Mechanisms of Ageing and Development 134 (2013) 261–269

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

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

The role of CSA and CSB protein in the oxidative stress response

Mariarosaria D'Errico [a], Barbara Pascucci [a,b], Egidio Iorio [c], Bennett Van Houten [d,e], Eugenia Dogliotti [a,*]

[a] Department of Environment and Primary Prevention, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[b] Institute of Cristallography, Consiglio Nazionale delle Ricerche, Via Salaria, Km 29,300, 00016 Monterotondo Stazione, Rome, Italy
[c] Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[d] Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA
[e] The University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, PA 15213, USA

ARTICLE INFO

Article history:
Available online 3 April 2013

Keywords:
Cockayne syndrome
Oxidative stress
Cellular redox balance
Mitochondrial dysfunction
Oxidative metabolism

ABSTRACT

Cockayne syndrome (CS) is a rare hereditary disorder in which infants suffer severe developmental and neurological alterations and early death. Two genes encoding RNA polymerase II cofactors, *CSA* and *CSB*, are mutated in this syndrome. CSA and CSB proteins are known to be involved in the transcription-coupled DNA repair pathway but the sensitivity of mutant cells to a number of physical/chemical agents besides UV radiation, such as ionizing radiation, hydrogen peroxide and bioenergetic inhibitors indicate that these proteins play a pivotal role in additional pathways. In this review we will discuss the evidence that implicate CS proteins in the control of oxidative stress response with special emphasis on recent findings that show an altered redox balance and dysfunctional mitochondria in cells derived from patients. Working models of how these new functions might be key to developmental and neurological disease in CS will be discussed.

1. Introduction

Reactive oxygen species (ROS) are constantly generated under normal conditions as a consequence of aerobic metabolism. The cell is endowed with an extensive antioxidant defense system to counteract ROS, either directly by interception of oxidative species or indirectly through reversal of oxidatively generated damage. When ROS overcome the defense systems of the cell and redox homeostasis is altered, the result is oxidative stress. Oxidative stress has been implicated in the development and progression of several diseases including: cancer; acquired immunodeficiency syndrome (AIDS); neurodegenerative diseases such as Huntington (HD), Parkinson (PD), amyotrophic lateral sclerosis (ALS), Alzheimer (AD), retinal degenerative disorders, as well as, in the process of aging (Visconti and Grieco, 2009; Torre et al., 2002; Lin and Beal, 2006). During the last decade, emerging evidence indicate that oxidative stress is also involved in several DNA repair related pathologies that present clinical features of neurodegeneration (Van Houten et al., 2006; Pascucci et al., 2011). These findings are consistent with the fact that the central nervous system uses large amounts of oxygen to fuel oxidative phosphorylation, and may be prone to oxidative stress. Cockayne Syndrome (CS) is an example of rare hereditary multisystem disease characterized by neurological and development impairment, with a rapid onset of features of aging. Clinical hallmarks of this syndrome include developmental delay, microcephaly, loss of subcutaneous fat, cutaneous photosensitivity, progressive hearing loss, and ocular anomalies. Mutations in *CSA* and *CSB* genes affecting transcription-coupled repair (TCR), the nucleotide excision repair (NER) subpathway dedicated to removal of transcription blocking lesions from the genome, cause the invariably fatal CS. CS-defective cells are sensitive to UV light, the recovery of RNA synthesis after UV-damage is delayed and apoptosis is triggered by the blocked transcription complex. Intriguingly, this photosensitivity does not lead to cancer (as in other NER-related diseases). The clinical symptoms of CS have been often explained as the consequence of hypersensitivity to oxidative stress. A large body of evidence indicates that upon oxidative stress CS-A and CS-B cells show increased cytotoxicity and accumulate oxidatively induced DNA damage (Tuo et al., 2001; D'Errico et al., 2007; Foresta et al., 2010). CS cells are defective in the repair of a variety of oxidatively generated DNA lesions including 8-oxoguanine (8-OH-Gua), 5-hydroxycytosine (5-OHCyt) and cyclopurines. Moreover, evidence has been provided that CSB might also participate to the repair of abasic sites (Wong et al., 2007). CS proteins have been shown to stimulate the activity of key base excision repair (BER) enzymes (e.g. Neil1, APE1) (Wong et al., 2007; Muftuoglu et al., 2009) and/or to affect their transcription (as in the case of OGG1) (Khobta et al., 2009). However, it is unlikely that the dramatic phenotype of patients with CS is solely due to the role of CS proteins as dispensable co-factors of BER in the removal of nuclear oxidatively generated DNA damage. A new hypothesis has recently emerged to explain CS pathology: CS proteins may be involved in the maintenance of

