DNA Repair 11 (2012) 511–521

Contents lists available at SciVerse ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

PCNA-dependent accumulation of CDKN1A into nuclear foci after ionizing irradiation

Claudia Wiese[b,*], Jeanette Heede Rudolph[a], Burkhard Jakob[a], Daniela Fink[a], Frank Tobias[a], Christine Blattner[c,1], Gisela Taucher-Scholz[a,**]

[a] GSI Helmholtzzentrum für Schwerionenforschung, Planckstrasse 1, 64291 Darmstadt, Germany
[b] Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[c] Karlsruhe Institute of Technology, Institute of Toxicology and Genetics, PO Box 3640, 76021 Karlsruhe, Germany

ARTICLE INFO

Article history:
Received 23 November 2011
Received in revised form 4 February 2012
Accepted 28 February 2012
Available online 26 March 2012

Keywords:
CDKN1A
PCNA
DNA repair
Ionizing radiation
DNA double-strand breaks
Base excision repair

ABSTRACT

The cyclin-dependent kinase inhibitor CDKN1A/p21 confers cell-cycle arrest in response to DNA damage and inhibits DNA replication through its direct interaction with the proliferating cell nuclear antigen (PCNA) and cyclin/cyclin-dependent kinase complexes. Previously, we reported that in response to *densely* ionizing radiation CDKN1A rapidly is recruited to the sites of particle traversal, and that CDKN1A foci formation in response to heavy ions is independent of its transactivation by TP53. Here, we show that exposure of normal human fibroblasts to X-rays or to H_2O_2 also induces nuclear accumulations of CDKN1A. We find that CDKN1A foci formation in response to radiation damage is dependent on its dephosphorylation and on its direct physical interaction with PCNA. Live cell imaging analyses of ectopically expressed EGFP-CDKN1A and dsRed-PCNA show rapid recruitment of both proteins into foci after radiation damage. Detailed dynamic measurements reveal a slightly delayed recruitment of CDKN1A compared to PCNA, which is best described by bi-exponential curve fitting, taking the preceding binding of PCNA to DNA into account. We propose a regulatory role for CDKN1A in mediating PCNA function after radiation damage, and provide evidence that this role is distinct from its involvement in nucleotide excision repair and unrelated to double-strand break repair.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

The spatiotemporal coordination of DNA damage repair is crucially important to maintain genomic stability and prevent carcinogenesis. To do so, eukaryotes have evolved a variety of complex DNA repair pathways that are targeted towards a plethora of endogenously and exogenously generated DNA lesions. These DNA repair pathways are tightly interwoven with transcription, apoptosis and cell cycle regulation within a network that is known as the DNA damage response (for review see [1]). Within this network the cyclin-dependent kinase (CDK) inhibitor CDKN1A (also known as p21/WAF1/CIP1 [2–4]) plays an important role. CDKN1A is involved in growth arrest through cell cycle checkpoints (for review see [5]), in regulating apoptosis [6–11] and gene expression/transcription [12–14], in inhibiting DNA replication by interacting directly with proliferating cell nuclear antigen (PCNA) and CDK complexes [15–18], and in the negative regulation of translesion DNA synthesis (for review see [19,20]). CDKN1A's pleiotropy is regulated by its expression levels, its sub-cellular localization, its protein binding partners and its phosphorylation status [21,22].

CDKN1A is a member of the CIP/KIP family of CDK inhibitors, which inhibit cyclin D-, E-, and A-dependent kinase activity [2,3,15,23,24]. In solution, CDKN1A is an intrinsically unstructured protein [25] presumably allowing it to adopt multiple conformations upon interaction with specific binding partners [26]. At its carboxy terminus (R143-S160), CDKN1A interacts with PCNA, the accessory protein of DNA polymerases δ and ε. Phosphorylation of CDKN1A at T145 leads to loss of the inhibition of DNA replication and to loss of PCNA-binding [27–29], and how the CDKN1A-PCNA interaction can affect DNA damage repair has been a subject of intense investigation (for review see [30]).

