



Invited Review Article

Emerging roles of Keap1/Nrf2 signaling in the thyroid gland and perspectives for bench-to-bedside translation

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ABSTRACT

The signaling pathway centered on the transcription factor nuclear erythroid factor 2-like 2 (Nrf2) has emerged during the last 15 years as a target for the prevention and treatment of diseases broadly related with oxidative stress such as cancer, neurodegenerative and metabolic diseases. The roles of Nrf2 are expanding beyond general cytoprotection, and they encompass its crosstalk with other pathways as well as tissue-specific functions. The thyroid gland relies on reactive oxygen species for its main physiological function, the synthesis and secretion of thyroid hormones. A few years ago, Nrf2 was characterized as a central regulator of the antioxidant response in the thyroid, as well as of the transcription and processing of thyroglobulin, the major thyroidal protein that serves as the substrate for thyroid hormone synthesis. Herein, we summarize the current knowledge about the roles of Nrf2 in thyroid physiology, pathophysiology and disease. We focus specifically on the most recent publications in the field, and we discuss the implications for the preclinical and clinical use of Nrf2 modulators.

1. Introduction

Follicular epithelial cells of the thyroid gland, an endocrine organ present in vertebrates, produce two hormones, triiodothyronine (T3) and thyroxine (T4), carrying respective numbers of iodine atoms per molecule of hormone. T3 and T4 act on all tissues of the organism through thyroid hormone receptors. Among their many important functions, thyroid hormones exert critical roles in cell differentiation during development and contribute to metabolic homeostasis by regulating the basal metabolic rate [1]. The synthesis of thyroid hormones takes place in the thyroid follicles, which are spherical structures lined by epithelial cells. Follicles are the basic structural and functional anatomical units of the thyroid gland. They are filled with colloid, a viscous substance that consists mainly of thyroglobulin (Tg), the thyroid hormone precursor protein that is produced by the follicular cells and serves as the backbone for the enzymatic synthesis of T3 and T4 (Fig. 1). Thyroid hormones are secreted in the bloodstream and they are transported to all tissues to exert their functions. They also mediate a negative feedback effect at the level of the hypothalamus and the pituitary gland, where they suppress the transcription of the genes encoding TRH (TSH-releasing hormone) and TSH (thyroid-stimulating hormone), respectively [2]. TSH is the major stimulus for thyroid hormone synthesis and

secretion.

Reactive oxygen species (ROS) can act as signaling molecules, and they participate in biochemical processes in various tissues. Thus, depending on the cell type, a baseline level of ROS is necessary to maintain normal function and preserve homeostasis [4]. This amount of ROS is generally low in most tissues, and when it exceeds the cell's ability to quench ROS, oxidative stress ensues. In that regard, the thyroid is unlike most other tissues, in that in order to function normally and to synthesize its hormones, it requires a continuous production of substantial amounts of ROS, and specifically hydrogen peroxide (H₂O₂). Once adequate iodine is available, H₂O₂ generation by the NADPH oxidases Duox1 and Duox2 for use by the enzyme thyroid peroxidase (TPO) is a crucial step for thyroid hormone production [5] (Fig. 1). Faced with a high baseline amount of ROS, follicular cells need to be able to coordinate the expression of a series of antioxidant and cytoprotective enzymes in order to prevent oxidative damage. Work by our group and others over the last few years has shown that the transcription factor nuclear erythroid factor 2-like 2 (Nrf2) lies central to the regulation of antioxidant and cytoprotective genes in the thyroid, as is the case also in other tissues [6]. As can be seen in Fig. 2 that shows mRNA and protein expression data extracted from the Human Protein Atlas database (<https://www.proteinatlas.org/>) [7], the mRNA expression

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levels and the protein abundance of NRF2 and its prototypical target NQO1 in the thyroid are among the highest observed in human tissues.

Under normal conditions, NRF2, encoded by the *NFE2L2* gene, is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (KEAP1), which facilitates the degradation of NRF2 by the proteasome. ROS react with the sulfhydryl groups of specific KEAP1 cysteines and cause allosteric modifications that render KEAP1 unable to target NRF2 for proteasomal degradation. Hence, under oxidative conditions, Nrf2 accumulates in the nucleus, where it binds to specific DNA sequences called Antioxidant Response Elements (AREs) in the regulatory regions of its target genes [8]. Nrf2 target genes include cytoprotective and antioxidant enzymes such as NAD(P)H quinone dehydrogenase 1 (*Nqo1*), glutathione peroxidase 2 (*Gpx2*) and thioredoxin reductase 1 (*Txnrd1*) (Fig. 1). Similar to ROS, chemical activators of the Nrf2 pathway such as the isothiocyanate sulforaphane (found in broccoli sprouts), dimethyl fumarate (used to treat multiple sclerosis and psoriasis) and the triterpenoid CDDO-Me (in clinical trials for various indications) can also cause modifications of specific Keap1 cysteines, thereby leading to induction of the Nrf2 antioxidant response [9]. Besides the classic cytoprotective Nrf2 target genes, Nrf2 can also crosstalk directly or indirectly with other pathways and factors such as Notch [10], Math1 [11], Cebpb [12], RXR α [13] and Fgf21 [14], thereby having effects on a variety of biological functions such as cell proliferation, differentiation, lipogenesis, etc. Through both its antioxidant effects and its interactions with other pathways, Nrf2 is implicated in the pathogenesis of various diseases in experimental models and in humans. Therefore, Nrf2 is considered a target for the prevention and treatment of various chronic diseases such as neurodegenerative disorders, chronic kidney disease, diabetes and its complications, cancer, and many others [15].