* Corresponding author. Tel.: +39 064990 2580; fax: +39 06 49903650.
E-mail address: Eugenia.dogliotti@iss.it (E. Dogliotti).

0047-6374/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.mad.2013.03.006

mitochondria that are the primary source of ROS. Elevated levels of oxidatively generated mitochondrial DNA (mtDNA) damage, hypersensitivity to bioenergetic inhibitors as well as altered organization of mitochondrial respiratory complexes have been reported in CS-B mouse cells (Osenbroch et al., 2009). Moreover, in human cells CSA and CSB localize to mitochondria and interact with mitochondrial BER proteins to protect from aging- and stress-induced mtDNA mutations and apoptosis-mediated loss of subcutaneous fat, a hallmark of aging (Kamenisch et al., 2010; Aamann et al., 2010). Recently, it was demonstrated that CS cells present an altered redox balance with increased steady-state levels of intracellular ROS and mitochondrial dysfunction (Pascucci et al., 2012; Scheibye-Knudsen et al., 2012). In addition, patient-derived CS-B deficient cells exhibited a defect in efficient mitochondrial transcript production and CSB specifically promoted elongation by the mitochondrial RNA polymerase in vitro (Berquist et al., 2012).

A large body of recent work on oxidative stress-related pathologies points to mitochondrial impairment as a central causative factor. Decreased activity of specific complexes of the electron transport chain (ETC), increased oxidatively generated damage, and altered activity of antioxidant defense enzymes have been shown in aging and neurodegenerative diseases (Beal, 2005; Van Houten et al., 2006). The hypothesis has been formulated that the neurodegeneration observed in CS patients might be aligned with that observed in other neurological disorders caused by dysfunctional mitochondria. In this model the well-established defect in transcription-coupled repair, which is the hallmark of CS cells, would account for the skin photosensitivity, while the key to developmental and neurological disease in CS is the loss of mitochondrial function upon oxidative stress (Cleaver, 2012).

In this review we will discuss the evidence that implicate CS proteins in the control of oxidative stress response and provide a working model of how defects in these proteins cause disease.

2. The redox balance: characterization and sources

ROS encompass a diverse range of species, including superoxide, hydrogen peroxide, nitric oxide, peroxynitrite, hypochlorous acid, singlet oxygen, and the hydroxyl radical. Each of these molecules represents a distinct chemical entity with its own reaction preferences, kinetics, rate and site of production, and degradation and diffusion characteristics in biological systems. Consequently the biological impacts of ROS depend critically on the particular molecule(s) involved and on the microenvironment and physiological or pathological context in which it is being generated. In general, many ROS are short-lived molecules that due to their high chemical reactivity can react with DNA, proteins, carbohydrates and lipids in a destructive manner (Winterbourn and Hampton, 2008). A common feature of neurodegenerative diseases, including CS, is oxidative stress, which arises when ROS exceed amounts required for normal redox signaling (i.e. alteration of redox balance).