We have shown previously that in nuclei of normal human fibroblasts the CDKN1A protein accumulates into foci at highly

Abbreviations: LET, linear energy transfer; DSBs, DNA double-strand breaks; SSBs, DNA single-strand breaks; CPD, cumulative population doublings; NHDF, neonatal human dermal fibroblasts; TLC, thin-layer cellulose; ICQ, Intensity Correlation Quotient; ICA, Image Correlation Analysis.

* Corresponding author. Tel.: +1 510 486 4024; fax: +1 510 486 6816.
** Corresponding author. Tel.: +49 6159 712606; fax: +49 6159 712106.
E-mail addresses: CWiese@lbl.gov (C. Wiese), G.Taucher-Scholz@gsi.de (G. Taucher-Scholz).
[1] Tel.: +49 721 60822634; fax: +49 721 60823354.

1568-7864/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2012.02.006

clustered DNA damage sites within minutes after traversal by accelerated charged particles [31,32]. CDKN1A foci formation is independent of functional TP53, NBS and ATM [31,33], and a strict spatial correlation for the signals of CDKN1A and MRE11, γH2AX and PCNA was observed at the sites of particle traversal [34]. However, it must be considered that DNA damage induced by high linear energy (LET) charged particle irradiation, besides predominantly DNA double-strand breaks (DSBs), likely additionally contains in close proximity multiple other lesions, including damaged DNA bases and DNA single-strand breaks (SSBs) [35]. We now show that CDKN1A also accumulates into foci after exposure to X-rays, and after treatment with H_2O_2. We provide evidence that recruitment of CDKN1A after radiation damage is dependent on its direct interaction with PCNA. Furthermore, live-cell imaging analysis of ectopically expressed EGFP-CDKN1A and dsRED-PCNA reveals that PCNA is recruited with slightly faster kinetics. Interestingly, recruitment of CDKN1A after radiation damage in nucleotide excision repair-deficient XPA cells is not different from wild type cells. These results support the notion that CDKN1A accumulation into foci in response to ionizing radiation is distinct from its role in nucleotide excision repair. We suggest that the local recruitment of CDKN1A by PCNA to DNA lesions induced by ionizing radiation or H_2O_2 is unrelated to DSB repair and serves to regulate the multiple protein interactions of PCNA required for faithful and efficient long-patch base excision repair.

2. Material and methods

2.1. Cell culture, transfection, CDKN1A expression plasmids and immunoprecipitations

Confluent normal human foreskin fibroblasts (AG1522C, Coriell Cell Repository, passage number 8-15, cumulative population doublings (CPD) 20-30), neonatal human dermal fibroblasts (NHDFs; CellSystems Biotechnologie, passage number 6-12, CPD 11-20) and XPA-deficient human GM00710B fibroblasts (Coriell Cell Repository, passage number 18-20, CPD 6-10) were grown in EMEM medium (BioWhitaker/Cambrex) with 15% fetal calf serum (FCS; Biochrom), 1% L-glutamine (Biochrom), 0.5% penicillin/streptomycin (Biochrom) at 37 °C and a 5% CO_2 atmosphere. Cells were grown 10–14 days with bi-weekly medium changes until confluent and arrested in G_0/G_1-phase. Cell cycle analysis was performed by flow cytometry as described [33] using a PAS III flow cytometer (Partec) to ensure that approximately 85–95% of growth-arrested cells were in G_0/G_1. Parental HCT116 cells and the CDKN1A/p21-deficient HCT116 cells were kindly provided by Dr. B. Vogelstein (Johns Hopkins University, Baltimore) and maintained as described previously [36]. HeLa cells and EM9 cells were maintained as described [37]. HeLa, EM9 and HCT116 cells were transfected using Lipofectamine2000 (Invitrogen) using standard procedures. Transfection of fibroblasts was done using AMAXA nucleofector (Lonza) according to the manufacturer's protocols.