Information about Nrf2 and its roles in thyroid physiology and pathophysiology has been emerging during the last few years via studies by our group and others; the state of knowledge in this area is evolving, and it was last reviewed over 2 years ago [3]. The purpose of the present

mini-review is to provide an overview, highlighting in particular the most relevant publications during the last 2 years on the roles of Nrf2 pathway in the thyroid and its diseases. Among the most common thyroid diseases in the general population are Hashimoto's thyroiditis (autoimmune thyroid disease often associated with decreased production of thyroid hormones – hypothyroidism); Graves' disease (autoimmune thyroid disease often associated with increased production of thyroid hormones – hyperthyroidism); goiter (thyroid enlargement, with or without thyroid nodules – usually benign tumors); and thyroid cancer (primarily papillary thyroid carcinoma, which usually has a good prognosis) [16].

2. Role of Nrf2 in thyroid physiology

2.1. Nrf2 as a key mediator of the antioxidant and cytoprotective response in the thyroid

As mentioned above, our group has shown previously that Nrf2 is an important regulator of antioxidant and cytoprotective responses in the thyroid gland, as it also happens in other tissues [17]. Specifically, in a rat thyroid follicular cell line (PCCL3) and in mice, the transcriptional activity of Nrf2 is positively associated with the expression of antioxidant and cytoprotective genes such as *Nqo1*, *Txnrd1* and *Gpx2* (Fig. 3). Further, in mice, Nrf2 protects the thyroid from lipid and protein oxidation induced by iodine overload [17]. Moreover, RNA sequencing analysis in thyroids of wild-type (WT) and Nrf2 knockout (*Nrf2*^{KO}) mice exposed to excess iodine via the drinking water, has given insights into the role of Nrf2 in the physiological transcriptional response to iodine [18]. Specifically, iodine overload upregulates in WT mice pathways related to inflammatory and immune responses (e.g., TNF- α , NFkB and IL-6), fibrosis (TGF β) and oxidative stress responses, including the Nrf2 pathway [18]. Lack of Nrf2 leads to a more pronounced inflammatory-autoimmune transcriptional response to iodine than the response observed in WT mice. The fact that the Nrf2 pathway is

