Redox Biology 6 (2015) 135–156

Contents lists available at ScienceDirect

Redox Biology

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

Review Article

Genetic disorders coupled to ROS deficiency

Sharon O'Neill [a], Julie Brault [b,c], Marie-Jose Stasia [b,c], Ulla G. Knaus [a,*]

[a] Conway Institute, University College Dublin, Dublin, Ireland
[b] Université Grenoble Alpes, TIMC-IMAG Pôle Biologie, CHU de Grenoble, Grenoble, France
[c] CGD Diagnosis and Research Centre, Pôle Biologie, CHU de Grenoble, Grenoble, France

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form
15 July 2015
Accepted 16 July 2015
Available online 17 July 2015

Keywords:
NADPH oxidase
Chronic granulomatous disease
Inflammatory bowel disease
Hypothyroidism
NOX
DUOX
Genetic disease
Reactive oxygen species (ROS)

ABSTRACT

Maintaining the redox balance between generation and elimination of reactive oxygen species (ROS) is critical for health. Disturbances such as continuously elevated ROS levels will result in oxidative stress and development of disease, but likewise, insufficient ROS production will be detrimental to health. Reduced or even complete loss of ROS generation originates mainly from inactivating variants in genes encoding for NADPH oxidase complexes. In particular, deficiency in phagocyte Nox2 oxidase function due to genetic variants (*CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*) has been recognized as a direct cause of chronic granulomatous disease (CGD), an inherited immune disorder. More recently, additional diseases have been linked to functionally altered variants in genes encoding for other NADPH oxidases, such as for *DUOX2*/*DUOXA2* in congenital hypothyroidism, or for the Nox2 complex, *NOX1* and *DUOX2* as risk factors for inflammatory bowel disease. A comprehensive overview of novel developments in terms of Nox/Duox-deficiency disorders is presented, combined with insights gained from structure–function studies that will aid in predicting functional defects of clinical variants.

Contents

* Correspondence to: Conway Institute, School of Medicine, University College Dublin, Belfield, Dublin 4, Ireland.
E-mail address: ulla.knaus@ucd.ie (U.G. Knaus).

http://dx.doi.org/10.1016/j.redox.2015.07.009

1. Introduction

Oxidative stress, the imbalance between the generation of reactive oxygen species (ROS) and the ability of antioxidant defense systems to scavenge ROS, has been recognized as a risk and contributing factor for various forms of pathophysiology, including inflammation and tissue injury, neurodegeneration, and carcinogenesis. Many enzyme systems can be the source of superoxide ($O_2^{\bullet}$) or hydrogen peroxide (H_2O_2), the initial ROS produced, and even more proteins are involved in adduct reactions involving oxygen radicals, generating for instance hypochlorous acid or peroxynitrite. A similar variety exists in antioxidant systems. The overall redox balance is critical for propagation and termination of essential signaling pathways in cells and tissues, while specialized functions in certain cells such as phagocytes require a regulated burst of ROS. Undesirable consequences of increased ROS, due to deregulated ROS overproduction or failure of antioxidant systems, can be detected by changes in cellular responses such as increased apoptosis or cell proliferation, and even in cases of overall cellular adaptation by the appearance of oxidative modifications on DNA, proteins or lipids.

