Best Practice & Research Clinical Endocrinology & Metabolism xxx (2017) 1–16

Contents lists available at ScienceDirect

Best Practice & Research Clinical Endocrinology & Metabolism

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

3

Disorders of H_2O_2 generation

Marina Muzza, Dr, PhD, Post Doc Researcher Fellow [a], Laura Fugazzolay, Prof, MD, Associate Professor of Endocrinology [b, *]

[a] Endocrine Unit, Fondazione IRCCS Ca' Granda Policlinico, Milan, Italy
[b] Division of Endocrine and Metabolic Diseases, IRCCS Istituto Auxologico Italiano, Dept. of Pathophysiology and Transplantation, University of Milan, Milan, Italy

ARTICLE INFO

Article history:
Available online xxx

Keywords:
DUOX
DUOXA
congenital hypothyroidism
dyshormonogenesis

After the identification of thyroid H_2O_2 generation system (DUOX) and of its maturation factors (DUOXA), defects in DUOX2 and/or DUOXA2 were rapidly recognized as the possible cause of congenital hypothyroidism (CH) due to thyroid dyshormonogenesis. The present Review reports data on the prevalence of *DUOX2* mutations, which is variable among different series but invariably high, pointing to DUOX2 defects as one of the leading causes of dyshormonogenesis. Differently, DUOXA defects seem to be rarely involved in the pathogenesis of CH. Genotype-phenotype correlations are also reported, highlighting the great intra- and inter-familial phenotype variability which appears to be a constant feature of the defects in the H_2O_2 generation systems. Finally, the hypotheses to explain the phenotypic variability of the *DUOX2/A2* mutations are discussed, such as the existence of other H_2O_2 generating systems, the age variability in thyroid hormones requirements, the differences in ethnicity, in iodine intake, and in the methodological approaches.

Background

Hydrogen peroxide (H_2O_2) formation represents a limiting step in thyroid hormone biosynthesis. Indeed, H_2O_2 is used by thyroid peroxidase (TPO) as final electron acceptor for thyroglobulin iodination and for coupling of iodinate tyrosine. In 1971 Björkman et al. [1] suggested that H_2O_2 would be produced at the apical plasma membrane of the thyrocyte by a complex that needs NADPH originating the

* Corresponding author.
E-mail address: laura.fugazzola@unimi.it (L. Fugazzolay).

http://dx.doi.org/10.1016/j.beem.2017.04.006
1521-690X/

Please cite this article in press as: Muzza M, Fugazzolay L,Disorders of H_2O_2 generation, Best Practice & Research Clinical Endocrinology & Metabolism (2017), http://dx.doi.org/10.1016/j.beem.2017.04.006

pentose phosphate pathway and requires calcium to acquire a functional conformation. Further biochemical studies showed that the enzyme responsible for H_2O_2 generation in the thyroid gland is a membrane-bound NADPH-dependent oxidase (NOX) [2–5]. NOXs are transmembrane electron carriers that use NADPH as an electron source and molecular oxygen as an acceptor to generate superoxide (O_2^-). However, no detectable O_2^- intermediate is released from thyroid cells [3], suggesting that NOX-mediated thyroidal H_2O_2 generation could result from intramolecular O_2^- dismutation.

The enzymes responsible for thyroidal H_2O_2 generation, the highly homologous genes dual oxidases 1 and 2 (*DUOX1* and *2*), originally called thyroid oxidases (*THOX1* and *2*), were cloned only 30 years later, by purification and partial sequencing of a thyroidal NOX flavoprotein [6] and via screening of a thyroid cDNA library for homologs of NOX2 [7].

DUOX1 and 2

Human *DUOX1* and *DUOX2* genes are located in opposite transcriptional orientation on chromosome 15 (15q15.3) and contain 35 and 34 exons, respectively, with 33 coding exons. They show 83% similarity in their sequences and encode for proteins with 1551 amino acids and 1548 amino acids, respectively. The NOX2 homologous region consists of six transmembrane oxidase domain, harboring four histidines, which are coordination sites for two heme prosthetic groups that provides an electron transport chain for transferring electrons from NADPH across the membrane and a C-terminal domain, including binding sites for NADPH and flavin adenine dinucleotide (FAD domains). In addition to other NOXs, DUOX proteins comprise a N-terminal peroxidase-like domain (hence named *dual* oxidases), followed by an additional transmembrane segment and a long cytosolic domain containing two calcium-binding sites (EF-hand motifs) [6,7], which could be involved in the direct activation of the H_2O_2 generator by calcium [8] (Fig. 1). The peroxidase-like domain has 43% similarity to TPO and is probably devoted to the binding to TPO [9]. Both *DUOXs* genes are located at the apical membrane of the thyroid cells, in close proximity to TPO [9]. At the thyroid level, DUOX2 is more expressed than DUOX1, and it is also more efficient in the production of peroxide [10,11]. At variance with other factors involved in thyroid hormone generation, DUOX1 and 2 are not expressed only in the thyroid gland; they are also expressed, though at a lower level, in the airway and tongue epithelia, cerebellum and testis (DUOX1), and in the salivary and rectal glands, gastrointestinal and airway epithelia, uterus, gallbladder and pancreatic islets (DUOX2) [10–15]. DUOX1/2 show two *N*-glycosylated states: the fully glycosylated mature form (190 kDa) expressed at the plasma membrane, and the high mannose glycosylated immature form (180 kDa) expressed exclusively inside the cell in the endoplasmic reticulum (ER) [16,17]. Despite their high level of sequence homology, DUOX1 and 2 are differently regulated by the two main signaling cascades in the thyroid: DUOX1 is stimulated by protein kinase A through Gs-PKA pathway, while DUOX2 activation is induced by protein kinase C through Gq-phospholipase C (PLC) pathway [8] (Fig. 1). Moreover, DUOX activity seems to be regulated by intracellular H_2O_2 concentrations [18,19]. Interestingly, initial experiments of DUOX2 proteins in heterologous systems failed to reconstitute H_2O_2 generation activity [11,20], since transfected cells express only the immature form of the protein retained in the ER, suggesting that the reconstitution of DUOX mediated H_2O_2 generation requires maturation factors. These factors have been identified by in silico screening of multiple parallel signature sequencing expression data [21], and have been called *DUOXA* (*DUOXA1* and *DUOXA2*).

DUOXA1 and 2

DUOXA1 and *2* genes are located between *DUOX* genes in a tail-to-tail orientation, and share the core of a bidirectionally active promoter with *DUOX1* and *DUOX2*, respectively, enabling co-expression of DUOX/DUOXA complexes [21–23]. The two DUOXA genes encode five transmembrane proteins, with a conserved *N*-glycosylated domain between the second and the third trasmembrane domains. Both DUOXA1 and 2 are more abundant in the thyroid, with lower expression in human respiratory epithelial cells and salivary gland, respectively [21,23]. DUOXA1 and 2 maturation factors form functional heterodimeric complexes with DUOX1 and DUOX2, that enable the exit of the dimer from ER and the translocation to the plasma membrane [16,24].

Please cite this article in press as: Muzza M, Fugazzolay L,Disorders of H_2O_2 generation, Best Practice & Research Clinical Endocrinology & Metabolism (2017), http://dx.doi.org/10.1016/j.beem.2017.04.006