Florence Nightingale | Demiroglu Science University Florence Nightingale Journal of Transplantation 2023;8(1-2):48-55
doi: 10.5606/dsufnjt.2023.16

NOX-4: A key player in protecting against cancer from free radicals

Şehri Çolak, Oytun Erbaş

Institute of Experimental Medicine, Gebze-Kocaeli, Türkiye

ABSTRACT

The oxygen (O_2) molecule, which is essential for every individual, is utilized by cells for their energy requirements. Harmful molecules released during the utilization of O_2 are referred to as free radicals. Free radicals are molecules with unpaired electrons in their outermost layers. Their tendency to attack surrounding molecules and strive for stability is the source of this behavior. To counter their instability, the body has developed antioxidant systems. These systems are obtained both within the body and through dietary intake. Excess free radicals and a deficiency in antioxidants lead to oxidative stress. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are molecules with a significant impact on the production pathway of free radicals. There are seven known types of NADPH oxidases, including five NADPH oxidases (NOX) and two dual oxidases (DUOX) varieties. The first discovered type, NOX2, plays a pivotal role in facilitating the discovery of other NOX species. The other species are classified based on their similarity to NOX2. Although NOX4, which is one of these NOX types, bears resemblance to others, the diseases it leads to, particularly cancer, exhibit differences. Digestive cancers resulting from free radicals are suppressed through NOX4. Research and studies on NOX4 continue due to its unique structure. This review discusses various free radical species, the functions and types of NOX enzymes, and the diseases that occur as a result of accumulations.

Keywords: Cancer, DUOX, free radicals, NADPH oxidase, NOX4, reactive oxygen species.

The harmful byproducts resulting from aerobic respiration are referred to as free radicals. Free radicals are molecules that have one or more unpaired electrons in their outermost orbits. Free radicals can be divided into two categories: reactive oxygen species (ROS) and reactive nitrogen species (RNS). The most prevalent type of free radical in the body is ROS.[1] Proteins responsible for the generation of free radicals can be found. The first of these is the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase; NOX) produced by mammalian phagocytic cells.[2] The NADPH oxidases are responsible for transporting electrons across the membrane. They facilitate the generation of superoxide ions ($O_2^{\bullet-}$) by donating electrons to O_2 molecules. Therefore, NOX proteins play a significant role in the formation of ROS. The excessive accumulation and activity of NADPH oxidase enzymes can lead to the development of various diseases, including cardiovascular diseases, psychological disorders, and cancer.[3] In this review, we have addressed free radical species, the roles, and variations of NOX enzymes, as well as the diseases that result from their accumulation.

FREE RADICALS

Free radicals are atoms, molecules, or ions in their outermost layers that contain unpaired electrons. These unpaired electrons, due to their high reactivity, are defined as harmful molecules as they readily engage in temporary but impactful reactions with surrounding molecules, leading to structural damage.[4]

Free radicals and ROS are often confused with each other. Reactive oxygen species, as defined, encompass unpaired O_2 species, just like in the definition of ROS. However, free radicals are more general and comprehensive, as they include not only ROS but also RNS and unpaired ions.[4,5]

Free radicals can originate from both endogenous and exogenous sources. The majority of endogenous free radical production is carried out by mitochondria, which are responsible for energy generation. During energy production in mitochondria, O_2 can become unstable and

Received: October 12, 2023
Accepted: October 23, 2023
Published online: November 07, 2023

Correspondence: Şehri Çolak.
E-mail: sehricolak07@gmail.com

Cite this article as:
Çolak Ş, Erbaş O. NOX-4: A key player in protecting against cancer from free radicals. D J Tx Sci 2023;8(1-2):48-55. doi: 10.5606/dsufnjt.2023.16.

transform into hydrogen peroxide (H_2O_2) and hydroxyl ($^{\bullet}OH$) molecules due to the presence of unpaired electrons. Exogenous sources of free radicals include factors such as ultraviolet radiation, environmental pollution (such as air and water pollution), harmful habits like smoking and drug use, and stress.[4,6]