We have shown (Pascucci et al., 2012) that the steady-state ROS levels, measured by different experimental approaches, such as dichlorodihydrofluorescein (DCFH) oxidation and electron spin resonance (ESR) analysis, were 2-fold higher in primary fibroblasts from both CS-A and CS-B donors as compared to normal cells. This increase was confirmed in another study (Scheibye-Knudsen et al., 2012) where ROS formation was measured by dihydroethidium in CS-B deficient CS1AN cells compared with cells expressing wild-type *CSB*. Moreover, induced pluripotent stem cells (iPSCs) derived from CS-B patient's fibroblasts exhibited higher ROS production as compared to iPSCs from normal fibroblasts (Andrade et al., 2012).

If the accumulation of ROS is a well-described feature of CS cells the identification of their sources is still incomplete. There are several potential sources of ROS within the cell (Fig. 1). An important generator of intracellular oxidants is a family of membrane-bound enzymes that rely on NADPH for their activity. Although the expression of these enzymes was initially thought to be confined to phagocytic cells, it now appears that this seven-member family (Nox1–5 and Duox1–2) is in fact widely expressed and evolutionarily conserved (Brown and Griendling, 2009; Aguirre and Lambeth, 2010). To date, the only clear function of these NADPH-dependent oxidases is the regulated generation of ROS. In this regard, it is interesting to note that Newman et al. (2006) by analyzing the genome transcriptional profile of a CS-B

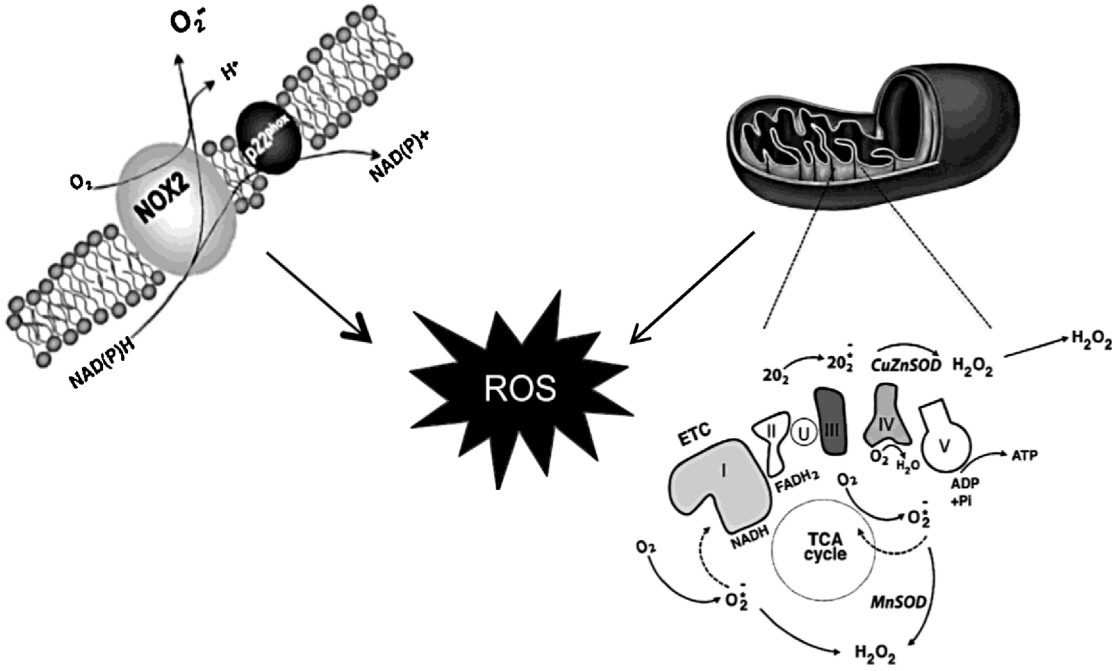

Fig. 1. Main sources of ROS. The enzymes of the mitochondrial electron transport chain as well as NAD(P)H oxidases (in particular NOX2 and its associated membrane subunit $p22^{phox}$) are indicated.