The dichotomy of ROS being vital signaling molecules in a plethora of physiological processes while also propagating disease often impedes a clear distinction between beneficial and harmful ROS, but a comprehensive study of genetic disorders can reveal the overall consequences for health when the redox balance is permanently altered. For example, sequence alterations in mitochondrial DNA, superoxide dismutases, catalase and glutathione synthetase usually augment ROS levels. In contrast, NADPH oxidases (Nox/Duox), the only enzyme family whose sole known purpose is the regulated generation of ROS, are downregulated or inactivated in genetic variants. NADPH oxidases have been associated with pathologically elevated ROS mainly by linking gene/protein expression profiles with ROS levels and oxidative modifications, but inferring a causal relationship of increased ROS with disease has been more challenging. The only potential gain-of-function variants of a gene directly required for terminal NADPH oxidase activation are certain *CYBA* ($p22^{phox}$) polymorphisms that may increase Nox1-4 activity and confer an elevated risk for cardiovascular disease [1–4]. On the other hand, loss-of-function variants in genes required for formation and catalytic activity of active Nox/Duox complexes are increasingly recognized as risk factors, or as origin of inherited or spontaneous genetic diseases that are characterized by reduced or abolished ROS production. The NADPH oxidase family comprises seven members (Nox1-5, Duox1-2) in humans, all of which assemble as multimeric complexes regulated by protein–protein interactions and by the small GTPase Rac (Nox1-3), requiring phosphorylation, calcium flux or lipid binding to generate O_2^- or H_2O_2 by catalyzing the transfer of electrons from NADPH to molecular oxygen. Their largely tissue-specific expression profiles correlate well with specific genetic diseases linked to Nox/Duox deficiency. Here, disorders associated with gene variants in NADPH oxidases including chronic granulomatous disease, inflammatory bowel disease and congenital hypothyroidism will be discussed in the context of functional consequences initiated by structural changes due to missense variants.

2. Chronic granulomatous disease – the new faces of the disease

2.1. Background

Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency syndrome (frequency 1/200,000 to 1/250,000) characterized by mutations in one of the genes encoding the components of the Nox2 NADPH oxidase complex in phagocytic cells. In most patients, diagnosis occurs early in childhood due to recurrent and life-threatening infections with bacterial and fungal pathogens (mainly catalase-positive bacteria, e.g. *Staphylococcus aureus*, Burkholderia and Nocardia species, and fungi e.g. Aspergillus and Candida species). These infections cannot be contained due to deficient generation of superoxide by a functionally impaired or structurally labile (and often absent) NADPH oxidase in innate immune cells, as pathogens cannot be killed even when phagocytosed efficiently [5,6].

Understanding the composition of the multimeric phagocyte Nox2 oxidase was greatly aided by studies on neutrophils collected from CGD patients [7–9]. CGD is a genetically heterogeneous disease with all ethnic groups equally affected. The molecular basis of CGD is characterized by two types of transmission and four main genetic forms. The major genetic form of CGD is X-linked CGD caused by mutations in the *CYBB* gene (OMIM number 306400) encoding $gp91^{phox}$ (renamed Nox2) (Fig. 1). X-CGD represents about 70% of the total cases reported to date [10]. The other forms of CGD are autosomal recessive (AR), characterized by mutations in *CYBA* (OMIM number 233690), *NCF1* (OMIM number 233700) and *NCF2* (OMIM number 233710) encoding $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$ respectively [11]. Whereas AR-$CGD22^0$ and AR-$CGD67^0$ are extremely rare (less than 5% of cases), AR-$CGD47^0$ occurs with high frequency (about 25% of CGD cases) due to the presence of two *NCF1* pseudogenes carrying the main mutation. Up to now only one case of AR-CGD in *NCF4*, encoding $p40^{phox}$, was described [12]. Nox2 oxidase activity additionally requires activation of the small GTP-binding protein Rac, which was discovered concomitantly by Knaus et al. [13] and Abo et al. [14] in neutrophils. The importance of Rac2 was underlined by a case of severe immunodeficiency diverging from classical CGD in a 5-week-old child that was traced back to a dominant negative mutation in *RAC2* [15,16]. For *CYBB*, *CYBA*, *NCF1* and *NCF2* many variants harboring deletions, frame shifts, missense, nonsense and splice site mutations have been identified and are accessible at the immunodeficiency (ID) bases (http://structure.bmc.lu.se/idbase/).

2.2. Are absence of ROS and hyperinflammation paradoxical in CGD?

The link between absent or decreased ROS production in CGD and defective killing mechanisms including autophagy is well established, but in contrast to the prevailing notion of ROS initiating or exacerbating tissue damage, hyperinflammation is often documented in CGD patients. Initially, decreased degradation of phagocytosed material in the absence of ROS production was considered the cause of the observed proinflammatory phenotype. Thereby, phagocytosed microorganisms could accumulate in NADPH oxidase deficient phagocytes leading to persistent cell