Reactive nitrogen species

Reactive nitrogen species are derivatives of nitric oxide (NO). Nitric oxide is the first discovered endogenous source of RNS. For many years, the presence of NO in biological systems and its therapeutic use have been subjects of debate. Drugs like nitroglycerin have been administered to patients to reduce blood pressure and induce vasodilation in the context of heart conditions. However, it was understood that, apart from its beneficial aspects, the NO molecule is considered a free radical due to its easy reactivity with O_2. Reactions between NO and O_2 occur even in the presence of the antioxidant superoxide dismutase (SOD). Therefore, NO has the potential to make O_2, which is normally not very harmful, up to two times more damaging.[7]

Studies involving cyclic guanosine monophosphate (cGMP) and endothelium-derived relaxing factor (EDRF) have revealed the significant role of the NO molecule in vasodilation. An increase in cGMP production by guanylate cyclases in vascular smooth muscle cells leads to the relaxation of blood vessels and, consequently, an increase in blood flow. The EDRF has been observed to enhance cGMP synthesis in isolated blood vessels and promote protein phosphorylation in smooth muscle.[7-9]

Reactive oxygen species

Reactive oxygen species are molecules that were initially discovered as oxygen derivatives in skeletal muscle. While they were originally believed to be generated as a result of aerobic respiration, it is now observed that ROS production occurs in many places, ranging from the gastrointestinal signaling pathways to the endoplasmic reticulum.[10] Reactive oxygen species come in the form of molecules that include H_2O_2, $O_2^{\bullet-}$, which is prone to rapid breakdown and instability, and $^{\bullet}OH$ molecules. Among these, H_2O_2 is the most well-defined ROS species to date.[11]

ANTIOXIDANTS

Antioxidants are molecules that stabilize free radicals by donating electrons and preventing the oxidation process. Like free radicals, antioxidants can also be of both endogenous and exogenous origins. The most important sources of antioxidants are vitamins A, C, and E. In addition to these, there are numerous antioxidant systems in the body, including coenzyme Q10, selenium, polyphenols, and internally produced antioxidants such as SOD, catalase (CAT), glutathione reductase, and glutathione peroxidase.[12,13]

Oxidative stress

Cells produce harmful free radicals as a byproduct of aerobic respiration, although they can be detrimental. To neutralize these radicals, cells have various antioxidant systems. Oxidative stress occurs as a result of either an insufficient antioxidant system or excessive production of free radicals. Oxidative stress, characterized by an excess of free radicals, is associated with neurodegenerative disorders, cardiovascular diseases, diabetes mellitus, and various other illnesses. However, molecules like glutathione and thioredoxin play a crucial role in counteracting oxidative stress, with NADPH being the molecule that enables them to do so.[14]

THE NADPH OXIDASES

The NADPH oxidase is found in various eukaryotic organisms but is not present in prokaryotic life forms. It plays a role in events such as the body's defense, signal transmission in tissues, and hormone synthesis. Additionally, NOX enzymes facilitate the conversion of O_2 into $O_2^{\bullet-}$, leading to the formation of various types of ROS. As a result of this formation, ROS can have harmful effects on the cell membrane and deoxyribonucleic acid (DNA) replication, contributing to the development of several related diseases.[2,15]

In the NADPH oxidase family, there are a total of seven types, including five NOX species and two dual oxidases (DUOX).[16] In addition to these seven units, two regulatory subunits (p47phox and NOXO1) and two activator subunits (p67phox and NOXA1) have been added, along with two DUOX-specific maturation factors (DUOXA1 and DUOXA2). Further genetic studies have

revealed the existence of two homologs of p40phox and p22phox.[3] The activator subunit p67phox moves to the membrane in association with the regulatory subunit p47phox upon cell stimulation. The p67phox contains two SH3 domains, both of which play a role in phagocytic NOX activation. The N-terminal SH3 domain on p67phox is known as the most conserved region. The C-terminal SH3 domain binds specifically to the pattern recognition receptor on p47phox, facilitating the membrane translocation of p67phox. In between the two SH3 domains, p67phox contains a PB1 domain, which allows p67phox to interact readily with p40phox in phagocytes. Human p40phox is not essential for oxidase activation; however, it has been acknowledged to play a crucial role in oxidase by enhancing the recruitment of p67phox and p47phox to the phagosomal membrane, thereby contributing to oxidase function.[17]

