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Cadmium treatment suppresses DNA polymerase δ catalytic subunit gene expression by acting on the p53 and Sp1 regulatory axis

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ABSTRACT

Cadmium (Cd) is a carcinogenic and neurotoxic environmental pollutant. Among the proposed mechanisms for Cd toxic effects, its ability to promote oxidative stress and to inhibit, in vitro, the activities of some Base Excision DNA Repair (BER) enzymes, such as hOGG1, XRCC1 and APE1, have been already established. However, the molecular mechanisms at the basis of these processes are largely unknown especially at sub-lethal doses of Cd and no information is available on the effect of Cd on the expression levels of BER enzymes. Here, we show that non-toxic treatment of neuronal cell lines, with pro-mitogenic doses of Cd, promotes a significant time- and dose-dependent down-regulation of DNA polymerase δ (POLD1) expression through a transcriptional mechanism with a modest effect on Polβ, XRCC1 and APE1. We further elucidated that the observed transcriptional repression on Polδ is acted by through competition by activated p53 on Sp1 at POLD1 promoter and by a squelching effect. We further proved the positive effect of Sp1 not only on POLD1 expression but also on Polβ, XRCC1 and APE1 expression, suggesting that Sp1 has pleiotropic effects on the whole BER pathway. Our results indicated that Cd-mediated impairment of BER pathway, besides acting on the enzymatic functions of some key proteins, is also exerted at the gene expression level of Polδ by acting on the p53–Sp1 regulatory axis. These data may explain not only the Cd-induced neurotoxic effects but also the potential carcinogenicity of this heavy metal.

1. Introduction

Cadmium (Cd) is a nonessential transition heavy metal, which is a ubiquitous environmental pollutant recognized as an occupational health hazard. It has recently gained greater public attention due to its increased use in modern industrial processes [1]. Since several epidemiological studies have demonstrated a causal association existing between Cd exposure and the risk of developing various type of cancers, Cd has been recognized as a potent human carcinogen and classified as a Group I carcinogen [2]. Major target organs of Cd deposition are liver and kidney but, in case of prolonged exposure, its accumulation results in physiological damage in lungs, brain, testes, heart, as well as in the central nervous (CNS) and peripheral neuronal system [3,4]. Cd can be uptaken from the nasal mucosa or from the olfactory pathway into peripheral and central neurons leading to CNS dysfunction. Although many evidences have clearly demonstrated its potential as etiological factor for neurological disorders, its precise mechanism of action is far from being completely clarified. Current understandings suggest that neurotoxicity associated with Cd-exposure may result from indirect multifactorial processes that can activate apoptotic cascades and can lead to the disruption of signaling pathways resulting in cell arrest [3]. A common set of mechanisms, by which most tissue types respond to Cd, has been defined [5]. Briefly, in vitro studies have demonstrated that Cd: (i) acts as a mitogen stimulating cell proliferation; (ii) induces the generation of reactive oxygen species (ROS); (iii) inhibits apoptosis and (iv) interferes with DNA repair [5–8]. The latter of these proposed mechanisms has been the subject of recent studies since interference with DNA repair enzymes in neuronal cells contribute to the onset of neurodegenerative diseases [9] and may lead to the genetic instability associated to cancer development. Cd has been shown to inhibit many DNA-repair systems such as BER (Base Excision Repair), NER (Nucleotide Excision Repair) and MMR (Mismatch Repair) [10]. However, the majority of data derived from in vitro experiments and showed that the inhibitory effect occurs on the enzymatic function of some of the

Abbreviations: AP, abasic site; APE1, apurinic/apyrimidinic endonuclease 1; BER, base excision repair; Cd, cadmium; CNS, central nervous system; DNA LigI, DNA ligase I; MMS, methyl methanesulfonate; OGG1, 8-oxoguanine DNA glycosylase; Polβ, DNA polymerase β; Polδ, polymerase delta 1, catalytic subunit; XRCC1, X-ray repair cross-complementing protein 1; ROS, reactive oxygen species; NER, nucleotide excision repair; MMR, mismatch repair; SSB, single-strand break.

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proteins involved [10]. Furthermore, information about the possible effect of Cd on the expression levels of these enzymes is still scanty. As mentioned above, induction of ROS is a key determinant of oxidative DNA damage. Among the most important cellular protection mechanisms involved in the removal of oxidized bases, the BER pathway is responsible for repairing most DNA non-distorting lesions: oxidized or alkylated bases, AP sites, as well as DNA single-strand breaks. BER is the most important pathway coping with oxidative DNA damage, having a prominent role in non-dividing cell and in neuronal pathophysiology [11]. In the BER pathway, small lesions are recognized and removed by specific glycosylases (such as hOGG1), leaving an abasic (AP) site which is further incised by the AP endonuclease 1 (APE1). Then, the resulting single-strand break (SSB) can be processed by either two pathways, the "short-patch" (SP-BER), in which a single nucleotide is replaced by DNA polymerase β (Polβ), or the "long-patch" BER (LP-BER) in which several new nucleotides are synthesized by DNA polymerase δ, ε, or β, along with proliferating cell nuclear antigen (PCNA) and replication factor-C (RF-C). Finally, a DNA ligase, i.e., DNA ligase III/XRCC1, can complete the repair process and restore the integrity of the helix by sealing the single-stranded DNA nick.

Several studies demonstrated that the efficiency of BER enzymes is affected in the context of Cd-induced stress response [10,12]. In particular, earlier works showed that Cd might inhibit the initial steps of BER pathway, such as the recognition and the removal of damaged DNA bases; however, Cd inhibitory effects on the additional steps of the pathway have not been extensively studied, yet.