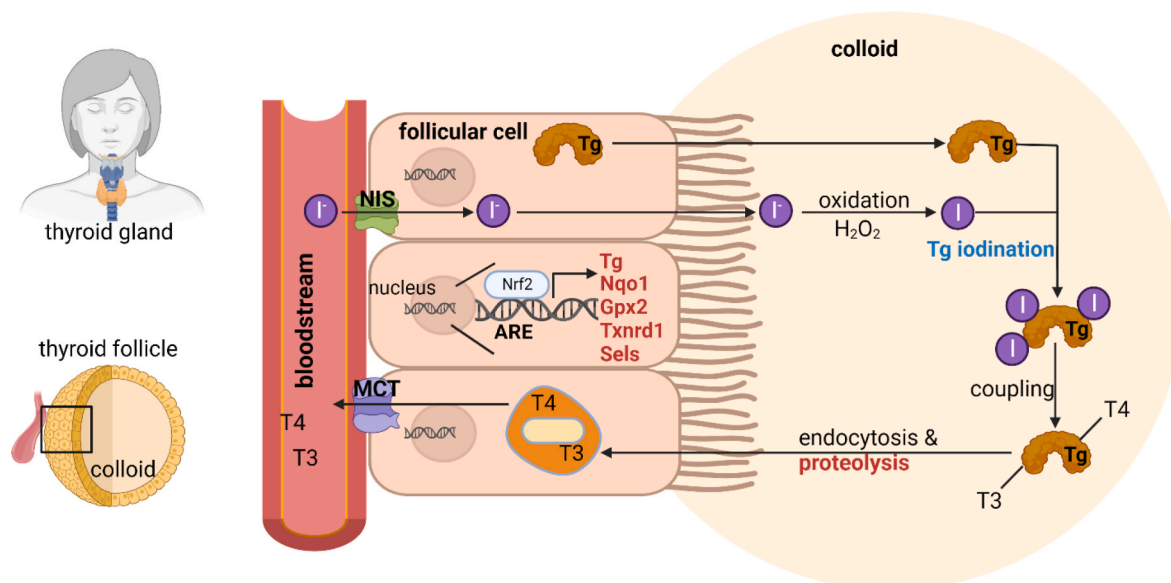


Fig. 1. Simplified representation of thyroid hormone synthesis. Iodide (I^-) enters thyroid follicular cells by active transport via the sodium (Na^+)-iodide symporter (NIS), and it is then exported via other channels into the colloid area at the center of the follicle. There, iodide is oxidized to iodine (I) and used in the iodination of thyroglobulin (Tg). Coupling between di- and/or mono-iodinated tyrosines of Tg produces the thyroid hormones T4 and T3 attached to the Tg backbone. The iodine oxidation, Tg iodination and coupling reactions are catalyzed by the enzyme thyroid peroxidase (TPO). Tg is then endocytosed into the thyroid follicular cells, where it is degraded inside lysosomes. T4 and T3 are ultimately released into the blood circulation via a monocarboxylate transporter (MCT). Effects of Keap1/Nrf2 signaling are indicated in red font (indicating upregulation) or in blue font (indicating downregulation). They include: (i) transcriptional induction of the gene encoding Tg; (ii) transcriptional induction of antioxidant and selenoprotein genes such as those encoding *Nqo1*, *Gpx2*, *Txnrd1* and *Sels*; (iii) suppression of Tg iodination; and (iv) increased Tg proteolysis in mice with constitutively activated Nrf2. The panel at the bottom left shows the focus area of the thyroid follicle (black rectangle) that is depicted enlarged in the main panel. Adapted from Thanas et al. [3] Created with BioRender (<https://biorender.com/>). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

physiologically relevant in the thyroid was also highlighted by studies in mice with ubiquitously decreased expression of Keap1 (Keap1^{flox/flox} mice, also described in the literature as hypomorphic Keap1 mice or Keap1 knockdown mice; Keap1^{KD}). In these mice, the floxed Keap1 allele is hypomorphic, which leads to constitutive Nrf2 pathway activation in various tissues such as liver, stomach, intestine and spleen [19]. We have also verified that this Keap1 hypomorphism leads to induction of Nrf2 cytoprotective target genes in the thyroid [20].

2.2. Effects of Nrf2 on Tg regulation

Besides its role in the regulation of antioxidant and cytoprotective enzymes in the thyroid, Nrf2 can have direct and indirect effects on the regulation of Tg expression and processing. In our previous research, we have characterized the Tg gene as a direct transcriptional target of Nrf2 in rodents and humans [17]. Specifically, there are two evolutionarily conserved AREs in the upstream regulatory region (distal enhancer) of the Tg gene that bind Nrf2 and are transcriptionally activated by it. Consistent with this, Nrf2^{KO} mice show decreased Tg mRNA expression