After the identification of NOX1 and NOX2 in phagocytic cells, it was observed that non-phagocytic cells also contain similar superoxide-producing oxidases. Shiose et al.[18] initially proposed that some similar oxidases play crucial roles in events like cell proliferation and oxygen response. Furthermore, they found that NOX4 was expressed in human embryonic kidney (HEK293) cells derived from the kidney, using real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

Most NOX enzymes are activated by the binding of specific proteins such as p22phox, Rac, and others. However, NOX4 is the only isoform suggested to be structurally active and negatively regulated by adenosine triphosphate binding.[19]

Despite being present throughout the whole body, NOX1 is abundant in the colon, NOX2 in phagocytic cells, NOX3 in the inner ear, NOX4 in the kidney and blood vessels, NOX5 in the testes and lymphoid tissues, while DUOX1 and DUOX2 are highly abundant in the thyroid gland.[3]

NOX2

It is the first NOX type identified in phagocytic cells. The discovery of the NOX2 enzyme has helped in understanding the other members of the NOX family. Thus, NOX2 has served as a prototype in terms of evolutionary and human health importance for other family members. While it is now known that NOX2 is effective in processes such as angiogenesis, signal transduction, and cell death, it was initially thought to be responsible only for immunity.[20]

The NOX2 functions as a transmembrane redox chain on the outer side of the membrane, connecting O_2 on the outer side of the membrane with the electron donor and acceptor in the vicinity of the cell membrane. It provides electrons through a flavin adenine dinucleotide (FAD) bound with two amide-linked histidine molecules, one on the inside and the other on the outside. Initially, electrons are transferred from NADPH to FAD through a process regulated by the activation protein of p67phox. In the second step, the reduced flavin adenine dinucleotide dihydrogen (FADH2) transfers one electron to the iron center of the inner heme. As the iron in the heme can only accept one electron, the second electron must be transferred from FADH, which is a partially reduced flavin, to the outer heme electron.[3]

Phagocytic NOX enzymes are composed of a multi-subunit complex that includes NOX2 and p22phox, supported by cytoplasmic protein factors p47phox, p67phox, p40phox, and small guanosine triphosphate-binding proteins (G proteins Rac1 or Rac2). This complex, which contains transmembrane flavocytochrome b558, is heterodimeric in nature. In the absence of microbial infection and various compounds, these structures are physically separated from each other. Activation regulatory subunits, upon binding to flavocytochrome b558 by translocating to the membrane, regulate NOX activation, preventing excessive $O_2^{\bullet-}$ production and harmful oxidation of biological macromolecules. After the assembly of the units, NOX2 activates the sequential transfer of electrons along the plasma membrane to reduce O_2, resulting in the synthesis of superoxide anion in the phagosome. The initial production of $O_2^{\bullet-}$ leads to the formation of various secondary oxidative metabolites. In particular, $O_2^{\bullet-}$ is typically converted to hypochlorous acid, a potent bactericidal compound primarily responsible for pathogen clearance in immune cells, especially myeloperoxidase-mediated conversion in phagocytes.[20]

NOX1

The NOX1 is the first homolog generated from NOX2. The genes for NADPH oxidase 1 and 2 appear to be the result of a relatively recent gene duplication, given the similar number and length of exons between the two genes. Similarly, the protein sequence identity shows a similarity of approximately 60%. The NOX1 gene found in humans and mice is located on the X chromosome. Alternatively, it has been found to have an additional form of NOX1 besides lacking exon 11. This additional variant has been observed to encode a protein that does not produce $O_2^{\bullet-}$. The presence of a very short second isoform of NOX1 has been suggested; however, it has been found to be artificial due to the formation of a stable loop in NOX1 messenger ribonucleic acid (mRNA). In mice, several other splice variants have been described based on the use of alternative promoters.[3]

