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Review Article

The role and mechanism of NADPH oxidase in the development and progression of thyroid carcinoma

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Abstract: Thyroid cancer is the fastest increasing cancer in both men and women and is the most common endocrine cancer. Researchers have gradually intensified their research on the mechanism of thyroid cancer development. Within this realm, Oxidative stress is often believed to play a causal and contributory role in thyroid cancer development. NADPH oxidase is one of the important sources of reactive oxygen species for tumor cell growth and is involved in the biological processes of thyroid tumor cell proliferation, migration, invasion and epithelial-to-mesenchymal transition. However, the mechanism of NADPH oxidase in the pathogenesis of thyroid cancer is still not very clear at present. Clarifying the role and mechanism of NADPH oxidase in the pathogenesis of thyroid cancer will help to develop new strategies for the prevention and treatment of thyroid cancer as early as possible, and improve the survival rates of thyroid tumor patients. This article reviews the research progress on the mechanism of NADPH oxidase in thyroid cancer.

Keywords: NADPH oxidase, thyroid cancer, mechanism of action, reactive oxygen species, epithelial-to-mesenchymal transition

Introduction

Thyroid cancer is the most common endocrine cancer, and its incidence has increased rapidly in the past 20 years. Papillary thyroid cancer is the most common histological type of human thyroid cancer and one of the fastest growing cancers. Although this is partly attributed to over diagnosis due to the heightened utilization of advanced imaging techniques, papillary thyroid cancer also degenerates into the more aggressive and lethal form of thyroid cancer [1]. Therefore, studying the underlying molecular mechanisms of PTC can provide promising biomarkers and therapeutic targets for early diagnosis and treatment, thereby improving the prognosis and quality of life of patients, especially those with aggressive tumor behavior and poor outcomes.

Reactive Oxygen Species (ROS) were detected on the apical surface of thyroid cells in a previous study, indicating a higher level of ROS oxidants in the thyroid [2]. NADPH oxidases (NOXs) can generate ROS in various tissues of the body. Cancer cells produce high levels of ROS, and in some cases, the source of these ROS is related to dysregulation of NOXs in thyroid cancer [3]. The dysregulation of NOXs plays an important role in thyroid cancer. Therefore, to clarify the regulatory mechanism of NOXs in thyroid cancer will be more conducive to understanding the pathological process of thyroid cancer. This review will be offered regarding the roles of NOXs family in the development of thyroid cancer, which can provide new reference for the targeted therapy of thyroid cancer.

The relationship between NADPH oxidase and thyroid cancerlationship

The NADPH oxidases comprise a group of enzymes that mediates electron transport through the intracellular membrane. The NOX family was originally found on the cell membrane of phagocytes, and 7 members of the

Table 1. Tissue distribution and functions of the NADPH oxidase family [12]

NOX	Tissue expression	Function
NOX1	Digestive tract, vascular system	Host defence, blood pressure regulation, proliferation of smooth muscle (speculative)
NOX2	Phagocytes, vascular system	Host defence (verified), signalling (speculative)
NOX3	Inner ear	Otoconia formation (verified)
NOX4	Kidney, thyroid, vascular system, heart, skeletal muscle, fibroblast	Vasoregulation, signalling (speculative)
NOX5	Lymph node, testis	Spermatogenesis (speculative)
DUOX1	Thyroid, respiratory tract, digestive tract, salivary glands, skin	Host defence, signalling (speculative)
DUOX2	Thyroid, respiratory tract, digestive tract, salivary glands	Thyroid hormone synthesis (verified), host defence (speculative)

NOX1 is also known as mitogenic oxidase 1 because its first identified function was in cell growth. NOX4 is also named Renox (renal oxidase) because it was first identified in kidney. The DUOX enzymes are called also Thox (thyroid oxidase), because they were first identified in the thyroid gland. DUOX, dual oxidase; NOX, NADPH oxidase.

