Mutation Research 782 (2015) 34–43

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

An altered redox balance and increased genetic instability characterize primary fibroblasts derived from xeroderma pigmentosum group A patients

Eleonora Parlanti[a], Donatella Pietraforte[b], Egidio Iorio[b], Sergio Visentin[b], Chiara De Nuccio[b], Andrea Zijno[a], Mariarosaria D'Errico[a], Valeria Simonelli[a], Massimo Sanchez[b], Paola Fattibene[c], Mario Falchi[d], Eugenia Dogliotti[a,*]

[a] Department of Environment and Primary Prevention, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[b] Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[c] Department of Technology and Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[d] National AIDS Center, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 9 October 2015
Accepted 11 October 2015
Available online 23 October 2015

Keywords:
Oxidatively generated damage
DNA repair
metabolism
mitochondria
micronuclei

ABSTRACT

Xeroderma pigmentosum (XP)-A patients are characterized by increased solar skin carcinogenesis and present also neurodegeneration. XPA deficiency is associated with defective nucleotide excision repair (NER) and increased basal levels of oxidatively induced DNA damage. In this study we search for the origin of increased levels of oxidatively generated DNA lesions in XP-A cell genome and then address the question of whether increased oxidative stress might drive genetic instability. We show that XP-A human primary fibroblasts present increased levels and different types of intracellular reactive oxygen species (ROS) as compared to normal fibroblasts, with $O_{2-}^{\bullet}$ and H_2O_2 being the major reactive species. Moreover, XP-A cells are characterized by decreased reduced glutathione (GSH)/oxidized glutathione (GSSG) ratios as compared to normal fibroblasts. The significant increase of ROS levels and the alteration of the glutathione redox state following silencing of XPA confirmed the causal relationship between a functional XPA and the control of redox balance. Proton nuclear magnetic resonance (^{1}H NMR) analysis of the metabolic profile revealed a more glycolytic metabolism and higher ATP levels in XP-A than in normal primary fibroblasts. This perturbation of bioenergetics is associated with different morphology and response of mitochondria to targeted toxicants. In line with cancer susceptibility, XP-A primary fibroblasts showed increased spontaneous micronuclei (MN) frequency, a hallmark of cancer risk. The increased MN frequency was not affected by inhibition of ROS to normal levels by *N*-acetyl-L-cysteine.

Abbreviations: acet, acetate; ala, alanine; ANOVA, analysis of variance; asp, asparagine; BER, base excision repair; BSO, buthionine sulphoximine; CAT, catalase; Cho, choline; CPD, cyclobutane pyrimidine dimers; CS, cockayne syndrome; CPH, 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine; DTPA, diethylenetriamine pentaacetic acid; EPR, electromagnetic paramagnetic resonance; GGR, global genome repair; gln, glutamine; glt, glutamate; GPCho, glycerophosphocholine; GS, glutathione; GSH, reduced glutathione; GSSG, glutathione disulfide; isol, isoleucine; lac, lactate; m-ins, myo-inositol; NADPH, nicotinamide adenine dinucleotide phosphate; NAC, *N*-acetyl-L-cysteine; NER, nucleotide excision repair; NMA, *N*-monomethyl-L-arginine; NMR, nuclear magnetic resonance; 8-oxoGua, 8-oxo-7,8-dihydroguanine; OGG1, 8-oxoGua DNA glycosylase; OXPHOS, oxidative phosphorylation; PCho, phosphocholine; PARP, poly ADP ribose polymerase; PCNA, proliferating cell nuclear antigen; PEG, polyethylene glycol; PI, proliferation index; RAD, radiation sensitive; ROS, reactive oxygen species; ROT, rotenone; SD, standard deviation; SEM, standard error of the mean; SOD, superoxide dismutase; tau, taurine; TCA, tricarboxylic acids; tCr, total creatine; TCR, transcription coupled repair; TMRE, tetramethylrhodamine ethyl ester perchlorate; UV, ultraviolet; val, valine; XPA, xeroderma pigmentosum group A protein; XPC, xeroderma pigmentosum group C protein.

* Corresponding author. Fax: +39 06 49903650.
E-mail address: dogliotti@iss.it (E. Dogliotti).

http://dx.doi.org/10.1016/j.mrfmmm.2015.10.002
0027-5107/

1. Introduction

Xeroderma pigmentosum complementation group A (XP-A) is a rare autosomal recessive disorder, characterized by dramatically increased incidence of sun-induced skin cancer often associated with progressive neurological abnormalities [1,2].

It is now well established that solar radiation, mostly represented by UVB and UVA, induces two major types of photolesions, cyclobutane pyrimidine dimers (CPDs) and oxidatively generated DNA lesions, mainly 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxoGua). It is still a matter of debate the relative contributions of these two types of lesions to photocarcinogenesis [3,4].

XPA is involved in the lesion recognition step of the nucleotide excision repair (NER), a repair pathway responsible for removal of a wide range of lesions leading to distortion of the DNA helix including CPDs. XPA plays a central role in both NER subpathways, the transcription-coupled repair (TCR) that repairs transcriptionally active domains and the global genome repair (GGR) that removes lesions from the genome overall [5]. There is mounting evidence that NER factors are also involved in the repair of oxidatively generated DNA lesions [6–9]. We have shown that XP-C [6] as well as Cockayne syndrome (CS)-A and CS-B [9] primary fibroblasts accumulate 8-oxoGua in their genome and, more recently, we have demonstrated that the same feature is shared by primary fibroblasts derived from XP-A patients [10]. These NER proteins seem to operate as cofactors in the repair of oxidative damage, either by increasing the loading and turnover of OGG1 as suggested for XPC-HR23B complex [6] or by participating, together with OGG1, to a transcription coupled repair of 8-oxoGua as in the case of XPA and CSB [11]. The mechanisms of these repair processes have not been fully clarified yet. It is of note that the same NER-defective cells present elevated levels of ROS [12–14] leaving open the hypothesis that this might contribute to the increased levels of DNA oxidation.