Unlike NOX2, NOX1 is not expressed in immune cells but plays a role in immunity. It is expressed in colon epithelial cells and is required for host defense, barrier function, and maintaining the balance of commensal bacteria. Stimulation of formyl peptide receptors on epithelial cells by bacteria induces NOX1, which not only protects the barrier through epithelial growth and repair but also induces ROS production. Additionally, it contributes to the prevention of overgrowth of commensal bacteria by promoting the production of H_2O_2 from $O_2^{\bullet-}$. In the presence of NOX1, there are bacteria that produce CAT, such as *Escherichia coli*, which supports respiration, as well as pathogenic bacteria like *Citrobacter rodentium* which use H_2O_2.[21]

NOX3

The NOX3 was identified in the year 2000 based on sequence similarities to other NOX isoforms, but research into its function began after 2004. NOX3 shares approximately 56% protein identity with NOX2. The human NOX3 gene is located on the 6th chromosome. While sequence alignment and hydropathy plot analysis have provided insights into the overall structure of NOX3, its NADPH- and FAD-binding regions, as well as the locations of heme histidines, are similar to NOX1 and NOX2. To date, no additional variants of NOX3 have been reported.[3]

Unlike NOX1 and NOX2, NOX3 does not play a role in immune cells and host defense. A defect in NOX3 results in head tilting in mice, known as otoconia morphogenesis defect. It is known that superoxides produced by NOX3 are responsible for noise-induced and cisplatin-induced hearing loss. Hearing loss caused by NOX3 can be corrected using NOX3-specific small interfering RNA and small-molecule inhibitors in the inner ear. Additionally, NOX3 can activate insulin in hepatocytes and lead to the production of vascular endothelial growth factor (VEGF) and the initiation of angiogenesis.[21]

NOX4

The NOX4, highly expressed in the kidney, osteoclasts, fibroblasts, and endothelial cells, shares a common core with NOX1 and NOX3, while it has only approximately 39% similarity with NOX2.[3] Studies on embryonic kidney cells have shown that similar to NOX2, the maturation of NOX4 is also dependent on the expression of p22phox. The NOX4 contains glycosylation regions and, unlike other isoforms, is structurally active. While the activity of NOX4 is regulated by its cellular localization, it is believed to occur with the participation of activating factors such as protein disulfide isomerase or polymerase δ-interacting protein 2. The complex of NOX4 and p22phox has been detected even *in vitro* in the absence of SOD.[16] Reactive oxygen species are mainly produced as H_2O_2. Mutations at the His222 position in the extracytoplasmic E-loop of NOX4 have been found to inhibit H_2O_2 production, thus playing an important role in the determination of ROS species through NOX4.[20]

NOX4 INHIBITORS

Endogenous compounds, natural compounds, and thiol-modifying compounds have shown an inhibitory effect on NOX4, but they haven't shown creative effects. Until now, there is still no specific NOX4 inhibitor. An inhibitor of NOX2 and NOX4, Fulvene-5, has been found to inhibit endothelial tumor growth in mice. Plumbagin, derived from plants, is known to inhibit NOX4 by affecting it. GKT137831 and GKT136901, developed by the biotechnology company GenKyoTex, have been shown to inhibit NOX1/4. While

the inhibitor GKT137831 has a phase I study, a phase II clinical trial is planned for diabetic nephropathy.[16] Additionally, S17834, 6-dimethylaminofulvene, and proton sponge have been used as NOX4 inhibitors to reduce cardiac arrhythmic phenotypes in zebrafish.[22] Although there are quite a large number of NOX4 inhibitors, their mechanism is still not fully understood. According to a research report, the presence of 73 compounds with NOX4 inhibitory effects has been identified, and these compounds belong to compounds such as oxalyl hydrazides, flavonoids, oxindoles, benzoquinolines, and benzothiophenes. Additionally, four pharmacophores have been identified that may contribute to the discovery of new NOX4-specific inhibitors in the future.[23] Peptides mimicking the regions of NOX2 and p22phox, while effective in developing specific inhibitors for NOX2, may not be as suitable for NOX4. In summary, while specific inhibitors for NOX4 are promising in combating various diseases, it's essential to precisely identify these compounds first.[16]