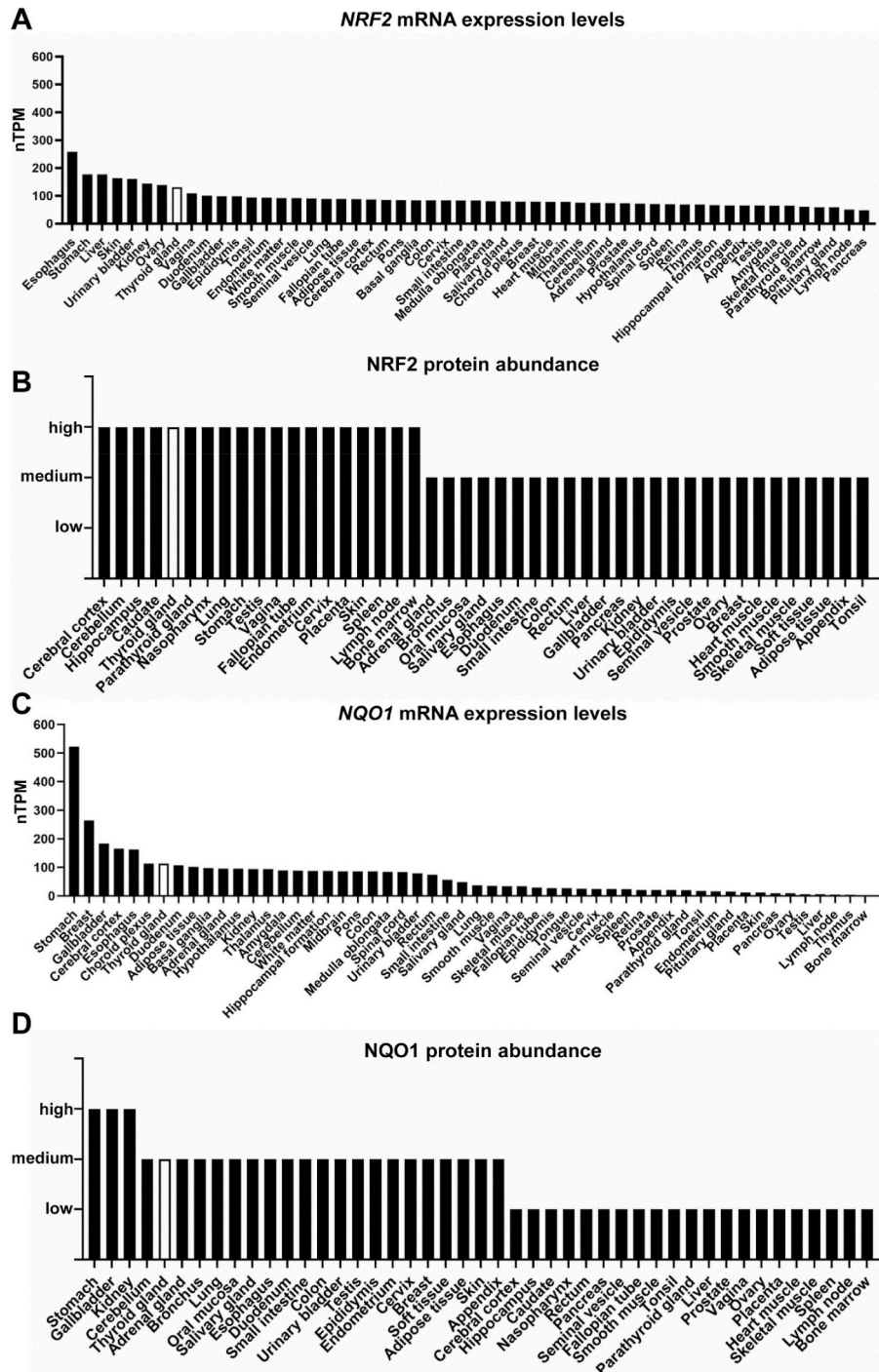


Fig. 2. Relative mRNA expression levels and protein abundance of NRF2 and NQO1 in various human tissues. Data extracted and adapted from “The Human Protein Atlas – HPA” (<https://www.proteinatlas.org/>). The RNA expression data shown are based on the consensus dataset that consists of normalized expression levels (number of Transcripts Per Million, nTPM) for 55 tissue types from the HPA and the Genotype-Tissue Expression (GTEx) projects.

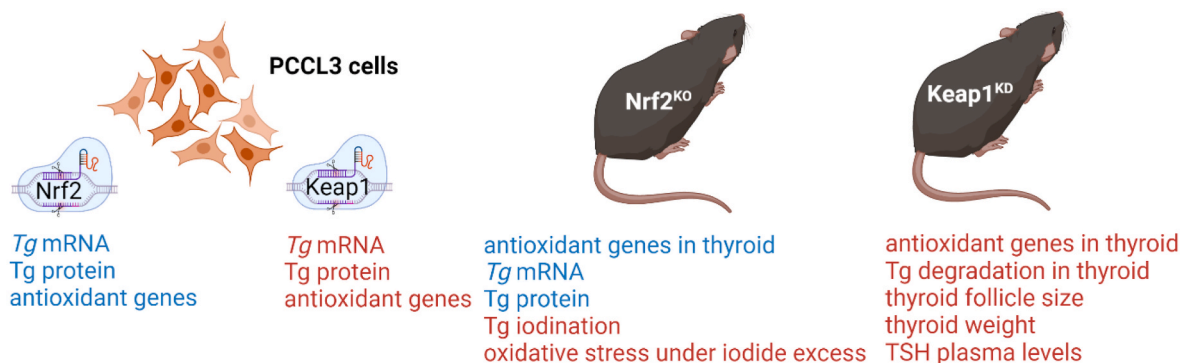


Fig. 3. Schematic representation of the phenotypes observed in models with altered Keap1/Nrf2 activity. Blue: downregulation; red: upregulation; Nrf2^{KO}, Nrf2 knockout; Keap1^{KD}, Keap1 knockdown. Adapted from Thanas et al. [3]. Created with BioRender (<https://biorender.com/>). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

