ROS Deficiency as Disease Risk – the Importance of Redox Balance

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Abstract

Preserving the balance between generation and elimination of reactive oxygen species (ROS) is crucial for health. Continuously elevated ROS can trigger oxidative stress and chronic inflammatory disease, but likewise insufficient ROS will impede physiologic functions and cause disease. Inactivating variants in genes encoding NADPH oxidase components have been linked to reduced or even complete loss of ROS generation. The most prominent case is deficiency in phagocyte NOX2 oxidase function due to inactivating gene variants (CYBB, CYBA, NCF1, NCF2, NCF4), resulting in the inherited immune disorder chronic granulomatous disease (CGD). More recently variants in genes encoding other NADPH oxidase family members have been linked to congenital hypothyroidism (DUOX2/DUOXA2) or constitute a risk factor for developing inflammatory bowel disease (IBD) (NOX1, DUOX2). Here, we provide a short summary of disorders associated with ROS deficiency due to genetic defects in NOX/DUOX with the view that this area will expand in the near future.

Introduction

The imbalance between the generation of ROS and the ability of antioxidant defence systems to scavenge or degrade ROS, is termed oxidative stress, a known risk and contributing factor to various pathophysiology including inflammation, tissue injury, carcinogenesis and neurodegeneration. Maintaining an overall redox balance is crucial for many regulatory circuits in cell signalling and tissue homeostasis.

Uncontrolled ROS production and/or failure of antioxidant systems will lead to oxidative damage such as the appearance of oxidative modifications on proteins, lipids or DNA, or will alter cell physiology with increased apoptosis and cell death or metabolic aberrations and augmented cell growth. NADPH oxidases (NOX/DUOX) comprise seven members and are the only known enzyme family whose sole function is to generate ROS. Several members of the family assemble as multimeric complexes that are regulated by protein–protein interactions and by the small GTPase Rac (NOX1-3). These complexes require phosphorylations, calcium flux or lipid binding to facilitate the generation of superoxide ($O_2^{-}$) or hydrogen peroxide ($H_2O_2$) by catalyzing the transfer of electrons from NADPH to molecular oxygen (Lambeth, 2004). While sequence alterations in mitochondrial DNA, superoxide dismutases, catalase and glutathione synthetases usually will increase ROS, the currently identified genetic variants of NADPH oxidases will lead to partial or complete inactivation. Loss-of-function variants in genes required for both the formation and catalytic activity of

Figure 1. Reduced generation of ROS is associated with disease
NOX/DUOX complexes are progressively accepted as risk factors, or indeed the main cause, of inherited or spontaneous genetic diseases that display reduced or abolished ROS production (O’Neill et al., 2015). The tissue-specific expression profiles of NADPH oxidases correlate well with the genetic disorders linked to NOX/DUOX deficiency.

Chronic Granulomatous Disease

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 NOX2 NADPH complex, which is mainly expressed in phagocytic cells. Diagnosis occurs in early childhood in the majority of cases due to recurrent and life-threatening bacterial (e.g. *Staphylococcus aureus*, Burkholderia and Nocardia species) and fungal (e.g. Aspergillus and Candida species) infections. Although bacterial uptake in innate immune cells is not compromised, pathogens cannot be destroyed due to deficient generation of O$_2^*$ by a functionally impaired or structurally labile NADPH oxidase (Holland, 2013).

CGD is a genetically heterogeneous disease with similar prevalence in all ethnic groups, albeit some geographic region-specific diversity seems to emerge. The molecular basis of CGD is characterized by two transmission types and four main genetic forms. X-linked CGD is caused by mutations in the *CYBB* gene encoding NOX2/gp91phox and represents 70% of the total cases reported to date. The other three genetic forms are autosomal recessive (AR) and are caused by mutations in *CYBA* encoding p22phox, *NCF1* encoding p47phox and *NCF2* encoding p67phox (O’Neill et al., 2015). AR-CGD22$^0$ and AR-CGD67$^0$ are usually rare and account for approximately 5% of world-wide CGD cases, while AR-CGD47$^0$ occurs with higher frequency (about 25% of cases) due to the presence of two NCF1 pseudogenes carrying the main mutation. Many variants harbouring deletions, frameshifts, missense, nonsense and splice site mutations have been identified for *CYBB*, *CYBA*, *NCF1* and *NCF2*, and can be accessed at the immunodeficiency (ID) data bases (http://structure.bmc.lu.se/idbbase/). As the components of the NOX2 complex are also expressed in cells and tissues other than phagocytes, inactivating mutations or deletions in NOX2, p22$^{phox}$, p47$^{phox}$ and p67$^{phox}$ have been also linked to diseases unrelated to immunodeficiency syndromes. In a 2009 study conducted with 25 X-CGD patients and 25 healthy subjects, NOX2 deficiency was associated with increased arterial dilation (Violi et al., 2009). Another study, conducted with 229 CGD patients at the National Institute of Health, concluded that diabetes, renal and cardiovascular diseases occurred more frequently and with greater severity in AR-CGD47$^0$ patients than in those with NOX2-deficient CGD (X-CGD) (Leiding et al., 2013), pointing to a NOX2 independent role of p47$^{phox}$.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is the common term for a group of chronic or recurring inflammatory conditions of the gut, comprising two main disorders, Crohn’s disease (CD) and ulcerative colitis (UC). Very early onset (VEO) IBD is classified as disease in children under 6 years old, according to the modified Paris classification (Ezster Muller et al., 2014), with patients usually harbouring rare genetic defects that cause primary immunodeficiency and that are often not detected in genome wide association studies. Within the rare gain-of-function and loss-of-function variants identified in VEOIBD through exome-targeted, candidate gene and whole genome sequencing (O’Neill et al., 2015), partially inactivated NADPH oxidases are now a recognized risk factor for IBD. In a study of 122 VEOIBD patients performed by Dhillon et al., targeted exome sequencing and single nucleotide polymorphism sequencing identified 11 NOX2-associated NADPH oxidase variants (Dhillon et al., 2014). Among these variants were NCF1 p.R90H and NCF2 p.H389Q variants, which both resulted in reduced ROS production. Mechanistically, NCF2 p.H389Q showed decreased binding to the Rac GTPase exchange factor VAV1, thus impeding an important process during FcγR-dependent NOX2 activation.

