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Enhanced DNA-PK-mediated RPA2 hyperphosphorylation in DNA polymerase η-deficient human cells treated with cisplatin and oxaliplatin

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ABSTRACT

The chemotherapeutic drugs cisplatin and oxaliplatin act by induction of DNA damage, including monoadducts, intrastrand and interstrand crosslinks. An increased understanding of the repair and replication of platinum-damaged DNA is required to improve the effectiveness of these drugs in killing cancer cells. We have investigated the effect of expression of DNA polymerase η (polη), a translesion synthesis (TLS) enzyme, on the response of human cell lines to cisplatin and oxaliplatin. Polη-deficient cells are more sensitive to both drugs than are normal cells. In polη-deficient cells, drug treatment leads to prolonged S-phase arrest, and increased phosphorylation of the phosphatidylinositol-3-kinase-related protein kinase (PIKK) substrates Chk1, p95/Nbs1 and RPA2, the 34 kDa subunit of replication protein A. Cisplatin- and oxaliplatin-induced hyperphosphorylation of RPA2, and association of the hyperphosphorylated protein with chromatin, is elevated in polη-deficient cells. Cisplatin-induced phosphorylation of RPA2 on serine 4/serine 8, but not on serine 33, is inhibited by the DNA-PK inhibitor, NU7441, but not by the ATM inhibitor, KU-55933. Cisplatin-induced DNA-PK-dependent hyperphosphorylation of RPA2 on serine 4/serine 8 occurs after recruitment of RPA to chromatin, as determined by immunofluorescence and by subcellular fractionation. ATR is required both for recruitment of RPA2 to chromatin and its subsequent hyperphosphorylation on serine 4/serine 8 by DNA-PK, since CGK733, an inhibitor of ATM and ATR, blocked both recruitment and hyperphosphorylation. Thus, increased sensitivity to cisplatin and oxaliplatin in DNA polη-deficient cells is associated with prolonged S-phase arrest, and enhanced PIKK-signalling, in particular activation of DNA-PK-dependent hyperphosphorylation of RPA2 on serines 4 and 8.

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Abbreviations: ICL, interstrand crosslink; ATM, ataxia telangiectasia mutated; ATR, ATM and Rad3-related; ATRIP, ATR interacting protein; PIKK, PI-3 kinase-related protein kinase; DNA-PK, DNA-dependent protein kinase; Polη, DNA polymerase η; RPA, replication protein A; XPV, xeroderma pigmentosum variant.

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1. Introduction

While novel targeted cancer therapies are being developed based on recent advances in cellular and molecular biology, DNA damaging agents, including ionising radiation and platinum-based chemotherapeutic drugs, continue to play a major role in the treatment of cancer [1]. The platinum-based compounds *cis*-diamminedichloroplatinum(II) [cisplatin] and (*trans*-*R*,*R*)1,2 diaminocyclohexaneoxalatoplatinum(II) [oxaliplatin] are effective and widely used in cancer chemotherapy. However, problems with their use include the development of resistant cells, and the formation of secondary tumours in surviving cells. A better understanding of the repair and replication of DNA damaged by platinum-based chemotherapeutic drugs is needed if the clinical efficacy of these compounds is to be further improved.

Cisplatin and oxaliplatin induce DNA damage, primarily at guanine residues, generating monoadducts, intrastrand crosslinks, and interstrand crosslinks (ICL). In an ICL, the covalent linkage of the two DNA strands prevents strand separation, inhibiting gene transcription and DNA replication. The ability to form ICLs correlates with the cytotoxicity of specific agents [1–4]. In an individual cell, the outcome of exposure to cisplatin can include cell cycle arrest and DNA repair, or induction of cell death [5]. By allowing DNA replication to occur even in the presence of unrepaired DNA damage, the cellular replication machinery may represent a potentially important mechanism by which tumour cells can tolerate and survive platinum-induced DNA damage. There has been considerable progress in understanding the process of replication of damaged DNA, including the identification of a number of specialised DNA polymerases involved in translesion synthesis (TLS) [6,7]. One of these proteins, DNA polymerase η (polη), a 78 kDa protein encoded by the human *POLH* gene, is required for error-free DNA replication across UV-induced cyclobutane pyrimidine dimers [8,9]. Mutations in *POLH* cause the disease xeroderma pigmentosum variant (XPV), characterised by increased UV-induced mutagenesis and elevated susceptibility to skin cancer [8–10]. Polη plays a key role in the replication of UV-damaged DNA, being required for the efficient and accurate translesion synthesis of lesions caused by UV light [8–15]. In the absence of active polη, lesions in the DNA are bypassed by, for example, DNA polymerase ζ, encoded by the *REV3/REV7* genes, leading to the greatly increased incidence of mutations observed in XPV cells [13,16]. A role for polη in 3′ strand extension during homologous recombination has also been proposed [17,18].

