Jpn. J. Infect. Dis., 57, 2004

Tissue Distribution and Putative Physiological Function of NOX Family NADPH Oxidases

Karl-Heinz Krause*

Biology of Ageing Laboratories, University of Geneva, Geneva, Switzerland

SUMMARY: The NOX family of ROS-generating NADPH oxidases consists of 7 members: NOX1 to NOX5, DUOX1 and 2. NOX1 is predominantly found in the colon, where it possibly plays a role in the host defense. NOX2 is the phagocyte NADPH oxidase, a clearly established host defense enzyme. NOX3 is almost exclusively expressed in the inner ear, where it is involved in otoconia morphogenesis, but based on its localization might also play a role in the auditory system. NOX4, widely expressed in kidney, vascular cells, osteoclasts etc.; it might be a constitutively active enzyme, regulated on the level of gene expression but its precise physiological function remains unknown. NOX5, a Ca^{2+} activated enzyme is predominantly expressed in lymphoid tissues and testis, where it might be involved in signaling processes. DUOX1 is expressed in the thyroid and in respiratory epithelia, and DUOX2 in the thyroid and in gastrointestinal glandular epithelia. Both DUOX enzymes are involved in thyroid hormone synthesis, but possibly also in epithelial host defense.

The NOX family of NADPH oxidases is a unique family of enzymes whose physiological function is the generation of reactive oxygen species (ROS). Based on our present state of knowledge, the family consists of 7 members: NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1, and DUOX2. All NOX family members share a core structure consisting of 6 transmembrane domains (which include two heme binding regions), and a relatively long cytoplasmic C-terminus (which contains FAD and NADPH-binding regions).

While NOX1, NOX2, NOX3, and NOX4 consist only of the above described NOX core structure, NOX5, DUOX1 and DUOX2 are characterized by N-terminal extensions (4,5). In the case of NOX5, the N-terminal extension consists of 4 EF-hand domains, in the case of DUOX1 and DUOX2, of 2 EF hand domains, an additional transmembrane domain, as well as a peroxidase homology domain. Consistent with their Ca^{2+}-binding EF hand domains, NOX5, DUOX1, and DUOX2 are Ca^{2+}-activated enzymes (4,5).

NOX1 tissue distribution: Colon: NOX1 was originally also referred to as mox1 or NOH-1. It shows by far the most abundant expression in the colon (6,7). Yet, additional sites of NOX1 expression have been described.

Stomach: NOX1 has been described to be expressed in the guinea pig stomach. Whether this also applies to other species remains unclear. At least in human stomach no relevant expression has been observed (6).

Uterus and prostate: NOX1 is expressed in uterus and prostate. This expression is markedly lower than expression in the colon, yet clearly detectable (6,7).

Inducible expression: Interestingly, NOX1 expression is found to be induced in some cell types, for example in PDGF-induced expression in aortic smooth muscle (7).

NOX1 function: At this point, the physiological function of NOX1 remains a matter of hypothesis. Basically two major suggestions have been discussed: host defense function, through ROS-dependent bacterial killing, and stimulation of cell division through activation of redox-sensitive intracellular signaling mechanism. At this point, it appears likely that NOX1 function depends on the cell type where it is expressed. In the colon, the organ of our body most heavily exposed to bacteria, a host defense function appears likely, and the upregulation through inflammatory mediators (see above) favor such an interpretation. However, the inducible expression in the vascular system most likely serves another purpose. Participation in blood pressure regulation appears a possibility. Angiotension-dependent NOX1 elevations would lead to increased superoxide generation in the vascular system; superoxide degrades NO and thereby would lead to an increase in blood pressure. As a more long-term effect, superoxide might also provide a stimulus for smooth muscle proliferation and - under pathological conditions - participate in the cascade leading to atherosclerosis.

NOX2 tissue distribution: Phagocytes: NOX2 is traditionally referred to as the $gp91^{phox}$ subunit of the "phagocyte NADPH oxidase". Clearly white blood cells of myeloid lineage are the predominant site of expression of NOX2, in particular neutrophil granulocytes, monocyte/macrophages, and eosinophils.

NOX2 function: NOX2 is beyond any doubt a enzyme of the host defense, as witnessed by the clinical presentation of patients with chronic granulomatous disease. Patients with this congenital disease lack either NOX2 (= $gp91^{phox}$) or one of its subunits ($p47^{phox}$ or $p67^{phox}$) and suffer from severe infections. As ROS do have a microbicidal action, the host defense function of NOX2 is in general attributed to a direct killing of microorganisms by the ROS, or the interaction of ROS with the myeloperoxidase system.