NOX family have been identified, including NOX1-5, DUOX1, and DUOX2. These enzymes share common structural features, including six transmembrane domains and a C-terminus with an NADPH-binding domain, and each member exhibits a specific tissue distribution [4] (**Table 1**). The regulatory mechanism of the activation of each NOX member is different, and multiple mechanisms have been reported to modulate the activity of NOXs, including post-translational modifications, lipids, calcium levels, and etc. [5, 6]. NOX also functions as a ROS-generating enzyme that regulates a range of biological functions, including redox-dependent signaling pathways, oxygen sensors, metabolic reprogramming, and immune defense [7, 8]. Furthermore, downstream ROS production emerges as a key regulator of cell differentiation, transformation, growth, and death. This active participation extends to the initiation and progression of various cancers, including thyroid cancer [9].

Oxidative stress is considered to be one of the contributors to DNA damage, which is the initial step in thyroid carcinogenesis [10], and the direct interaction between ROS and DNA can lead to the formation of oxidative damage to DNA bases, including base sites, single-strand DNA breaks, glycosyl modifications, deaminated bases, and adduced bases. If left unrepaired, these DNA lesions may alter some genetic information, thereby affecting genome integrity by causing mutations, inhibiting replication or transcription [11].

Over the past decade, NADPH oxidase, given its pivotal role in ROS production as its primary and exclusive function, has become a focal point of research attention within the realm of thyroid malignancies. Unlike other ROS-producing enzymes, NADPH oxidase produces superoxide (O_2) or hydrogen peroxide (H_2O_2) in a tightly controlled manner, and can act locally in redox control of specific signaling pathways [12]. The currently identified NOXs are NOX1-5, DUOX1 and DUOX2, among which the NADPH oxidase expressed in the thyroid gland includes NOX1, NOX4, DUOX1, and DUOX2. The dysregulation of different NOXs corresponds to different pathways regulated by redox, different NOXs can act on the occurrence and development of thyroid cancer through various pathways.

The mechanism of NADPH oxidase in the occurrence and development of thyroid cancer

The mechanism of action of NOX1 in thyroid cancer

NOX1 is a member of the NADPH oxidase family. Its main function is to generate superoxide and rapidly convert it into hydrogen peroxide. NOX1 can be stimulated and activated by various agonists including pro-inflammatory factors and oxidized low-density lipoprotein [13]. Extensive evidence reveals the heightened expression of NOX1 in many types of cancer, and its involvement in tumor cell proliferation, apoptosis and cell migration. The study found that mTORC1 is one of the downstream effectors of NOX1. NOX1 and mTORC1 are located in the same VPS39-/VPS41-positive lysosome, and the spatial proximity of these proteins allows ROS generated by NOX1 to affect the function of mTORC1, which in turn affects the progression of related cancers [14]. Studies have confirmed that the NOX1/ROS signaling pathway can deactivate Protein Tyrosine Phosphatase (PTP) and activate the autophosphorylation of Receptor Tyrosine Kinase (RTK). PTP's catalytic framework undergoes oxidative modification at cysteine residues that is sensitive to

redox stimulation, leading to hyperphosphorylation of RTKs and activation of downstream oncogenic signaling, which in turn can promote tumor progression [15]. The activation of NOX1 complex is regulated by NOXO1 protein levels and phosphorylation regulation [16]. Echizen [17] found that after the NOXO1 gene was disrupted, the NOX1/ROS pathway could participate in the activation of EGFR signaling by inhibiting the functions of PTP, which may be a major tumor-promoting mechanism of NOX1/ROS signaling.

At present, there are few studies on the role of NOX1 in thyroid cancer. Cancer stem cells are responsible for the occurrence, proliferation and recurrence of tumors [18]. The elevated intracellular levels of ROS will activate tumorigenesis-related pathways. However, the impact of ROS on the self-renewal capability of thyroid cancer stem cells is still unclear. A recent study found that CD133+ thyroid cancer cells had a higher transcription level of the ROS-generating oxidase NOX1. Knockout of NOX1 reduced ROS levels and inhibited the self-renewal activity and tumorigenicity of CD133+ thyroid cancer cells. This effect is believed to be facilitated by the partial activation of the threonine kinase (AKT) signaling pathway, thereby modulating the self-renewal properties of CD133+ thyroid cancer cells [19].