Specifically, functional interference of Cd with BER proteins has been established for hOGG1, APE1, PARP, DNA Polβ and DNA Lig I [10,13–18]; however, most of these works were carried out in vitro with purified recombinant proteins or with cell extracts using high concentrations of Cd that do not resemble those required for cultured cells. Moreover, except the case of hOGG1 [14], no information is still available on the effects of Cd on the expression levels of BER enzymes in cells.

In this study, we investigated the effect of sub-lethal doses of Cd on the expression of the enzymes downstream of the recognition/removal step of the DNA lesion. We observed that neuronal cells (i.e., SH-SY5Y and SF-767) exposure to low Cd-concentrations exerted a mitogenic effect. Notably, under these conditions, we showed, for the first time, that Cd-treatment induced a concentration- and time-dependent down-regulation of the expression of the catalytic subunit of the DNA polymerase δ, with a milder effect on the other BER enzymes tested (i.e., APE1, XRCC1, Polβ). Interestingly, Polδ down-regulation is also apparent in differentiated SH-SY5Y cells. Furthermore, we identified in p53 a negative regulator of Polδ expression and suggested that p53 exerts its inhibitory effect by interfering with Sp1-mediated transcriptional activation on Polδ promoter. Cd-mediated effect on p53–Sp1 axis could represent a general mechanism to regulate BER enzymes expression. Our results provide new insights on the molecular mechanisms by which Cd inhibits DNA repair thus contributing to the explanation of Cd-induced mutagenic and carcinogenic effects.

2. Materials and methods

2.1. Cell lines and materials

In this study, we used the neuronal cell SH-SY5Y (neuroblastoma cell line) and SF-767 (glioblastoma cell line) as models for neuronal tumoral cell lines. SH-SY5Y cells were maintained in EMEM/F12 1:1 supplemented with 15% FCS (EuroClone, Milan, Italy) and 1% non-essential amino acids (EuroClone); while SF-767 cells were cultured in Dulbecco's modified Eagle's medium (EuroClone) supplemented with 10% FCS. To induce differentiation, SH-SY5Y cells were treated with 10 μM all trans-retinoic acid (RA) (Sigma–Aldrich, St. Louis, MO) for 7 days. Medium supplemented with RA was changed every 2 days. The isogenic HCT116 cell lines were a gift from Prof. L. Collavin (University of Trieste, Trieste, Italy). HCT116 were grown in Dulbecco's modified Eagle's medium containing 10% FCS. All cells were grown in medium added with 1% penicillin streptomycin solution (100 U/mL penicillin, 100 mg/mL streptomycin), 2 mM L-glutamine (Euroclone) and cultured in a humidified incubator at 5% CO_2 at 37 °C.

2.2. Transient transfection and silencing experiments

The constructs of pN and pN3-Sp1 carrying Sp1 cDNAs were kindly provided by prof. Annunziato (Federico II University of Naples, Naples, Italy) while the construct of pcDNA3-p53-FLAG carrying p53 wild type form was provided by Prof. L. Collavin (University of Trieste, Trieste, Italy). For transient transfections, cells were plated onto 10-cm plates and grown overnight. Transfection of Sp1 and p53 on SH-SY5Y cells were performed with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions and harvested 24 h after transfection. Transfection of p53 in HCT116 cell lines were performed with Lipofectamine 3000 (Invitrogen, Carlsbad, CA).

For siRNA experiments, SH-SY5Y were transfected with 150 pmol siRNA siGENOME SMART pool or the scramble control siRNA (Dharmacon, Lafayette, Co.) using DharmaFECT reagent (Dharmacon). After 72 h upon transfection, the cells were treated with $CdCl_2$ at the indicated concentrations and time of treatments.

2.3. $CdCl_2$ treatment

In order to prepare stock solutions, $CdCl_2$ (Sigma) was dissolved in sterile distilled water at a concentration of 1 M and stored at −20 °C. Working solutions were diluted directly in the complete media. SH-SY5Y, SF-767 and HCT116 cells were exposed to $CdCl_2$ at concentrations ranging from 10 to 100 μM for 24 h at 37 °C. Differentiated SH-SY5Y cells were exposed to $CdCl_2$ at concentrations ranging from 1 to 50 μM for 24 h at 37 °C.

2.4. Cell viability and proliferation

Cell viability was measured by using the 3-(4-5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS) assay (Celltiter 96 Aqueous One solution cell proliferation assay, Promega) on cells grown in 96-well plates and treated with $CdCl_2$ for 24 h. Upon treatment, the MTS solution was added to each well and the plates were incubated for additional 2 h at 37 °C. Then, absorbance was measured at 490 nm by using a multiwell plate reader. The values were standardized to wells containing media alone.

Cell proliferation was evaluated by trypan blue exclusion test. Trypan blue-excluding cells were counted in a hemocytometer in four randomly selected fields and averaged.

2.5. Protein extraction

For preparation of total cell lysates, cells were harvested by trypsinization and centrifuged at 250 × *g* for 5 min at 4 °C. Supernatant was removed, and the pellet was washed once with ice-cold phosphate-buffered saline (PBS) and then centrifuged again as described before. Cell pellet was resuspended in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, and 1% (wt/vol) Triton X-100 supplemented with 1× protease inhibitor cocktail (Sigma), 0.5 mM phenylmethylsulfonyl fluoride (PMSF),