Wild type and mutant (T145A and T145D) CDKN1A expression plasmids in the pcDNA3.1-*myc*-his$_6$ vector (Invitrogen) are described elsewhere [27] and were kindly provided by Dr. S. Dimmeler (Goethe University, Frankfurt am Main). EGFP-CDKN1A was generated by amplifying the CDKN1A ORF from the wild type CDKN1A expression plasmid [27] and by cloning the amplified CDKN1A ORF into pEGFP-C2 (Clontech) from *Hin*dIII to *Xba*I. The PCNA construct was generated as described in [38], but using a DsRed-tag and was kindly provided by Dr. M. Cardoso (Technical University, Darmstadt).

Immunoprecipitations were carried out in RIPA buffer (1% NP40, 50 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxycholate) with protease inhibitor cocktail (Roche) and essentially as described [27], using anti-cMyc rabbit polyclonal antibody (Santa Cruz; A14) or polyconal rabbit anti-CDKN1A (Ab-5 antibody, Oncogene).

Table 1
Calculated charged particle doses absorbed per nucleus.

Ion species	Mean energy on target (MeV/u)	LET (keV/μm)	Dose (Gy)
Nickel	4.5	3790	18
Argon	7.4	1550	5.0
Zinc	4.8	4120	20
Gold	4	13,030	42
Chromium	5.6	2810	225
Uranium	4.2	14,925	48–72
Xenon	4.7	8650	28–42
Samarium (suppl. Fig. 2C)	4.2	10,290	49
Iron (suppl. Fig. 2B)	175	337	3.2

2.2. Irradiation and H_2O_2 treatment

For experiments involving peptide mapping the cells were irradiated with γ-rays from a Gammacell 220 irradiator (A.E.C.L. Atomic energy of Canada limited, Ottawa, Canada) with a ^{60}Cobalt source. Cells were irradiated with dose rates of 2 Gy/min at room temperature.

Exposure to X-rays was performed using an industrial X-ray generator (Isovolt DS1, type IV320-13 (Seifert) at 250 kV and 16 mA. This X-ray tube is equipped with a 7 mm beryllium filter and one aluminium and one copper filter of 1 mm thickness each to eliminate soft X-rays. All samples were irradiated at room temperature at dose rates of 2–3.5 Gy/min. Exposure to heavy ions was carried at the UNILAC facility at the Helmholtzzentrum für Schwerionenforschung (Darmstadt) as described previously [31]. All ion species used throughout this study are depicted in Table 1.

UV micropore irradiation was based on the method described previously [39]. The medium was removed, the cells were rinsed twice with PBS and immediately after covered by polycarbonate foils containing pores of 3 μm diameter in size. These filters were produced in-house by pre-irradiation with charged particles and subsequent etching in NaOH solution for 80 min. Cells covered by polycarbonate filters were irradiated with 100 J/m^2 UV-C light (254 nm) which took about 10 s. Following irradiation, polycarbonate filters were removed and immediately filled with warm growth medium.

For H_2O_2 treatment, cell were rinsed with PBS and incubated with 0.2 mM H_2O_2 in PBS for 20 min at room temperature. H_2O_2 was removed, cells were washed several times in warm PBS and then incubated at 37 °C for 15 min in regular growth medium before fixation.

2.3. Immunofluorescence assay

Cells were fixed in 4% paraformaldehyde and immunostained as described previously [31]. For CDKN1A detection, cells were permeabilized in Hepes buffer before fixation as described [33]. PCNA immunostaining was performed as described in [34] using cytoskeleton buffer for pre-extraction. The following primary antibodies were used: mouse anti CIP1/WAF1 (BD; dilution 1:80); mouse anti PCNA (Chemicon; 1:50); mouse/rabbit anti γH2AXser139 (Millipore; 1:500); mouse/rabbit anti 53BP1 (Bethyl Laboratories; 1:2000) and rabbit anti XRCC1 (Alexis Biochemicals; 1:1000). Alexa 488 goat anti-mouse IgG F(ab')2 and Alexa 568 goat anti-mouse IgG F(ab')2 (Molecular Probes) were used at 5 μg/ml. DNA was stained with 1 μM ToPro-3 (Molecular Probes). The samples were air-dried and mounted in Vectashield solution (Vector Laboratories).

Download English Version:

https://daneshyari.com/en/article/10823454

Download Persian Version:

https://daneshyari.com/article/10823454

Daneshyari.com