There is increasing evidence that NOX2 is involved in a variety of pathological processes, including the development of cardiovascular disease; neurodegeneration (8), and HIV pathogenesis (9).

NOX3 tissue distribution: NOX3 appears to be the NOX isoform with the most restricted and specialized tissue dis-tribution. Indeed, NOX3 is - at relevant amounts - found almost exclusively in the inner ear (1,10). Within the inner ear, it appears to have a ubiquitous tissue distribution, found within sensory epithelia and ganglia both of the auditory and the vestibular system (1).

NOX3 function: In the NOX3-deficient *het* (head-tilt) mouse, lack of otoconia formation and subsequent troubles of equilibrium are the most obvious phenotype (10). Yet, the abundant expression of NOX3 in the auditory system (organ of Corti and spiral ganglion) (1) raise the possibility that additional functions might exist.

NOX4 tissues distribution: NOX4 was initially also referred to

*Corresponding author: E-mail: Karl-Heinz.Krause@medecine.unige.ch

as Renox, because of its abundant expression in the kidney cortex (2,3). Yet, by now it appears to be probably the most widely expressed among the various NOX isoforms. Besides its predominant distribution in the kidney, NOX4 expression has been described - among others - in endothelial cells, smooth muscle cells, in the heart, pancreas, placenta, skeletal muscle, ovary, testis, osteoclasts, fibroblasts and astrocytes.

NOX4 function: NOX4 function remains elusive. The most popular working hypothesis is a role in oxygen sensing in the kidney cortex (2). Yet, available data concerning this option are rather contradictory and there is no convincing experimental proof. Other options include a role in the regulation of cell proliferation; again until now, there is no convincing evidence for this suggestion.

The most striking feature of NOX4 function in terms of biochemistry is that fact that so far, no activation mechanism has been found. Thus, it is conceivable (but net yet proven) that NOX4 is a constitutively active enzyme, regulated on the level of gene expression rather than on the level of enzyme activity. It would thus be comparable to iNOS (inducible nitric oxide synthetase). Obviously, the physiological function of the enzyme cannot be derived from such biochemical considerations, yet the possibility that NOX4 might be a constitutively active, inducible enzyme should provide food for thought.

NOX5 tissue distribution: NOX5 is essentially found in lymphoid tissues and in testis (5). Strikingly, NOX5 mRNA, while abundant in tissue lymphocytes, is almost absent in circulation blood lymphocytes . Within lymphoid tissues, NOX5 is enriched in B cell-rich regions surrounding germinal centers, but is also found in T-cell rich regions; macrophages and dendritic cells however seem to be NOX5-negative (5). In testis, NOX5 mRNA was mostly observed in pachytene spermatocytes (5). I should however be noted that this enrichment of mRNA in the early stages of spermatogenesis does not exclude a function of NOX5 in more mature sperm cells, as mRNA is synthesized at early stages of spermatogenesis, even if the protein is required at later stages.

NOX5 function: Quite obviously, the full length form of NOX5, as found in adult tissues, mediates Ca^{2+}-dependent ROS-generation in tissue lymphocytes and in testis. Yet, the physiological function of such a Ca^{2+}-dependent ROS generation in these tissues is poorly understood. A host defense function appears rather unlikely, so does a function in biosynthesis. Thus, ROS-dependent signaling and regulation of transcription factors is the most likely explanation for the function NOX5 in testis. Indeed, a role of ROS in lymphocyte differentiation, potentially through NFkappaB activation is conceivable. In spermatogenesis, a role for ROS has been proposed at different stages, from induction of apoptosis in early spermatogenesis to capacitation reaction and sperm oocyte fusion.

Tissue distribution of DUOX1 and DUOX2: DUOX1 and DUOX2 are both expressed in the thyroid and have therefore been also referred to as thyroid oxidases, ThOX (4). This is clearly a misnomer, as already relatively primitive organisms such as *C. elegans* express DUOX enzymes (11). Thus, from an evolutionary point of view, the appearance of DUOX enzymes precedes the appearance of the thyroid gland. But even in mammals, DUOX enzymes are not restricted to the thyroid with a predominant expression of DUOX1 in the respiratory epithelia and DUOX2 in salivary and rectal gland epithelia (12).

DUOX1 and DUOX2 functions: In mammals, both DUOX1 and DUOX2 are thought to be involved in thyroid hormone synthesis. This is most convincingly demonstrated by hypothyroidism in rare patients with DUOX2 mutations (13). DUOX mutants of *C. elegant* have an abnormal extracellular matrix and a role of DUOX enzymes in cross-linking of the extracellular matrix proteins has been proposed (11). The extra-thyroid function in mammals remains poorly understood, however based on their localization on respiratory and gastrointestinal epithelia, a host defense function is conceivable (12).