After the discovery of the impact of NOX4 on tumor formation, the development of therapeutic drugs has begun. Currently, the drug GKT137831 is the only therapeutic drug tested in clinical trials. The GKT137831 can reverse the myofibroblastic-cancer-associated fibroblast (myCAFs) phenotype and support the infiltration of cytotoxic (CD8) T cells. The GKT137831 reduces stromal-tumor interaction by reducing prostatic cancer-derived fibroblastic activation. In non-small cell lung carcinoma, GKT137831 limits tumor growth by reducing the pro-tumoral M2-like macrophage and immune infiltration via NOX4 blockade. Tumors with high CAF levels enhance the immune therapy response by GKT137831. Immune therapy resistance is prevented by NOX4 blockade, which hinders multiple cancer prognoses. The potential NOX4 inhibitor Fulvene-5 has been confirmed to inhibit NOX4 activity in hemangioma and inhibit *in vivo* hemangioma growth. While NOX4 inhibitors have been tested *in vitro* and *in vivo* for various anticancer types, further research is needed to find a definitive solution. In doing so, the tumor-sensitizing properties of NOX4 inhibitors should be utilized.[24]

NOX5

The NOX5 was discovered by two groups of researchers in 2001. Cheng et al.[25] described it as a complementary DNA (cDNA) predicting a protein with 565 amino acids, while Bánfi et al.[26] described it as a cDNA predicting a protein with over 700 amino acids. The human NOX5 gene is located on the 15th chromosome. The NOX5 isoforms, as described by Bánfi et al.,[26] distinguish themselves from other NOX enzymes due to their long intracellular amino radical (NH2) containing a Ca2-binding ejection fraction-hand motif (EF-hand motif). The isoform described by Cheng et al.[25] showed similarity to other NOX species as it lacks the EF-hand motif. In immunoblots, they identified NOX5 as an 85 kDa protein. This confirmed its molecular mass and indicated that the protein is not glycosylated. Like NOX2, nicotinamide adenine dinucleotide (NADH) cannot replace NADPH as the cytoplasmic electron donor for NOX5.[3]

When Ca^{2+} binds to the extra EF-hand motif of NOX5, it induces a conformational change in the region, leading to the emergence of hydrophobic regions that interact with the core, thus enabling efficient electron transfer. While NOX5 has low sensitivity to Ca^{2+} by itself, binding of calmodulin to the C-terminal region at constant pressure increases its Ca^{2+} sensitivity, resulting in conformational change and elevated levels of ROS production at low Ca^{2+} concentrations. NOX5, like other NOX species, undergoes regulation through various translational events such as phosphorylation and oxidation-induced modifications. Finally, co-precipitation experiments have shown that NOX5 has the ability to form a functional homodimer through interactions between its two dehydrogenase domains, although it differs from other NOX types.[20]

DUOX1 AND DUOX2

For several NOX isoforms, the characterization of the protein came before understanding its function. However, the process was reversed for dual oxidase 1 (DUOX1) and DUOX2. It was already known that they produced H_2O_2 bound to Ca^{2+} and NADPH at the apical plasma membrane of thyroid epithelial cells. Researchers studying the thyroid were actively looking for an active

