BER) or long patch BER (LP-BER) pathways. Inhibition of the dRP lyase activity of Polβ has been known to favor the long patch BER pathway [13].

X-ray repair cross-complementing gene 1 (XRCC1) is a molecular scaffold protein which plays a critical role in BER and single strand break repair pathways [14]. XRCC1 is recruited to single strand breaks by Poly-ADP ribose polymerase 1 (PARP1) where it provides a platform for the interaction of numerous BER proteins. Lack of XRCC1 can lead to accumulation of single strand breaks, resulting in replication stress and potential formation of DNA DSBs [15]. Studies show that down-regulation of XRCC1 leads to increased sensitivity to cisplatin in the hepatocellular carcinoma cell line, HepG2 [16]. In these cells, reduced DNA repair capacity and increased reactive oxygen species (ROS) production have been associated with enhanced cellular sensitivity to cisplatin. Moreover, increased XRCC1 expression has been associated with cisplatin resistance in gastric cancer cells [17,18]. The thioredoxin like protein-1 (TXNL-1) mediated downregulation of XRCC1 has been shown to be a novel mechanism for cisplatin sensitivity in gastric cancer cells [17]. XRCC1 deficiency has also been shown to sensitize ovarian cancer cells to cisplatin and this sensitivity is further potentiated by ATR inhibition [19].

In our previous studies, we have shown that the extrahelical cytosines adjacent to cisplatin ICL sites are more prone to undergo oxidative deamination to uracil. The resulting uracils activate the BER pathway. We observed that the polymerase activity of Polβ is essential for maintaining cisplatin sensitivity, and error prone DNA synthesis by Polβ leads to misincorporation of nucleotides adjacent to cisplatin ICL sites [5]. Our results showed that Polβ misincorporates dATP at ~1.6 fold higher frequency as compared to the correct nucleotide base dCTP opposite guanine near the ICL site [5]. The generation of these mismatches leads to increased recruitment of MMR proteins and a subsequent role of MMR in blocking productive cisplatin specific ICL repair [12,20]. The binding of BER and MMR proteins results in a futile cycle at the ICLs by blocking the productive ICL DNA repair pathways, resulting in the persistence of DNA ICLs which give rise to enhanced cisplatin cytotoxicity. Unlike the changes observed in cisplatin ICL DNA repair following BER or MMR loss, there are no differences in cisplatin intrastrand adduct DNA repair [5,12]. Despite cisplatin intrastrand adducts playing a role in the efficacy of the drug, when it pertains to the BER and MMR pathways, the mediation of cisplatin efficacy is via ICL non-productive processing.

In this manuscript, the contribution of BER proteins in mediating cisplatin sensitivity and ICL processing was further assessed. UNG was found to be required to mediate cisplatin sensitivity while other uracil DNA glycosylases have no effect on cisplatin efficacy. In addition, we studied the individual roles of the DNA repair and redox domains of APE1 in mediating cisplatin sensitivity in human breast cancer cells. The data indicates that both activities of APE1 are important for mediating cisplatin sensitivity. It was also established that the 5′ dRP lyase activity of Polβ plays no role in cisplatin efficacy, while XRCC1 activity can mediate cisplatin sensitivity. Overall, these results highlight a role for UNG initiated canonical BER processing of uracils adjacent to cisplatin ICLs to mediate drug efficacy.

2. Materials and methods

2.1. Chemicals, antibodies and enzymes

Cisplatin, oxaliplatin, methoxyamine, methyl methanesulfonate (MMS) and myricetin were purchased from Sigma Aldrich. APE1 redox inhibitor E3330 was purchased from Novus biologicals. All other chemicals were from standard suppliers. APE1 antibody

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