Scientific Final Report

Title of the project: Characterization of the function of Duox2-derived hydrogen peroxide in gallbladder epithelia

Type of grant: OTKA-PD103960

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1. Published results

The aim of the project is the characterization of the potential antimicrobial, epithelial cell protecting or signal transduction modulator effect of Duox2-produced H_2O_2 in gallbladder. To date there is only one solely Duox2-deficient mouse strain that enables the examination of Duox2 function. These mice carry a single nucleotide mutation in exon 16 that results in a highly conserved valine 674 to glycine amino acid exchange leading to the loss of function and consequently to congenital hypothyroidism. The V674 mutation is located between the first transmembrane helix and the calcium-binding EF-hand motifs of Duox2, which region was suggested to contain an ER retention signal in the human Duox2 enzyme. To understand how the V674G mutation cause the loss of Duox2 function we expressed it in Flp-in HEK cells.

We showed that the V674G mutant Duox2 fails to release extracellular H_2O_2 and it does not localize to the plasma membrane, but retains in the endoplasmic reticulum, where it still forms a stable complex with its maturation factor Duoxa2. On salivary gland sections we showed that the mutant Duox2 is less intense along the apical plasma membrane and forms dot-like vesicular structures within intracellular sites. With a cytosol-targeted FRET-based H_2O_2 sensor we also measured that the mutant Duox2 does not produce reactive oxygen species (ROS) intracellularly either. To further characterize the 674 amino acid position of Duox2 we prepared the alanine, leucine, threonine mutants as well and measure extracellular H_2O_2 production. Our findings revealed a strong requirement for bulky aliphatic amino acid side chain at position 674 in Duox2, as both the glycine and alanine mutant proteins appeared to be considerably less stable without detectable ROS production while H_2O_2 production and stability of the leucine and threonine substituted Duoxes were similar to the wild type.

Publication: **Donkó Á**, Morand S, Korzeniowska A, Boudreau HE, Zana M, Hunyady L, Geiszt M, Leto TL. Hypothyroidism-associated missense mutation impairs NADPH oxidase activity and intracellular trafficking of Duox2. Free Radic Biol Med. 2014 Aug; 73:190-200. **IF: 5.71**

2. Results prepared for manuscript

In our further experiments we addressed the signal transduction events upstream and downstream of Duox-mediated ROS production. Although we could culture primary gallbladder epithelial cells successfully for a few days, these cells were not dividing and our attempts to set up the long term culture was serially unsuccessful. Since the number of obtainable mouse gallbladder epithelial cells is strongly limited by the dimensions of this organ, therefore we focused to another cell system where we could examine the function of Duox in signal transduction. One of our earlier observation was that A431 (human epidermoid carcinoma cell line) produce high level of ROS under basal conditions and for calcium mobilizing stimuli like thapsigargin as well. We showed that the ROS production of A431 cells can be suppressed by Duox1 specific siRNA along with the decrease of Duox1 protein detected by a polyclonal anti-Duox antibody. In mammalian cells there are multiple stimuli that can trigger the formation of H_2O_2 and among them growth factor induced ROS production is thought to be a particularly important signaling event. Although the signaling pathways provoked by EGF are mostly well-understood, the enzymatic source of EGF-stimulated H_2O_2 production was unknown.

Extending our experiments we showed in A431 and HaCaT cells (immortal keratinocyte cell line from human skin) that the long sought source of EGF-induced H_2O_2 production in epidermal cells is Duox1. Our observations provide evidence for a new signaling paradigm in which EGF-stimulated changes of the intracellular calcium concentration are transformed into redox signal through the activation of Duox1. In HaCaT cells we also demonstrated that Duox1-mediated H_2O_2 production is sensed by the thioredoxin-peroxiredoxin system, because activation of the Duox1 enzyme increased the amount of peroxiredoxin 1 and peroxiredoxin 2 dimers, indicating the oxidation of these proteins. Albeit previous reports suggested that EGF-induced H_2O_2 can increase tyrosine phosphorylation of proteins through the inhibition of protein tyrosine phosphatases, in our experiments we did not observe a major change in overall tyrosine phosphorylation pattern, when Duox1 expression was suppressed.

Manuscript: Sirokmány G, Pató A, Zana M, **Donkó Á**, Bíró A, Nagy P, Geiszt M. Epidermal growth factor-induced hydrogen peroxide production is mediated by Dual oxidase 1 – under review at BMC Biology

During the grant period we were also studying the antimicrobial function of Duox2 and we have promising preliminary results, but to collect these together into a manuscript further experiments are needed, that is part of an ongoing project.