NADPH oxidase. Despite this knowledge, it took 15 years from the initial discovery to be officially characterized, and in the early years of the discovery, it was referred to as thyroid oxidase. This characterization was carried out by two different groups using different methods on the thyroid gland. The purification of the DUOX2 enzyme and partial sequencing, followed by the rapid amplification of cDNA ends (RACE) PCR using cDNA ends and low-temperature hybridization with a NOX2 probe to a thyroid cDNA library, ultimately led to the identification. The DUOX proteins not only exhibit similarity to NOX1-4 but also possess a seventh transmembrane domain with an outward-facing peroxidase-like domain at the NH2 terminal, in addition to the EF-hand motif. Among the NOX groups, DUOX isoforms share approximately 50% similarity with NOX2. It is also known that there is a truncated form of DUOX2 mRNA at the NH2 terminal in rat thyroid cell sequences. In fact, the peroxidase homology domains of DUOX lack many amino acids necessary for peroxidase function. The common co-expression of peroxidase with DUOX in DUOX-expressing systems questions the peroxidase function of DUOX, especially since thyroid peroxidase deficiency is well-documented to lead to severe hypothyroidism due to a lack of peroxidase-dependent hormone synthesis. Nonetheless, the peroxidase homology region of DUOX2 seems to be functionally important since hypothyroidism has been reported in individuals with mutations in this extracellular area.[3]

CANCER

Several NOX and regulatory subunits have been found to exhibit increased expression in different parts of tumor formation, tumor types, or cancer sequences, and NOX species are considered a contributing factor. Similarly, studies conducted in patients with gastric cancer have shown that high levels of NOX2/4 and DUOX1 in the tumor area, compared to adjacent tissues, can serve as reliable markers for gastric cancer.[20] The expression of exogenous NOX1 in wild-type fibroblasts from the NIH3T3 cell line, a cell type of mouse embryonic fibroblasts, has led to a noticeable increase in cell growth and the formation of new tumor areas in mice. In these experiments, cells combined with NOX1 (with overexpression of NOX-1 by 10 times in NIH3T3 fibroblasts) showed increased growth and transformation despite limited superoxide production, indicating that a high level of ROS is not effective in the initial stages of tumorigenesis. However, it was observed that NOX1 led to a significant increase in intracellular H_2O_2 when combined with the expression of CAT responsible for O_2 dismutation and the reversion of the initial phenotype. This suggests that H_2O_2 still plays a role in the induction of these mechanisms. After the identification of H_2O_2-dependent activation of apoptosis following treatment with doxorubicin or camptothecin drugs, it is also considered that H_2O_2 generated as a result of NOX may have a potential role as an antitumor agent.[20,27]

Recent reports suggest that the ROS-producing activities of NOX4 are primarily associated with specific cell signaling molecules, particularly pro-angiogenic VEGF, cell growth and invasion (mitogen-activated protein kinase; MAPK), phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), and transforming growth factor beta (TGF-β). In different tumor types, NOX4 appears to impact tumor progression through various signaling pathways. While NOX4 is generally considered to support tumor development in most tumor types, some studies have also identified situations where NOX4 may provide protection against tumors, particularly in digestive cancers. In non-digestive tumors, tumor cells can survive and proliferate through the NOX4/ROS/hypoxia-inducible factors (HIF)/VEGF pathway. For example, in ovarian and prostate cancer cells with high NOX4 expression, ROS production (H_2O_2) regulates the expression of HIF. The HIF-1 is essential for tumor-associated angiogenesis and tumor growth through the VEGF pathway. It has been found that in the context of carcinoma, NOX4 upregulates HIF-2a in a manner dependent on von Hippel-Lindau tumor suppressor protein and promotes cell stimulation.[15]

Cellular senescence is the condition where cells cease to divide and lose their ability to replicate. Therefore, it plays a crucial role in suppressing cancerous cells. Reactive oxygen species are thought to be signaling molecules in this process. However, ROS can also cause DNA damage at the same time.[28] Ogrunc at all.[29] observed in their study that NOX4 generates ROS, which can

transform pancreatic cells into cancerous ones, while also inducing a DNA damage response that leads to cellular senescence.