However, according to the mechanism of NOX1 in other cancers, it is speculated that the mechanism of NOX1 in the genesis and development of thyroid cancer not only mediates the involvement of CD133+ thyroid cancer cells in the tumor process, but also reveals the role of NOX1 in the progression of thyroid cancer, orchestrating its effects through several interlinked pathways.

*What is the role of NOX4 in thyroid cells and how is it regulated? (**Figure 1**)*

NOX4: a versatile protein involved in ROS generation and regulation: Thyroid cells express NOX4 in abundance, which is a subtype of NADPH oxidase proteins responsible for producing ROS via its constituent gene [20]. NOX4 requires the presence of p22phox, a protein that forms a heterodimer with NOX4, to facilitate ROS production. Knockdown of p22phox leads to a loss of NOX4 activity, and different mutations in p22phox have varying effects on NOX4 maturation and activity [21]. NOX4 is active not only in the plasma membrane but also in different organelles including the endoplasmic reticulum, mitochondria, and nucleus [22-24]. Unlike other NOX isoforms, NOX4's E-Loop has a highly conserved histidine that can switch enzyme activity from generating H_2O_2 to generating O_2^- [25]. This is because the E-Loop acts as a proton source, which accelerates the spontaneous disproportionation of O_2^- to form H_2O_2. Thus, the E-Loop's change is critical to the final product, either O_2^- or H_2O_2 [26]. NOX4's presence in the nuclear environment promotes redox modifications that not only affect DNA binding of transcription factors and gene expression, but also regulate interactions and enzymatic activities related to DNA damage signaling, DNA replication, and repair [27]. Rac1, a GTPase, also seems to regulate NOX4 activity, but this regulation depends on the cell context [28].

NOX4 is also known as Renox, as it was originally discovered as an oxidase homolog that is highly expressed in the kidney [29]. Abnormal expression patterns of NOX4 has been observed in various human cancers, including kidney cancer, thyroid cancer, liver cancer, breast cancer, and melanoma. Interestingly, in normal human kidney tissue, expression of NOX4 is significantly surpasses that found in other tissues. Notably, in kidney chromophobe, renal clear cell carcinoma, and renal papillary cell carcinoma, NOX4 expression is lower than in normal tissues, implicating a plausible protective function for NOX4 in kidney health. However, in 23 other types of tumors, NOX4 transcription is highly expressed, and high NOX4 expression has been associated with better patient prognosis in both KICH and KIRC renal cancers, as determined by GEPIA analysis [30].

NOX4 expression in thyroid cancer and its role in thyroid cell dedifferentiation: NOX4, an abundantly expressed protein within thyroid cells, demonstrates a particularly robust presence in Papillary Thyroid Cancer (PTC). Its expression is governed by thyroid-stimulating hormone (TSH), which also regulates the expression of its heterodimer partner p22phox [31]. In BRAF-mutated PTC cells, NOX4 transcription experiences marked elevation and is mediated by the TGF-β/Smad3 signaling pathway, contributing to the role of BRAF V600E inhibition of Sodium/Iodide Symporter (NIS) [32]. Studies