levels and decreased Tg protein abundance in their thyroids [17]. However, the thyroidal protein abundance of iodinated Tg, which is the precursor of thyroid hormones, is increased in Nrf2^{KO} mice at the basal state; after treatment with iodine, it increases further, instead of decreasing as is the case in WT mice [17]. Unlike the direct effect of Nrf2 on Tg transcription, the mechanisms for its effect on iodinated Tg are currently unknown. Interestingly, the opposite effects on Tg protein abundance and Tg iodination may explain why Nrf2^{KO} mice are euthyroid, showing no differences in plasma levels of TSH and T3 in comparison to WT mice [17]. A difference observed in the plasma levels of T4 between the two genotypes is ostensibly due to a difference in T4 transport in the plasma via an effect of Nrf2 in other tissues such as the liver. This hypothesis is supported by the observation that, when we generated mice with genetic ablation of Nrf2 restricted to the thyroid and the kidney, their plasma T4 levels were the same as in their control counterparts [17]. All the other physiological and molecular phenotypes that were seen in the global Nrf2^{KO} mice were also observed in these tissue-restricted Nrf2^{KO} mice.

2.3. Effects of Keap1 on thyroid structure and function

Theoretically, one might expect that activation of the Nrf2 signaling pathway and Nrf2 deletion should have the exact opposite effects on the thyroid. In practice, however, it is not as straightforward to predict the effects of Nrf2 deletion/activation, because these can be mediated by the regulation of Nrf2 target genes related with cytoprotection and antioxidant pathways, or by the crosstalk with other pathways. Moreover, Nrf2 appears to show a “hormetic response” [21], which suggests that the effects of too high or too low Nrf2 activity could potentially converge. This notion is similar to what has been described with ROS; very high ROS levels lead to increased oxidative stress and toxic effects, but very low ROS levels are also detrimental, because, in such conditions, it is not possible to maintain a baseline activation of the antioxidant pathways [22]. As mentioned above, we have previously used the Keap1^{KD} mice as a model of Nrf2 pathway activation. Interestingly, it was found that Tg mRNA levels in the thyroid were decreased [20], which was unexpected in view of the direct positive regulation of Tg transcription by Nrf2 and the decreased Tg mRNA expression levels in Nrf2^{KO} mice [17]. Whereas Nrf2^{KO} mice did not show major morphological differences in their thyroids compared to WT mice, Keap1^{KD} mice developed diffuse goiter (i.e., thyroid enlargement) from early adult life, and this phenotype became fully penetrant and more pronounced with age [20]. This goiter was characterized by increased size of thyroid follicles and absence of thyroid nodules or hyperplasia [20]. In contrast to mice lacking Nrf2 in the thyroid, which showed no differences in plasma TSH or thyroid hormone levels [17], Keap1^{KD} mice showed decreased T4 levels with no difference in TSH levels in early adult life, that were apparently compensated by increased TSH levels at an older age. The presence in

the thyroids of these mice of several peptides with molecular weights lower than that of monomeric Tg indicated the presence of Tg fragments [20]. One potential mechanism that could explain these changes in Tg could be its post-translational processing by proteolytic enzymes such as cathepsins. Consistent with this hypothesis, increased protein abundance of cathepsin D was observed in the thyroids of Keap1^{KD} mice [20]. Whether the changes in cathepsin D and other Tg-processing cathepsins are a direct result of regulation by Nrf2 or a result of adaptation to avoid overt hypothyroidism warrants further investigation. Another potential explanation is that increased ROS quenching due to higher Nrf2 activity may impair normal thyroid hormone synthesis. The generation of double Keap1^{KD}-Nrf2^{KO} mice would also be useful to examine whether the thyroidal phenotypes seen in Keap1^{KD} mice are exclusively Nrf2-dependent, or whether other binding partners of Keap1, such as Ikkb for example [23], may also play a role. Last but not least, generation of thyroid-specific Keap1^{KO} would address the possibility that changes in thyroid hormones observed in Keap1^{KD} mice might be affected by changes in thyroid hormone transport or peripheral processing.

3. Roles of Keap1/Nrf2 signaling in thyroid diseases in humans

3.1. Nrf2 and goiter

To date, there are only two reports of families with loss-of-function germline mutations in *KEAP1*. Based on these reports, such mutations have been associated with familial nontoxic multinodular goiter [24, 25], a rare genetic disease. In the first family of patients with *KEAP1* mutations [24], thyroid function was preserved, whereas the index patient in the second family (which was characterized much less thoroughly) [25] had suppressed TSH (indicative of hyperthyroidism); in both cases, multiple nodules were found in the thyroid. The goiter phenotype observed in Keap1^{KD} mice in our previous study partially recapitulates this phenotype, but thyroid enlargement in the mice was diffuse and it was not accompanied by the presence of nodules [20]. It would therefore be relevant to characterize more families with *KEAP1* mutations to facilitate more extensive genotype-phenotype correlations. The mouse and human data available to date indicate that over-activation of Nrf2 in the thyroid by genetic means causes goiter, but the precise phenotypic manifestation of this goiter may be variable. Thus, albeit rare, germline *KEAP1* mutations have the potential to yield valuable insights into the impact of Keap1/Nrf2 signaling on thyroid structure and function in humans.