Aerobic metabolism constantly generates ROS that control fundamental cellular functions but, when excessive, can damage DNA, RNA and proteins [15]. Mitochondria are major sites of ROS production and energy metabolism [16,17]. Mitochondrial dysfunction has been associated with neurological diseases and cancer [18]. Recently, several studies have shown that defects in DNA repair proteins belonging to different pathways determine alterations in the redox balance, mitochondrial dysfunction and perturbed metabolism [12–14,19–22]. These changes, in the case of XPC silencing in normal human keratinocytes, trigger neoplastic transformation [13].

In this study we thoughtfully characterized the redox balance of XP-A primary fibroblasts and the mitochondria bioenergetics machinery. We then addressed the question of whether increased oxidative stress might drive genetic instability as a hallmark of cancer risk.

We show that XP-A human primary fibroblasts present an altered glutathione redox balance and a more glycolytic metabolism. This metabolic perturbation is associated with an altered response of mitochondria to low doses of the mitochondrial toxin rotenone. Finally, we provide evidence that XPA deficiency is associated with increased genetic instability that is unaffected by ROS scavenging by *N*-acetyl-L-cysteine (NAC).

2. Materials and methods

2.1. Cell culture and treatment

The N1RO, N2RO and N3RO primary fibroblasts were derived from three healthy donors [23]. The XP-A primary fibroblasts, XP20PV, were a kind gift of Dr. M. Stefanini (CNR, Pavia, Italy). The XP-A primary fibroblasts, XP25RO and XP132AA, were a kind gift of Dr. K. Jaspers (Erasmus University Medical Center Rotterdam, The Netherlands). The EUE-siXPA cell line and its normal counterpart EUE-pBD650 have been previously described [10]. Briefly, EUE-siXPA cell line presents 80% silencing of the XPA gene and is hypersensitive to the killing effects of UV. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen Life Technologies S.r.l., Milan, Italy), supplemented with 10% foetal calf serum. When required cells were exposed for 1 h to 1 mM buthionine sulphoximine (BSO).

2.2. Electron paramagnetic resonance (EPR) measurement of ROS levels

The spin probe 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (CPH; ENZO Life Sciences Inc.) was dissolved in degassed phosphate buffer (PBS) without Ca^{2+} and Mg^{2+} (Sigma), pH 7.4, extensively treated with Chelex-100 (Bio-Rad, Richmond, CA) to avoid metal contamination. 0.5 mM CPH was added to 100 μl suspensions of primary fibroblasts (20×10^6 cells/mL) in PBS, pH 7.4. The oxidation of CPH was monitored by the formation of the characteristic 3-line spectrum with hyperfine coupling constant of 1.63 ± 0.04 mT attributable to the corresponding nitroxide radical 3-carboxyproxyl radical ($CP^{\bullet}$). The characterization of ROS was performed by pre-incubating cell suspensions with the suitable scavengers and the metal chelating agent (15 min, 37 °C), or with the inhibitor of nitric oxide synthase (1 h, 37 °C) before CPH addition. It should be noted that since the used scavengers and chelating compounds do not cross the plasma membrane, the measurements relate to oxidant species that are released in the extracellular compartment. Samples were drawn up into a gas-permeable teflon tube with 0.81 mm internal diameter and 0.05 mm wall thickness (Zeuss Industrial Products, Raritan, NJ). The Teflon tube was folded four times, inserted into a quartz tube and fixed to the EPR cavity. Spectra were acquired 30 min after the addition of the spin probe at room temperature. The low field shoulder of this spectrum was chosen to quantify the $CP^{\bullet}$ because the middle component centered at *g* 2.0 overlaps with other free radical signals found in biological systems. Spectra were acquired with a Bruker ELEXSYS continuous wave X-band spectrometer equipped with a high sensitivity cylindrical microwave cavity (Bruker SHQ), with: modulation frequency, 100 kHz; microwave frequency, 9.7 GHz; microwave power, 10 mW; modulation amplitude, 0.1 mT; conversion time, 80 ms; time constant, 80 ms; sweep time, 80 s; number of scans, 3.

2.3. GSH and GSSG measurement

Sample aliquots (20×10^6 cells/mL) were deproteinized by adding 1.22 M iced trichloroacetic acid (1:2 v/v), kept 5 min in ice and centrifuged at 10.000 rpm, 5 min, 4 °C. GSH was determined spectrophotometrically at 412 nm, in the clear supernatants 5 min after the addition of 0.1 mM 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) reagent to 0.1 mM phosphate buffer/1 mM diethylenetriaminepentaacetic acid, pH 7.4, containing aliquots of samples or GSH standard curve. GSSG was measured in 25 μL cleared acidified supernatants by using DTNB-GSSG reductase recycling assay [24]. GSH and GSSG content was normalized for protein content measured in each sample by using BCA assay.

2.4. NMR spectroscopy

Cells grown to 60–70% confluence were trypsinized 24 h after culture medium change, counted, and assessed for viability (80–90%) and membrane integrity by trypan blue staining. Cells were washed twice with ice-cold physiological saline solution and pellets resuspended in 0.5 mL of ice-cold twice-distilled water. Aqueous extracts (from 10×10^6 cells/sample) were pre-