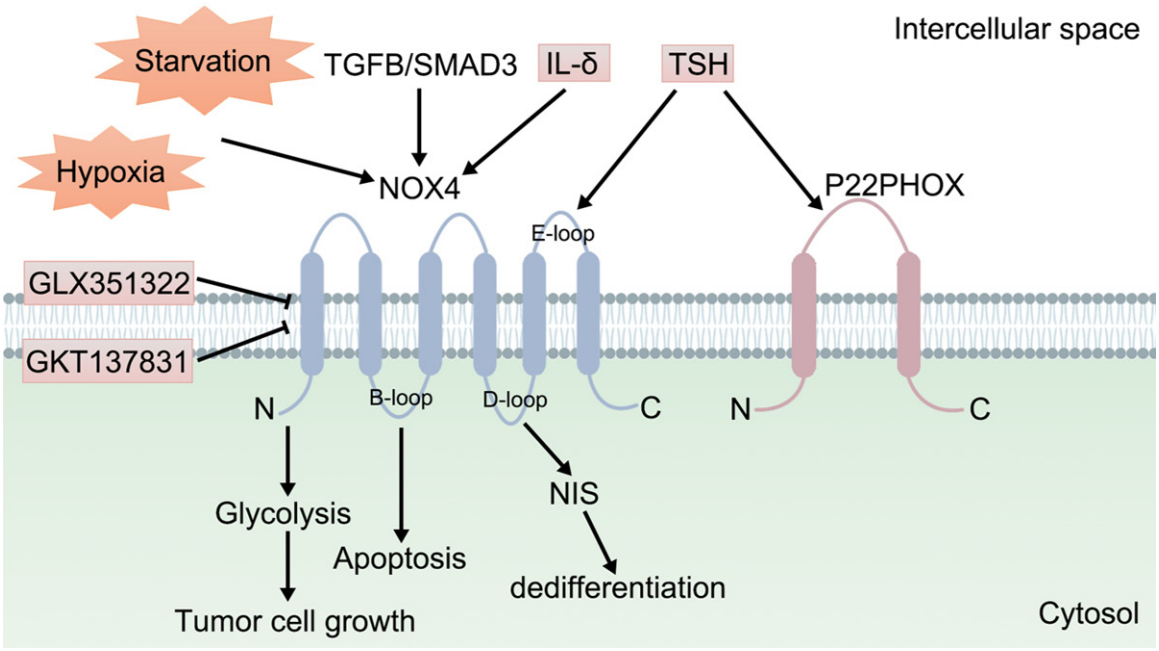

Figure 1. NOX4's structure, regulation, and therapeutic implications. This figure illustrates the simplified model of NOX4, outlining its transmembrane domains and plasma membrane orientation. NOX4 requires p22phox to form a heterodimer, essential for ROS production. The extracytosolic loop (E-loop) with a conserved histidine facilitates proton supply, accelerating superoxide dismutation into H_2O_2. The Nox4 B-loop, particularly arginine residues, plays a crucial role in NOX4 activity. NOX4 D-loop mutations optimize NADPH oxidase activity. TSH regulates NOX4 expression and its heterodimer partner p22phox. In WRO thyroid cancer cells, NOX4 mediates IL-δ-induced responses, influencing proliferation and apoptosis. In BRAF-mutated PTC cells, TGF-β/Smad3 signaling upregulates NOX4, impacting NIS inhibition and thyroid dedifferentiation. Under hypoxia, NOX4 interference reduces mitochondrial ROS in PTC cells, leading to decreased glycolysis and inhibited cell growth. Therapeutically, NOX4 knockdown inhibits PTC proliferation and promotes apoptosis, especially under low serum conditions. NOX4-derived ROS inhibitors, like GLX351322, yield consistent effects. Additionally, GKT137831, a NOX4 inhibitor, reverses the CAF phenotype, enhancing CD8+ T cell infiltration. Combining GKT137831 with a CTLA-4 antibody significantly improves patient survival. This concise overview sheds light on NOX4's structural insights, regulatory mechanisms, and therapeutic potential in thyroid cancer, offering valuable implications for further research and clinical applications.

have shown that NOX4 overexpression in PTC patients with BRAF mutation is associated with thyroid cell dedifferentiation and reduced expression of thyroid iodine metabolism genes such as NIS, suggesting a possible role of NOX4 in thyroid dedifferentiation [33]. Additionally, in WRO human poorly differentiated thyroid cancer cells, NOX4 is highly expressed in response to IL-δ stimulation, and plays a key role in mediating the cellular response to IL-δ signal, as NOX4 interference leads to ineffective inhibition of WRO cell proliferation and reduction of IL-δ-induced apoptosis [34]. Further research is needed to determine whether increased NOX4 expression is a cause or a consequence of thyroid dedifferentiation in cancer.