3.2. Nrf2 and thyroid cancer

We have shown previously that Nrf2 is activated in papillary thyroid carcinoma [26], the most common type of thyroid cancer, and we have

catalogued the somatic mutations of *KEAP1* that have been detected in thyroid cancers [27]. Most of these mutations have been described in other contexts to cause stabilization of Nrf2 and activation of Nrf2 signaling. In general, somatic *KEAP1* mutations are more likely to enhance the survival of various cancer cell types than to serve as *bona fide* drivers of tumorigenesis, but this issue is still under debate [28,29]. Persistent antioxidant response, crosstalk with proliferation pathways and metabolic adaptations are some of the proposed mechanisms through which Nrf2 pathway activation can confer survival advantages to cancer cells in general. The importance of *KEAP1* mutations in the prognosis of thyroid cancer has not been thoroughly evaluated, considering also that the majority of papillary thyroid carcinomas have excellent prognosis. However, the evaluation of the activation status of the Keap1/Nrf2 pathway and the possible presence of mutations could be studied with reference to the progression of thyroid cancers (relapse, resistance to radioactive iodine, dedifferentiation etc.).

Specifically, three original research studies have been published since 2020 on the implication of Nrf2 in thyroid cancer. Two of them focused on anaplastic thyroid carcinoma, which represents <2% of all thyroid cancers but is one of the most aggressive human cancers, with a historical overall median survival of 4 months with conventional treatments. In one of these studies, anaplastic thyroid carcinomas from 6 patients were used to generate cell lines that then served as heterotopic xenografts in athymic mice [30]. One of these tumors carried the *NFE2L2*^{D27Y} somatic mutation that has been previously described in esophageal cancers and results in non-proper binding of NRF2 to KEAP1, thereby preventing the KEAP1-mediated degradation of NRF2 [31]. This was the first time that this *NFE2L2* mutation was reported in a thyroid tumor [30]. These findings provide a rationale for the preclinical testing of Nrf2 inhibitors in the treatment of anaplastic thyroid carcinoma.

Indeed, another study employed the anaplastic carcinoma cell line KAT-18 to assess the effect of Nrf2 downregulation on proliferation, growth and response to chemotherapy [32]. When KAT-18 cells with Nrf2 knockdown were transplanted in nude mice, smaller tumors were observed. KAT-18 cells with Nrf2 knockdown were also more sensitive *in vitro* to treatment with lenvatinib, a tyrosine kinase inhibitor commonly used for radioiodine-refractory differentiated thyroid carcinoma and tested also for anaplastic thyroid carcinoma. This preclinical study proposes the possibility of inhibiting the Nrf2 pathway as a treatment strategy to slow the progression of anaplastic thyroid carcinoma and to render it more sensitive to other treatments [32].

Finally, in another preclinical study, the papillary thyroid carcinoma cell line K1 was treated with the commonly used antidiabetic drug saxagliptin, an inhibitor of dipeptidyl peptidase-4 (DPP4); saxagliptin treatment promoted the nuclear translocation of Nrf2 [33]. When K1 cells were injected in the tail vein of nude mice, more lung metastases were observed in saxagliptin-treated mice. Similar observations were made in a previous study where cell line models of various cancers (hepatoma, colorectal adenocarcinoma, breast, lung and ovarian carcinoma) were treated with a DPP4 inhibitor [34]. A study using sitagliptin, another DPP4 inhibitor, also showed activation of the Nrf2 pathway in the intestine [35]. These findings suggest that DPP4 inhibitors, a widely used class of antidiabetic drugs, could activate the Keap1/Nrf2 pathway, thereby potentially leading to enhanced migration of already developed thyroid cancer cells. Further research is warranted to verify these results and to investigate whether they apply at the clinical level. Currently, there is no contraindication to introduce DPP4 inhibitors in patients with active thyroid cancer, and there is no recommendation to screen for thyroid cancer in patients considered for treatment with DPP4 inhibitors. A previous population-based cohort study in South Korea did not find a significant association between use of DPP4 inhibitors and metastases of various cancers, with the notable exception of thyroid cancer [36]. However, the number of thyroid cancer samples analyzed in that study was very limited; thus, further clinical studies on this topic are needed.