Intestinal epithelial cells (IECs) are important in maintaining barrier function and mucosal homeostasis, and compromising their interaction with the intestinal environment through genetic alterations can be considered the gateway for inflammatory processes. IECs...
express the NOX1 and DUOX2 NADPH oxidases. NOX1 activity is stimulated by immune receptor signalling and promotes tissue restitution, while expression of the DUOX2/DUOX2 complex is upregulated by the microbiota, intestinal pathogens and in certain inflammatory disease processes (O'Neill et al., 2015). Recently, the first inactivating NOX1 and DUOX2 variants in VEOIBD patients have been identified (Hayes et al., 2015). Two variants of X-linked NOX1 were identified in three VEOIBD patients presenting with severe pancolitis - NOX1 p.P330S, a missense variant located directly upstream of the FAD binding domain, which reduced ROS generation by 50-60%, and NOX1 p.D360N, a variant placed in the second FAD binding domain, resulting in a 60-80% reduction in ROS production in both a model cell line and in in vivo reconstituted crypts of Nox1 knockout mice (Hayes et al., 2015). In the same study, two DUOX2 patients were identified in VEOIBD patients with recurrent pancolitis. Both patients harboured monoallelic missense variants (DUOX2 p.R1211C, DUOX2 p.R1429C), which impaired H₂O₂ production but not intracellular localization in a cell-based model. In a recent genome wide association study involving two large Ashkenazi Jewish families with a high prevalence of CD, exome sequencing identified the DUOX2 p.P303R variant, which was associated with adult IBD and displayed reduced H₂O₂ generation (Levine et al., 2016). A CH-associated patient study reported the same variant in their normal control population, and showed not significantly decreased activity in a comparable cell system (Muzza et al., 2013). None of the patients harbouring IBD-associated DUOX2 variants presented with CH at diagnosis.

Congenital Hypothyroidism

Congenital hypothyroidism (CH) is caused by thyroid hormone deficiency present at birth and is the most common congenital endocrine disorder, occurring in 1/3000-4000 births. The majority of CH cases are due to defects in the development of the thyroid gland, with only 15-20% of cases resulting from thyroid dysshormogenesis. H₂O₂ produced by DUOX2 at the apical membrane of thyroid follicular cells is necessary for the organification of iodide, the rate-limiting step in thyroid hormone synthesis. The crucial role of the DUOX2 complex in thyroid hormone synthesis is evidenced by the growing number of DUOX2 and DUOX2 variants identified in CH. To date, in excess of 40 DUOX2 and 4 DUOX2 variants have been reported that result in transient to permanent congenital hypothyroidism (O'Neill et al., 2015). More than 20 of these DUOX2 mutations are clustered in the peroxidase(PO)-like domain. Several are nonsense mutations or frameshifts that generate a premature stop codon, or missense mutations that result in improper folding and structural instability of the PO domain. These variants lead to retention in the endoplasmatic reticulum, to degradation or to truncated, mislocalized and usually functionally deficient protein (O'Neill et al., 2015). Aside from mutations in this extracellular domain, a number of DUOX2 variants have been identified in the first cytosolic loop in the vicinity of the EF-hand domains. Two particular variants, DUOX2 p.R842X and p.E879K, were identified in patients with compound heterozygosity. Their location inside the EF-hand domains resulted in transient CH (DUOX2 p.R376W /R842X and p.K530X/E879K) (Muzza et al., 2013). These mutations likely reduce calcium binding, thereby diminishing DUOX2 activation. Heterodimerization is fundamental to the correct localization and catalytic activity of the DUOX2/DUOX2 complex, and therefore it is not surprising that variants in DUOX2, the obligatory DUOX2 dimerization partner, have been associated with CH. To date, four variants, DUOX2 p.I26M, p.Y131X, p.C189R and p.Y246X have been described (O'Neill et al., 2015).

Discussion

Pathologies linked to ROS deficiency triggered by loss-of-function mutations in genes encoding NADPH oxidase family members and their partner proteins highlight the importance of ROS in specialized functions such as microbial killing and thyroid hormone synthesis. Often not recognized is the regulatory role of ROS in signal transduction, exemplified by persistent hyperinflammation as a result of decreased or absent ROS generation. The causal relationship between NOX2 and DUOX2 with CGD and CH,
respectively, is conserved between rodents and man, while the risk potential for disease due to inherited polymorphisms or mutations in NOX-associated genes will require further investigation into epigenetic and environmental risk factors.

References