Inhibition of NOX4 expression and its effects on PTC cell growth and apoptosis: NOX4 has been studied extensively for its molecular function in thyroid cancer cells. In hypoxic conditions, NOX4 interference can inhibit the increase of mitochondrial ROS in Papillary Thyroid Cancer (PTC) cells, resulting in the destabilization of HIF1a and a downstream reduction in glycolytic levels, ultimately hindering PTC cell growth [35]. Additionally, knockdown of NOX4 expression can inhibit PTC cell proliferation and promote apoptosis in the treatment of chemotherapeutic or targeted agents, especially when serum levels are modest. The effect of NOX4-derived ROS inhibitors, such as GLX351322, has been found to be consistent with NOX4 knockdown [3]. These findings indicate that NOX4 plays a crucial role in the proliferation of PTC cells under hypoxic and starved conditions. Future studies should comprehensively investigate the expression of NOX4 in various subsets of thyroid cancer cells within the tumor microenvironment in vivo and characterize the effects of NOX4 deficiency on various subsets of thyroid cancer cells. Furthermore, new direc-

tions for thyroid cancer treatment with NOX4 targets or in combination with other drugs should be explored.

Potential applications of NOX4 in cancer therapy and immunotherapy: Immunotherapy, particularly checkpoint inhibitors, has revolutionized clinical outcomes in numerous clinical trials. The impressive strides achieved in this realm have stimulated a fervent interest among researchers, compelling them to explore the application of NOX4 in the context of tumor immunotherapy. When fibroblasts are subjected to stress by tumor cells, they become Cancer-associated fibroblasts (CAFs), which promote tumor growth, spread, and evasion of the immune response, making tumors difficult to treat. Studies have found that the higher the distribution of CAF cells in tumor tissues is, the lower the survival rate of tumor patients will be. Researchers have found that NOX4 plays an essential regulatory role in CAF differentiation, with healthy fibroblasts relying on NOX4 expression to transform into CAFs. In *vivo* experiments have shown that inhibiting NOX4 expression in CAFs results in a reduction in tumor volume by 30.6 to 64.0% [36]. A NOX4 inhibitor, GKT-137831, has effectively reversed the CAF phenotype and promoted lymphocyte infiltration, such as CD8+ T cells. The combination of GKT137831 and an immune checkpoint inhibitor (CTLA-4 antibody) has engendered a remarkable enhancement in patient survival rates. These findings suggest that targeting NOX4 can overcome tumor resistance and immune escape (evasion) caused by CAF [37]. Although GKT137831 and other NOX4 inhibitors have not yet been tested in the treatment of cancer, scientists continue to advance immunotherapy research around NOX4, with the hope of expanding its uses.

Based on the evidence presented, NOX4 appears to be a promising target for the treatment of thyroid cancer. While these strides are undoubtedly promising, further exploration is imperative to glean a holistic comprehension of both the merits and potential limitations. The success of future clinical trials will ultimately determine the role of NOX4 in the fight against this disease. Nevertheless, continued dedication and relentless pursuit of innovation, underpin the confidence that we can unravel the full potential of NOX4 and make significant strides towards conquering thyroid cancer.

The mechanism of action of DUOX1 in thyroid cancer

In the NOXs family, different from other counterparts, NOX4, DUOX1, and DUOX2 produce hydrogen peroxide (H_2O_2) as their reactive oxygen species (ROS) of choice. DUOX1 and DUOX2 play a pivotal role as key sources of airway ROS production. Notably, the expression patterns of DUOX1 vary across different tumor types. DUOX1 is underexpressed in human lung cancer tissues and liver cancer groups [38, 39], and up-regulated in prostate cancer [40]. Studies have found that the loss of DUOX1 expression is related to EMT. Silencing the expression of DUOX1 in lung cancer cells can induce the reverse transformation of EMT, thereby reducing the migration ability of cells and restoring epithelial characteristics. Therefore, DUOX1 may mediate tumor progression in tumors by regulating the process of EMT process [41]. More and more recent evidence supports that chronic oxidative stress may drive the progression of radiation-induced late effects, radiation-induced ROS generation and increased oxidative stress.