3.3. Nrf2 and autoimmune thyroid disorders

The *NFE2L2* promoter harbors three functional single-nucleotide polymorphisms (SNPs) that impact the promoter's transcriptional activity. These SNPs have been associated with various diseases whose pathogenesis involves oxidative stress [37–40]. We have previously described a genetic interaction between the *NFE2L2* promoter genotype and a functional SNP in the promoter of *SELENOS* [41]. This gene encodes selenoprotein S, a protein that promotes resistance to endoplasmic reticulum stress, but also shares common functions with the Nrf2 pathway, including suppression of pro-inflammatory cytokines. A previous study in a Portuguese population had shown that the *SELENOS* functional SNP increased the risk of Hashimoto's thyroiditis [42]. However, when the *NFE2L2* promoter genotype was also considered, only individuals harboring minor alleles in both *SELENOS* and *NFE2L2* had increased risk for Hashimoto's [41]. Further studies showed that, at the cellular level, this interaction may be explained by a bidirectional positive feedback between the selenoprotein S and Nrf2 pathways [41].

Regarding Graves' disease, in our analysis of the transcriptomic response of mouse thyroid to excess iodine we have found that the induction of inflammatory mediators (e.g., NFκB, IL-6, etc.) and antioxidant pathways, including the Nrf2 antioxidant response, was similar to the transcriptomic profile observed in a mouse model of Graves' disease [18]. This indicates that inflammatory/autoimmune pathways and Nrf2 signaling could be part of both the physiological response of thyroid to iodine and the pathogenesis of Graves' disease. However, the role of Nrf2 in Graves' disease pathogenesis remains to be addressed in dedicated studies using relevant experimental models and patient cohorts. Interestingly, in a recent multicenter observational case-control study, treatment with DPP4 inhibitors was associated with a significantly higher risk of Graves' disease exacerbation, with an odds ratio of 7.39 (95% confidence interval: 1.30–42.1) [43]. However, the study was relatively small, with 80 participants in total, of whom only 16 showed exacerbation of Graves' disease. Therefore, further clinical research is needed into the possible thyroidal side effects of DPP4 inhibitors and the potential involvement of Nrf2.

The potential effect of Nrf2 on autoimmune diseases in general, and on Hashimoto's thyroiditis and Graves' disease in particular, has been the focus of patents on compounds targeting Nrf2 [44]. Specifically, indoline and benzimidazole compounds that activate Nrf2 [45] have been proposed as adjunct treatments for Hashimoto's and Graves' (patent US20200039933A1). This is based mainly on the general concept that increased ROS levels in the thyroids of subjects with Hashimoto's and Graves' participate in the pathogenesis and exacerbation of these diseases [46]. However, specific studies to document benefits from pharmacological targeting of Nrf2 in thyroid autoimmune diseases are currently lacking.

Shifting the focus from the thyroid gland to the peripheral target tissues of thyroid hormones, both hypothyroidism (commonly associated with Hashimoto's) and hyperthyroidism (commonly associated with Graves') have been described as states that cause increased levels of ROS in peripheral tissues. Specifically, the levels of the oxidative stress markers malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS) were increased in the serum of hypothyroid patients [47–49]. Increased levels of oxidative stress markers were also seen in patients with Graves' disease [50], including urinary levels of the oxidized guanosine species 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) [51] and plasma levels of MDA [52]. Patients with hypothyroidism can be treated with T4 to restore thyroid hormone levels; conversely, patients with hyperthyroidism associated with Graves' disease or other causes of increased thyroid hormone synthesis can be treated with anti-thyroid drugs that inhibit TPO. Even though these treatments can restore thyroid hormone levels to normal, there are patients whose treatment proves to be challenging or lengthy, or who, despite normalization of thyroid hormone levels, continue to present symptoms and to experience worse

health-related quality of life [53]. In this context, it is interesting to explore whether activation of the Nrf2 pathway may ameliorate symptoms and/or restore thyroid function to normal levels more rapidly. To date, there is some supportive evidence for this concept from preclinical studies in rodents. For example, the Nrf2 activator quercetin was found to alleviate hyperthyroidism-induced liver damage via the Nrf2 signaling pathway [54]. An earlier study had shown that, in rats with experimentally induced hypothyroidism, broccoli sprouts exerted a beneficial influence on the antioxidant balance of the thyroid gland [55]. These encouraging data provide a foundation and a rationale for additional preclinical studies to test whether Nrf2 activators can translate into adjunct treatments for disorders of thyroid function. The roles and potential effects of the Nrf2 pathway in thyroid diseases in humans are summarized in Table 1.

