Genetic defects of hydrogen peroxide generation in the thyroid gland

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ABSTRACT. Hydrogen peroxide (H₂O₂) is a key element in thyroid hormone biosynthesis. It is the substrate used by thyroid peroxidase for oxidation and incorporation of iodine into thyroglobulin, a process known as organification. The main enzymes composing the H₂O₂-generating system are the dual oxidase 2 (DUOX2) and the recently described DUOX maturation factor 2 (DUOXA2). Defects in these reactions lead to reduced thyroid hormone synthesis and hypothyroidism, with consequent increased TSH secretion and goiter. Since the first report in 2002 of DUOX2 mutations causing congenital hypothyroidism (CH), to date 25 different mutations have been described. Affected patients show a positive perchlorate discharge test and high phenotypic variability, ranging from transient to permanent forms of CH. Up to now, only two cases of CH due to DUOXA2 defects have been published. They also suggest the existence of a great genotype-phenotype variability. The phenotypic expression is probably influenced by genetic background and environmental factors. DUOX and DUOXA constitute a redundant system in which DUOX1/DUOXA1 can at least partially replace the function of DUOX2/DUOXA2. Furthermore, increased nutritional iodide could ensure a better use of H₂O₂ provided by DUOX1.

INTRODUCTION

Congenital hypothyroidism (CH) is the most common inborn endocrine disorder in childhood. It can originate from developmental defects of the thyroid gland, known as thyroid dysgenesis, or from defects in one of the steps of thyroid hormone synthesis, also known as thyroid dyshormonogenesis.

In recent years, the worldwide lowering of neonatal screening thresholds was followed by an increase in incidence of CH, with reports of up to 1:1500 newborns per year in Italy (1), USA (2), Greece (3), and UK (4). This is mainly the consequence of an improved detection of milder forms of CH, which were previously missed at neonatal screening. Therefore, the etiological spectrum of CH, previously characterized by predominance of patients with thyroid dysgenesis, has changed in favor of patients presenting an orthotopic thyroid gland [accounting for 68% of total CH cases in a recent report by Corbetta (1)]. CH with in situ thyroid gland represents a very heterogeneous group of disorders, including transient and permanent forms of thyroid dysfunction, with a wide spectrum of severity degrees. Understanding of the molecular mechanisms underlying thyroid dyshormonogenesis has become particularly important for the development of appropriate clinical and therapeutic strategies.

THYROID HORMONE BIOSYNTHESIS

Iodide (I⁻) is actively transported from the blood circulation into thyrocytes by a NIS (NIS/SLC5A5; MIM#601843) on the basolateral membrane, while pendrin (PDS/SLC26A4; MIM#605646) facilitates the efflux of I⁻ into the follicular lumen across the apical membrane. The central steps in thyroid hormones synthesis take place at the apical membrane of the follicular thyroid cells. First, TPO (TPO; MIM#606765) oxidizes I⁻ and links it covalently to tyrosine groups of TG (TG; MIM#188450) on the luminal side of the apical membrane. Then, iodinated tyrosyl residues (mono- and di-iodinated tyrosine, MIT and MIT) are linked via phenoxy-ether bonds to form T₄ and T₃.
T3. This second step is also catalyzed by TPO. The activity of TPO is limited by the supply of \( \text{H}_2\text{O}_2 \) as final electron acceptor. The \( \text{H}_2\text{O}_2 \)-generating system in the thyroid is constituted by dual oxidases (DUOX1 and DUOX2), expressed at the apical plasma membrane, and their maturation factors (DUOXA1 and DUOX2A). Thyroid hormones are released into the circulation after lysosomal digestion of the TG matrix protein. Concomitantly, released MIT and DIT are deiodinated by the iodotyrosine dehalogenase 1 (DEHAL1; MIM#612025) allowing recycling of \( I^- \) for further hormone synthesis (10). Thyroid hormone formation is predominantly regulated by TSH. The binding of TSH to the TSH receptor (TSHr; MIM#603372) activates both Gs and Gq proteins. The former activates the growth regulation, differentiation and thyroid hormone secretion, whereas the latter activates \( \text{H}_2\text{O}_2 \) generation and iodide binding to protein through the phospholipase C-dependent inositol phosphate Ca\(^{2+}\)/diacylglycerol pathway (11).

**ROLE OF \( \text{H}_2\text{O}_2 \) IN THYROID PHYSIOLOGY**

In vertebrates \( \text{H}_2\text{O}_2 \) is generated in response to insulin and growth factors in many systems. At physiological levels it enhances proliferation through several biochemical effects, like activation of different kinases and inhibition of their phosphatases. In normal cell biology, \( \text{H}_2\text{O}_2 \) is also produced as a toxic metabolite in host defence by neutrophils, monocytes, and macrophages, and it may have a similar role in gastrointestinal mucosa and lung epithelium (12).