At present, there is a limited amount of research available regarding the DUOX1 mechanism in thyroid cancer. In a previous study, Ameziane-El-Hassant et al. [42] found that the expression of DUOX1 in human thyroid cells was up-regulated after the exposure to radiation, and for the first time identified DUOX1-dependent H_2O_2 production plays a key role in persistent radiation-induced DNA damage and thus in DDR signaling. They found that DUOX1 knockdown resulted in a significant reduction in DNA damage in thyroid cells after radiation, further exploring the mechanism is down-regulation of DUOX1 expression affecting p38 activation, IL-13 expression, and DNA damage. The findings strongly support the notion that DUOX1-derived H_2O_2 is involved in the maintenance of DNA damage response in irradiated thyroid cells.

The mechanism of action of DUOX2 in thyroid cancer

DUOX2 is a core member of the NOXs family. The gene is located in the long arm 21 region 1 of chromosome 15. The protein encoded by DUOX2 is a glycoprotein containing 1548 amino acids. In addition to being abundantly

expressed in thyroid cells, it is expressed in the airway epithelium. It is widely expressed in many parts, such as the gastrointestinal tract and the prostate [43]. Cytokines regulate the expression of thyroid DUOXs, TH1-dominant cytokines [interleukin alpha (IL-1α) and interferon gamma (IFN-γ)] and TH3-dominant cytokines [transforming growth factor beta (TGF-β) and IL-10] decreased the expression of DUOX2 mRNA and protein in human thyroid cells and rat thyroid cells. Studies have shown that DUOX2 is involved in the regulation of hypothyroidism, cases of hypothyroidism caused by DUOX2 or DUOXA2 mutations have been identified, and DUOX2 mutation diagnosis has been applied to thyroid function screening, mainly based on neonatal thyroid function screening [44].

DUOX2 activity plays a crucial role in the binding of iodine-binding thyroid peroxidase (TPO) to thyroglobulin, and Ginabreda et al. [45] found that there is a significant relationship between DUOX2 and TPO in the pathophysiology of thyroid cancer. Lacroix et al. [46] reported that DUOX2 protein expression level was positively correlated with tumor cell differentiation, and was more prevailing in thyroid cancer tissues. In addition, the promoter of DUOX2 in thyroid carcinogenesis was induced by TSH and insulin, and the enrichment results showed that DUOX2 gene-related biological processes were activated in the PDMTC group [47]. These evidences reinforce the potential role of DUOX2 in the mechanism of metastatic thyroid cancer, and DUOX2 could serve as a prognostic biomarker or therapeutic target in patients with metastatic thyroid cancer. In addition, et al. used an unbiased approach to identify novel germline mutations in DUOX2 associated with hyperpenetrant familial non-medullary thyroid cancer and found that combining the FOXE1 rs965513 polymorphism also amplified DUOX2 expression. The data suggesting that DUOX2 is involved in H_2O_2 metabolism. These findings propose that the deregulation of proteins may be a common mechanism by which common high-penetrance and rare low-penetrance genetic factors contribute to an increased susceptibility to thyroid cancer [48].

Oxidative stress in thyroid cancer

In the thyroid gland of mouse models, levels of oxidative DNA damage are higher than in other organs, with a high level of OGG1 mRNA expression [49]. An increase in nuclear levels of 8-oxodG was found in both human follicular adenomas and carcinomas, which reinforces the concept that oxidative stress might be an early event during thyroid cell transformation to tumour cells. Although individual DNA lesions are generally repaired efficiently, some clustered oxidative DNA lesions might be difficult to resolve and in some circumstances can lead to the formation of DNA double-strand breaks (DSBs). DSBs can promote genetic instability and chromosomal rearrangements. Interestingly, exposure of human thyroid cells to H_2O_2 induces DSBs and consequently an oncogenic RET/PTC rearrangement, which is an early event in thyroid follicular cell transformation. Upon H_2O_2 exposure, thyroid cells increase expression of antioxidant response genes. Inhibition of this response markedly decreases cell resistance to H_2O_2 and promotes DNA damage [50], which indicates that weakening the detoxification process due to the dysregulation of gene expression or epigenetic changes might favour the mutagenic effect of H_2O_2. Consequently, an imbalance in redox homeostasis that leads to oxidative stress and oxidative DNA damage might represent an initial step in thyroid carcinogenesis (**Figure 2**).