4. Nrf2 pathway activators and thyroid

The use of Nrf2 pathway activators is expanding in clinical practice (e.g., use of dimethyl fumarate in multiple sclerosis [56]) and in clinical trials (e.g., use of the isothiocyanate sulforaphane in the form of broccoli sprout extracts in cancer chemoprevention [57], and in type 2 diabetes [58], and use of CDDO-Me in chronic kidney diseases [59,60]). In this context, issues of safety can be critical [15], as illustrated also by the premature termination of a phase 3 clinical trial of CDDO-Me for diabetic nephropathy due to an increased rate of cardiovascular adverse events [59]. Current considerations of the safety of Nrf2 activators usually revolve around toxicity, the risk of promoting malignancy and the risk of cardiovascular disease. On the other hand, the effect of Nrf2 modulators on thyroid function is rarely examined, and this is the case not only in clinical trials but also in preclinical pharmacological tests in animals.

Because we had found that activation of Nrf2 by genetic means (*Keap1* hypomorphism) can cause goiter and hypothyroidism [20], we hypothesized that pharmacological activation of Nrf2 might have similar effects. To address this question, we analyzed sera collected before and after a 12-week treatment with a broccoli sprout beverage rich in sulforaphane, and we evaluated the effects on thyroid function and thyroid autoimmunity by measuring the levels of TSH, free T4, Tg and anti-TPO and anti-Tg auto-antibodies [61]. The data showed no effect on the thyroid hormonal and autoimmunity status, indicating the safety of this treatment for the thyroid.

In the past, we had catalogued all naturally-occurring compounds with potential effects on thyroid function and morphology and/or on the Keap1/Nrf2 pathway [62]. Glucosinolates, which are found in broccoli sprout extracts, can be hydrolyzed to isothiocyanates; the best-known example is probably sulforaphane [63]. This class of compounds also includes pro-goitrin that can be hydrolyzed to goitrin, a cyclic thiocarbamate named after its goitrogenic action that results from an inhibitory effect on thyroid hormone synthesis [64]. Therefore, it would be interesting in future clinical trials with broccoli sprout extracts to measure the concentrations of goitrin in the extract, as well as to evaluate any changes in the volume of the thyroid using ultrasound.

Regarding dimethyl fumarate, a case of possible acute exacerbation of Hashimoto's thyroiditis has been reported [65]. Recently, we evaluated retrospectively the safety of dimethyl fumarate in patients with multiple sclerosis who received this drug in our hospital [66]. Out of 163 patients treated with dimethyl fumarate (mean treatment duration of 23 months), none developed structural thyroid disease, and only two developed functional thyroid disease. During treatment, one patient presented transient mild hypothyroidism with negative thyroid auto-antibodies. After treatment discontinuation, another patient developed hyperthyroidism due to Graves' disease. After analysis of the cases, a causal link between the thyroid hormonal imbalances and the drug was considered unlikely [66]. We concluded that dimethyl fumarate shows a very good thyroidal safety profile.

Table 1
Involvement of Keap1/Nrf2 signaling in human thyroid diseases.

Disease	Status and role of Keap1/Nrf2 pathway
Goiter	Germline <i>KEAP1</i> mutations (Nrf2 pathway activation)
Papillary thyroid carcinoma	Somatic <i>KEAP1</i> mutations (Nrf2 pathway activation)
Anaplastic thyroid carcinoma	Somatic <i>NFE2L2</i> mutations (Nrf2 pathway activation)
Hashimoto's thyroiditis	Genetic interaction of minor alleles in <i>SELENOS</i> and <i>NFE2L2</i> increases the risk of Hashimoto's thyroiditis
Autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis)	Potential of Nrf2 pathway activation as adjunct treatment to restore thyroid function and alleviate symptoms

5. Conclusions and perspectives

The characterization of the roles of Nrf2 in the normal function of the thyroid gland and in its various pathophysiological states is still in its early stages. Nevertheless, it is already evident that Nrf2 has pleiotropic roles that concern a wide range of phenomena, including antioxidant defense, iodine metabolism, protection from thyroid autoimmunity, promotion of thyroid cancer cell survival, etc. On the one hand, these pleiotropic roles highlight the need for further studies on the topic to identify additional areas of relevance for Nrf2 in the normal and abnormal thyroid, and to elucidate the underlying mechanisms. On the other hand, the multiplicity of thyroidal aspects involving Nrf2 might increase the complexity and risk inherent in targeting Nrf2 to prevent or treat thyroid diseases. Ostensibly due to its physiologic significance, the thyroid gland has a rich blood supply [67]; hence, it may be expected that systemically administered compounds can be delivered to the thyroid by the blood circulation at pharmacologically relevant concentrations. While this is known to be true for many drugs, it is not yet known whether it also holds true for current Nrf2-modulating compounds. Conversely, the few human data available to date on sulforaphane and dimethyl fumarate suggest that Nrf2 activators are safe for the thyroid. Nevertheless, this may also depend on the specific drug, its dose, the treatment duration and the mode of administration. Therefore, thyroidal safety should ideally be assessed for each Nrf2-modulating compound entering animal preclinical testing and human clinical trials.

Data availability

No data was used for the research described in the article.

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