Although both UV light and platinum-based drugs cause DNA damage that is repaired by nucleotide excision repair, the types of damage induced by these agents are structurally quite distinct. Purified polη can bypass cisplatin-induced guanine-guanine adducts in oligonucleotide templates *in vitro* [16,19], and cells lacking polη are more sensitive to cisplatin [2,20–22]. While polη expression reduces carcinogenesis resulting from UV-induced DNA damage, it may also reduce the effectiveness of platinum-based anti-cancer drugs, by allowing cells to carry out DNA replication in the presence of drug-induced lesions in the DNA. Thus, a better understanding of the consequences of polη-deficiency on DNA replication, cell cycle progression and activation of downstream DNA damage responses is important in efforts to enhance the efficacy of widely-used cancer treatments that are based on cisplatin and related drugs.

Processing of DNA damage induced by cisplatin and oxaliplatin is complex, and involves proteins from a number of cellular pathways, including nucleotide excision repair, mismatch repair, homologous recombination (HR) and the Fanconi anemia (FA) protein complex [2,5]. In S-phase cells, unrepaired DNA damage can lead to replication fork arrest, and fork collapse generating DNA strand breaks [23]. Repair of ICLs may also generate single or double-strand breaks [24–26], and lead to the activation of DNA damage response pathways [2,5]. Both replication arrest and DNA strand breaks activate DNA damage responses mediated by the PI-3 kinase-related protein kinases (PIKKs), including ATM, ATR and DNA-PK. In response to DNA damage, these serine/threonine kinases phosphorylate a large number of effector proteins, including Chk1, Chk2, Nbs1 and p53 [27–30], resulting in cell cycle arrest or in apoptosis [2,5].

Replication protein A (RPA), the major single-stranded DNA binding protein in eukaryotic cells, is also a PIKK substrate following DNA damage. RPA is a heterotrimeric protein comprising of subunits of 70 kD (RPA1), 34 kD (RPA2) and 14 kD (RPA3) that plays a key role in DNA replication, repair and recombination [31]. RPA2 is phosphorylated at a number of sites in the N-terminal in a cell cycle-dependent manner [32–34], and following exposure of cells to UV- and γ-irradiation [34–40]. PIKK-mediated hyperphosphorylation of RPA2 may be involved in coordination of DNA damage responses and cell cycle arrest [41], for example during activation of the ATR-dependent S-phase checkpoint [38]. We have recently shown that UV-induced phosphorylation of RPA2 on serine 4 and serine 8 is increased in cells lacking polη [42], and that this process requires DNA-PK, a component of the non-homologous end joining (NHEJ) pathway for repair of DNA double-strand breaks. Damage-induced RPA2 hyperphosphorylation may represent an important mechanism for regulating the interaction of RPA with cisplatin-damaged DNA. PIKK-mediated RPA2 hyperphosphorylation reduces the affinity of RPA for purine-rich single-stranded DNA, for double-stranded DNA and for duplex DNA containing a single cisplatin guanine-guanine intrastrand crosslink [39,43,44]. RPA2 phosphorylation also alters the interaction of RPA with other proteins [37,39]; PIKK-dependent RPA2 hyperphosphorylation reduces RPA interaction with DNA polymerase α, but not with the repair protein XPA [39].

Using polη-deficient and normal human cell lines, we have investigated the effect of polη-deficiency on cisplatin and oxaliplatin-induced DNA replication arrest, and activation of PIKK-mediated DNA damage responses, characterising cisplatin-induced RPA2 hyperphosphorylation in detail. Cisplatin and oxaliplatin sensitivity in polη-deficient XP30RO cells is associated with prolonged S phase arrest, and increased activation of PIKK-mediated DNA damage responses, in particular hyperphosphorylation of RPA2. Using specific inhibitors of DNA-PK, ATM and ATR, we show that in response to cisplatin-induced DNA damage, RPA2 is recruited to chromatin in an ATR-dependent manner, and is then hyperphosphorylated on serine 4/serine 8 in a reaction requiring

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