Oncogenes in thyroid cancer

NOX4 expression is increased in thyroid cancers, and particularly in PTC. Interestingly, in TCGA data, NOX4 expression levels are significantly increased in PTCs with a BRAF mutation. BRAF Val600Glu is associated with thyroid cell dedifferentiation with a decreased expression of thyroid iodide-metabolizing genes reported, including NIS. The mechanism by which BRAF Val600Glu controls NOX4 is currently under investigation. Strikingly, analysis of human thyroid tumours with the BRAF Val600Glu mutation shows that the level of NOX4 expression is inversely correlated to thyroid differentiation [33]. Further investigation is needed to determinate whether the increase of NOX4 expression is a cause or a consequence of the thyroid dedifferentiation process.

Hypermethylation is a mechanism that promotes silencing of gene expression that might lead to cell dedifferentiation. ROS induce an oxidative-stress-mediated epigenetic imbalance, which alters genomic integrity in numerous injuries induced by oxygen radicals, disturbing the DNA demethylation mechanisms

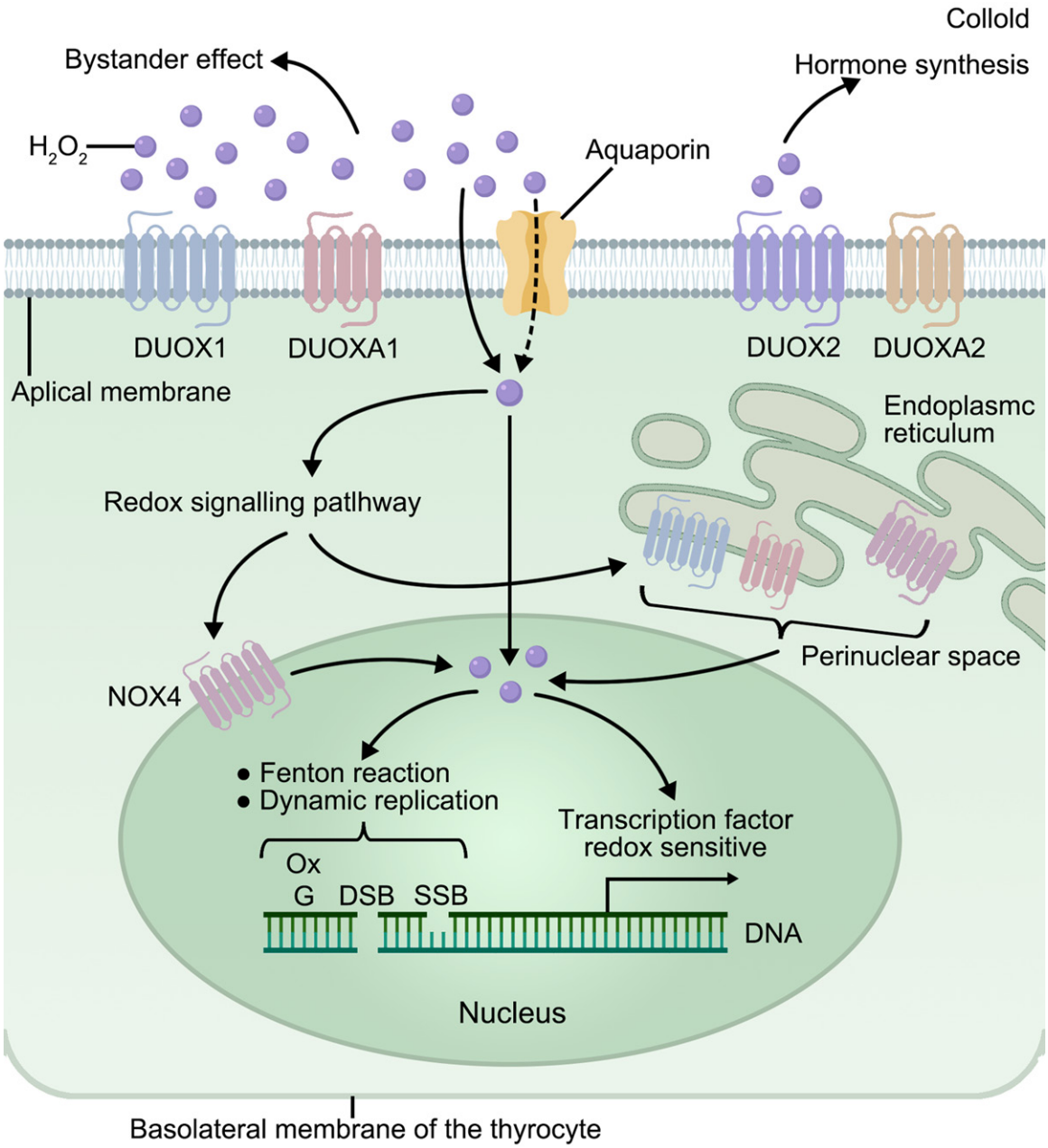

Figure 2. Hypothetical model of an imbalance in redox homeostasis that leads to oxidative stress and oxidative DNA damage in the thyroid. In a pathological context, hydrogen peroxide (H_2O_2) produced by dual oxidase (DUOX) enzymes in the colloidal lumen can diffuse through the apical membrane of the human thyrocyte either passively or possibly through aquaporins that are not yet identified. Inside the cells, H_2O_2 could reach the nucleus directly or via a redox signalling pathway that might activate intracellular H_2O_2-generating DUOX1 and/or NADPH oxidase 4 (NOX4), which are detected at the perinuclear level in human thyrocytes. In the nucleus, H_2O_2 can modulate the nuclear redox status and the expression of some redox-sensitive genes. Nuclear oxidative stress might promote DNA damage via hydroxyl radicals generated by the iron-mediated Fenton reaction. Indeed, oxidative stress might also compromise replication fork stability, which leads to replication stress (a risk factor for thyroid cancer). Finally, H_2O_2 produced by unstable thyroid cells propagates the stressful effects to other cells (known as the bystander effect). DSB, double-strand break; DUOXA, dual oxidase activator; G, guanine; Ox, oxidative damage; SSB, single-strand break.