Cancer and Vitamin D

Reactive oxygen species should be suppressed by antioxidant systems in order to prevent DNA mutation, cell proliferation, proinflammatory responses, and cell death that promote tumor formation. Vitamin D, as an antioxidant, is known for its role in protecting cells from DNA damage resulting from oxidative stress. Studies have shown its significant role in preventing DNA damage-induced colon cancer in mice and in calcitriol treatment in rats. Daily vitamin D supplementation in humans is believed to reduce cancer risk associated with DNA damage. Vitamin D may potentially induce antioxidants and activate antioxidant defenses, as it is associated with nuclear factor erythroid 2-related factor 2, a transcription factor that increases the expression of various antioxidant enzymes. This suggests that vitamin D has the potential to induce antioxidants and implement antioxidant defenses, not only in preventing DNA damage but also in the processes of DNA damage repair.[30,31] Ting et al.[32] has shown that the D vitamin increases the expression of genes involved in DNA damage repair, such as p53, proliferating cell nuclear antigen, and breast cancer susceptibility gene 1, in breast cancer and ataxia-telangiectasia mutated.

Cancer and many diseases are now known to be primarily caused by free radicals, especially ROS, which are involved in the formation pathway. The NADPH oxidases play a role in the formation of these ROS, and it affects cancer development. However, NOX4, unlike other species, is shown to increase rather than decrease the occurrence of different cancer types, such as digestive cancers, and sometimes even stop them. Unlike other species, attention should be paid to NOX4, and its suppressive effect should be examined on other cancer types. The use of this feature is important, and the development of technologies plays a significant role in making research more accessible.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Burke SL, Cobb J, Agarwal R, Maddux M, Cooke MS. How robust is the evidence for a role of oxidative stress in autism spectrum disorders and intellectual disabilities? J Autism Dev Disord 2021;51:1428-45. doi: 10.1007/s10803-020-04611-3.
2. Takemoto D, Tanaka A, Scott B. NADPH oxidases in fungi: diverse roles of reactive oxygen species in fungal cellular differentiation. Fungal Genet Biol 2007;44:1065-76.
3. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. Physiol Rev 2007;87:245-313. doi: 10.1152/physrev.00044.2005.
4. Wang XQ, Wang W, Peng M, Zhang XZ. Free radicals for cancer theranostics. Biomaterials 2021;266:120474. doi: 10.1016/j.biomaterials.2020.120474.
5. Wang XQ, Gao F, Zhang XZ. Initiator-loaded gold nanocages as a light-induced free-radical generator for cancer therapy. Angew Chem Int Ed Engl 2017;56:9029-33. doi: 10.1002/anie.201703159.
6. McAllister MJ, Basham SA, Waldman HS, Smith JW, Mettler JA, Butawan MB, et al. Effects of psychological stress during exercise on markers of oxidative stress in young healthy, trained men. Physiol Behav 2019;198:90-5. doi: 10.1016/j.physbeh.2018.10.013.
7. Di Meo S, Venditti P. Evolution of the knowledge of free radicals and other oxidants. Oxid Med Cell Longev 2020;2020:9829176. doi: 10.1155/2020/9829176.
8. Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. Circ Res 1983;52:352-7. doi: 10.1161/01.res.52.3.352.
9. Rapoport RM, Draznin MB, Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. Nature 1983;306:174-6. doi: 10.1038/306174a0.
10. Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD. ROS and the DNA damage response in cancer. Redox Biol 2019;25:101084. doi: 10.1016/j.redox.2018.101084.
11. Moloney JN, Cotter TG. ROS signalling in the biology of cancer. Semin Cell Dev Biol 2018;80:50-64. doi: 10.1016/j.semcdb.2017.05.023.
12. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging 2018;13:757-72. doi: 10.2147/CIA.S158513.