At high concentrations, \( \text{H}_2\text{O}_2 \) induces oxidative stress, DNA oxidation and damage, leading to mutagenesis and apoptosis. \( \text{H}_2\text{O}_2 \) is therefore potentially carcinogenic and has been found to play a role in several human cancers (13).

In the thyroid, \( \text{H}_2\text{O}_2 \) is necessary for TPO to oxidize iodide and synthesize thyroid hormones. Defects in these reactions lead to reduced thyroid hormone synthesis, hypothyroidism, and consequent increased TSH secretion and goiter. On the other hand, \( \text{H}_2\text{O}_2 \) exerts on the thyrocytes the same toxicity described for other cell types: at high concentrations, \( \text{H}_2\text{O}_2 \) induces apoptosis in thyroid cells, and at even higher levels necrosis (14, 15). The main protection against the generated \( \text{H}_2\text{O}_2 \) relies on mechanisms finally protect the cell in case of intracytoplasmic leakage of \( \text{H}_2\text{O}_2 \): these mechanisms are based on selenoproteins, like glutathione peroxidases and thioredoxin reductases (17).

**DUOX2**

About 30 years ago it was already known that the enzyme generating \( \text{H}_2\text{O}_2 \) for TPO activity should be a membrane-bound NADPH-dependent flavoprotein (18). Fifteen years later two highly homologous genes, the dual oxidases DUOX1 and DUOX2, were cloned and identified as the catalytic core of the thyroidal \( \text{H}_2\text{O}_2 \) generator (19, 20). They were originally called thyroid oxidases, or THOX.

DUOX1 (MIM#606758) and DUOX2 (MIM#606759) are glycoproteins located on chromosome 15q15.3, 16 kb apart from each other, with opposite transcriptional orientations. They include 33 coding exons spanning, respectively, 36 and 21.5 kb and having 1551 and 1548 amino acids for open reading frame. They share 83% similarity in their DNA sequences, but have different promoters.

DUOX proteins contain seven transmembrane helices, an extracellular peroxidase-like domain, a long intracellular loop containing two EF-hand calcium-binding motifs responsible for their Ca\(^{2+}\)-regulated activity, a NADPH-oxidase catalytic core, and a binding cavity for flavin adenine dinucleotide (FAD) (19, 20). DUOX co-localizes at the cell membrane with TPO and its peroxidase-like domain has 43% similarity to TPO, but there is up to now no evidence for peroxidase activity. The DUOX2 mRNA expression in the thyroid gland is 1.5-5 times more abundant than that of DUOX1, and it is also more efficient in the production of peroxide (21). Only the fully glycosylated mature forms of DUOX are transported to the apical membrane of thyrocytes and generate \( \text{H}_2\text{O}_2 \) (22-25).

DUOX differs from the other factors involved in thyroid hormone biosynthesis pathway for some aspects. In particular, the DUOX1 and DUOX2 expression is not restricted to the thyroid gland (26). In fact, DUOX1 is present in other epithelial tissues like the prostate, testis, placenta, heart, kidney, brain, pancreas, and skin, while DUOX2 is found in the salivary gland, stomach, duodenum, colon, rectum, pancreas, and tests (26-28). Both DUOX1 and 2 are expressed in human airway epithelial cells (28-30). Furthermore, DUOX homologs are evolutionary conserved molecules, also found in invertebrates like Drosophila and C. Elegans (31).

**DUOXA**

In 2006, Grasberger et al. identified the DUOX maturation factors (DUOXA1 and DUOXA2); the dimervation of a DUOX with their corresponding DUOXA proteins enables the proper folding of DUOX, exit from ER and trafficking to the cell surface (24). The DUOX genes are located adjacent within the 16 kb intergenic region between DUOX1 and DUOX2 genes in a tail-to-tail orientation to each other. The DUOXA1 protein consists of 343 amino acids and DUOXA2 of 320 amino acids. Both proteins are predicted to include five transmembrane spans, with an extended extracellular loop where three N-glycosylation sites are present.

The evolutionary conserved genetic linkage of DUOX1/DUOXA1 and DUOX2/DUOXA2 into transcriptional units leads to a strict coexpression of each DUOX with the corresponding maturation factor. The DUOX2 mRNA is more abundantly expressed in the thyroid gland than in the salivary gland while the DUOX1 mRNA is also predominantly distributed in the thyroid gland, with a lower level in the esophagus and in human respiratory epithelial cells (24, 32).

Recently a novel murine model with global DUOXA deficiency has been created, by simultaneous targeting of the contiguous DUOXA1 and DUOXA2 genes (Duoxa\(^{-/-}\))