[51]. Oxidative damage contributes to the relocalization of a silencing complex containing DNA methyl transferases. Consequently, by generating DNA damage, NOX4 might promote recruitment of silencing proteins in thyroid cells.

Summary

The incidence of thyroid cancer is steadily on the rise year by year. While most patients with thyroid cancer can respond well to treatments like radiotherapy, chemotherapy or surgery, there are still some patients experience unfavorable outcomes, and there is the possibility of recurrence and distant metastasis to a certain extent. It is necessary to further identify potential therapeutic targets for thyroid cancer. Members of the NADPH oxidase family have different expression levels in different cancers. Currently, NOX1, NOX4, DUOX1 and DUOX2 have been studied more in thyroid cancer. The NOXs family is a hotspot in the research of molecular markers, and a large number of studies have proved that NOXs are involved in biological processes such as thyroid cancer cell migration, apoptosis and EMT. These studies all indicate that NOXs are expected to become potential therapeutic targets. However, comprehensive insights into the mechanisms through which NOX enzymes contribute to the initiation and progression of thyroid cancer are still limited. The mechanism of action of many NOXs in thyroid cancer is still unclear, such as the remaining members of the NOXs family NOX3 and NOX5, etc. Still need a lot of experimental proof to give theoretical support. Based on the existing understanding of oxidative stress in thyroid carcinoma, upcoming treatments with drugs targeting NOX4, either as a single agent or in combination, have to be tested [52].

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Disclosure of conflict of interest

None.

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