13. Diaz de Barboza G, Guizzardi S, Moine L, Tolosa de Talamoni N. Oxidative stress, antioxidants and intestinal calcium absorption. World J Gastroenterol 2017;23:2841-53. doi: 10.3748/wjg.v23.i16.2841.
14. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative stress in cancer. Cancer Cell 2020;38:167-97. doi: 10.1016/j.ccell.2020.06.001.
15. Tang CT, Gao YJ, Ge ZZ. NOX4, a new genetic target for anti-cancer therapy in digestive system cancer. J Dig Dis 2018;19:578-85. doi: 10.1111/1751-2980.12651.
16. Guo S, Chen X. The human Nox4: Gene, structure, physiological function and pathological significance. J Drug Target 2015;23:888-96. doi: 10.3109/1061186X.2015.1036276.
17. Sumimoto H. Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. FEBS J 2008;275:3249-77. doi: 10.1111/j.1742-4658.2008.06488.x.
18. Shiose A, Kuroda J, Tsuruya K, Hirai M, Hirakata H, Naito S, et al. A novel superoxide-producing NAD(P)H oxidase in kidney. J Biol Chem 2001;276:1417-23. doi: 10.1074/jbc.M007597200.
19. Ogboo BC, Grabovyy UV, Maini A, Scouten S, van der Vliet A, Mattevi A, et al. Architecture of the NADPH oxidase family of enzymes. Redox Biol 2022;52:102298. doi: 10.1016/j.redox.2022.102298.
20. Vermot A, Petit-Härtlein I, Smith SME, Fieschi F. NADPH oxidases (NOX): An overview from discovery, molecular mechanisms to physiology and pathology. Antioxidants (Basel) 2021;10:890. doi: 10.3390/antiox10060890.
21. Taylor JP, Tse HM. The role of NADPH oxidases in infectious and inflammatory diseases. Redox Biol 2021;48:102159. doi: 10.1016/j.redox.2021.102159.
22. Cai WX, Liang L, Wang L, Han JT, Zhu XX, Han H, et al. Inhibition of Notch signaling leads to increased intracellular ROS by up-regulating Nox4 expression in primary HUVECs. Cell Immunol 2014;287:129-35. doi: 10.1016/j.cellimm.2013.12.009.
23. Borbély G, Szabadkai I, Horváth Z, Markó P, Varga Z, Breza N, et al. Small-molecule inhibitors of NADPH oxidase 4. J Med Chem 2010;53:6758-62. doi: 10.1021/jm1004368.
24. Gong S, Wang S, Shao M. NADPH oxidase 4: A potential therapeutic target of malignancy. Front Cell Dev Biol 2022;10:884412. doi: 10.3389/fcell.2022.884412.
25. Cheng G, Cao Z, Xu X, van Meir EG, Lambeth JD. Homologs of gp91phox: Cloning and tissue expression of Nox3, Nox4, and Nox5. Gene 2001;269:131-40. doi: 10.1016/s0378-1119(01)00449-8.
26. Bánfi B, Molnár G, Maturana A, Steger K, Hegedûs B, Demaurex N, et al. A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes. J Biol Chem 2001;276:37594-601. doi: 10.1074/jbc.M103034200.
27. Arnold RS, Shi J, Murad E, Whalen AM, Sun CQ, Polavarapu R, et al. Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. Proc Natl Acad Sci U S A 2001;98:5550-5. doi: 10.1073/pnas.101505898.
28. Bi Y, Lei X, Chai N, Linghu E. NOX4: A potential therapeutic target for pancreatic cancer and its mechanism. J Transl Med 2021;19:515. doi: 10.1186/s12967-021-03182-w.
29. Ogrunc M, Di Micco R, Liontos M, Bombardelli L, Mione M, Fumagalli M, et al. Oncogene-induced reactive oxygen species fuel hyperproliferation and DNA damage response activation. Cell Death Differ 2014;21:998-1012. doi: 10.1038/cdd.2014.16.
30. Jeon SM, Shin EA. Exploring vitamin D metabolism and function in cancer. Exp Mol Med 2018;50:1-14. doi: 10.1038/s12276-018-0038-9.
31. Erbaş O, Solmaz V, Aksoy D. Inhibitor effect of dexketoprofen in rat model of pentylenetetrazol-induced seizures. Neurol Res 2015;37:1096-101. doi: 10.1179/1743132814Y.0000000391.
32. Ting HJ, Yasmin-Karim S, Yan SJ, Hsu JW, Lin TH, Zeng W, et al. A positive feedback signaling loop between ATM and the vitamin D receptor is critical for cancer chemoprevention by vitamin D. Cancer Res 2012;72:958-68. doi: 10.1158/0008-5472.CAN-11-0042.