CONCLUSION

At this point, two physiological functions of NOX enzymes can be considered as proven:

i) Host defense: This has been demonstrated beyond doubt for the phagocyte NADPH oxidase. It has also been indirectly suggested for NOX1, DUOX1, and DUOX2. However, based on presently available data, host defense is rather unlikely as a function of NOX3, NOX4, and NOX5.

ii) Biosynthetic processes: This is best documented for DUOX enzymes. In mammals, it involves biosynthesis of thyroid hormones and in *C. elegans* the crosslinking of extracellular matrix. The lack of otoconia in NOX3-deficient mice might also reflect a role of NOX3 in the biosynthesis of otoconia, although the direct biochemistry of such an involvement is less understood. Note however, that at this point it is not clear whether such an involvement of DUOX1, DUOX2 or NOX3 in biosynthetic processes is the predominant biological function of these enzymes.

Signaling function of ROS, has been received wide attention over that last years. It is therefore likely that certain, or possibly all, NOX enzymes are involved in signaling. At this point, such a signaling can been documented in in vitro situation, but the confirmation of its physiologically relevant in vivo function requires further experimental proof.

ACKNOWLEDGMENTS

This work was supported by the grant 3100A0 - 103725, attributed to KHK by the Swiss National Foundation.

REFERENCES

1. Banfi, B., Malgrange, B., Knisz, J., Steger, K., Dubois-Dauphin, M. and Krause, K. H. (2004): NOX3: A superoxide-generating NADPH oxidase of the inner ear. J. Biol. Chem.
2. Geiszt, M., Kopp, J. B., Varnai, P. and Leto, T. L. (2000): Identification of renox, an NAD(P)H oxidase in kidney. Proc. Natl. Acad. Sci. USA, 97, 8010-8014.
3. Shiose, A., Kuroda, J., Tsuruya, K., Hirai, M., Hirakata, H., Naito, S., Hattori, M., Sakaki, Y. and Sumimoto, H. (2001): A novel superoxide-producing NAD(P)H oxidase in kidney. J. Biol. Chem., 276, 1417-1423.
4. De Deken, X., Wang, D., Many, M. C., Costagliola, S., Libert, F., Vassart, G., Dumont, J. E. and Miot, F. (2000): Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. J. Biol. Chem., 275, 23227-23233.
5. Banfi, B., Molnar, G., Maturana, A., Steger, K., Hegedus, B., Demaurex, N. and Krause, K. H. (2001): A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes. J. Biol. Chem., 276, 37594-37601.
6. Banfi, B., Maturana, A., Jaconi, S., Arnaudeau, S., Laforge, T., Sinha, B., Ligeti, E., Demaurex, N. and Krause, K. H. (2000): A mammalian H+ channel generated through alternative splicing of the NADPH oxidase homolog NOH-1. Science, 287, 138-142.
7. Suh, Y. A., Arnold, R. S., Lassegue, B., Shi, J., Xu, X., Sorescu, D., Chung, A. B., Griendling, K. K. and Lambeth, J. D. (1999): Cell transformation by the superoxide-generating oxidase Mox1. Nature, 401, 79-82.
8. Zekry, D., Epperson, T. K. and Krause, K. H. (2003): A role for NOX NADPH oxidases in Alzheimer's disease and other types of dementia? IUBMB Life, 55, 307-313.
9. Vilhardt, F., Plastre, O., Sawada, M., Suzuki, K., Wiznerowicz, M., Kiyokawa, E., Trono, D. and Krause, K. H. (2002): The HIV-1 Nef protein and phagocyte NADPH oxidase activation. J. Biol. Chem., 277, 42136-42143.
10. Paffenholz, R., Bergstrom, R. A., Pasutto, F., Wabnitz, P., Munroe, R. J., Jagla, W., Heinzmann, U., Marquardt, A., Bareiss, A., Laufs, J. et al. (2004): Vestibular defects in head-tilt mice result from mutations in Nox3, encoding an NADPH oxidase. Genes Dev., 18, 486-491.
11. Edens, W. A., Sharling, L., Cheng, G., Shapira, R., Kinkade, J. M., Lee, T., Edens, H. A., Tang, X., Sullards, C., Flaherty, D. B. et al. (2001): Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91phox. J. Cell. biol., 154,879-891.
12. Geiszt, M., Witta, J., Baffi, J., Lekstrom, K. and Leto, T. L. (2003): Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J., 17, 1502-1504.
13. Moreno, J. C., Bikker, H., Kempers, M. J., van Trotsenburg, A. S., Baas, F., de Vijlder, J. J., Vulsma, T. and Ris-Stalpers, C. (2002): Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. N. Engl. J. Med., 347, 95-102.