

REVIEW

Thyrotropin receptor antagonists and inverse agonists, and their potential application to thyroid diseases

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Abstract. The thyrotropin receptor (TSHR) plays critical roles in thyroid growth and function and in the pathogenesis of several thyroid diseases including Graves' hyperthyroidism and ophthalmopathy, non-autoimmune hyperthyroidism and thyroid cancer. Several low-molecular weight compounds (LMWCs) and anti-TSHR monoclonal antibodies (mAbs) with receptor antagonistic and inverse agonistic activities have been reported. The former binds to the pocket formed by the receptor transmembrane bundle, and the latter to the extracellular TSH binding site. Both are effective inhibitors of TSH/thyroid stimulating antibody-stimulated cAMP and/or hyaluronic acid production in TSHR-expressing cells. Anti-insulin-like growth factor 1 inhibitors are also found to inhibit TSHR signaling. Each agent has advantages and disadvantages; for example, mAbs have a higher affinity and longer half-life but are more costly than LMWCs. At present, mAbs appear most promising, yet the development of more efficacious LMWCs is desirable. These agents are anticipated to be efficacious not only for the above-mentioned diseases but also for resistance to thyroid hormone and have utility for thyroid cancer radionuclide scintigraphy/therapy as a new theranostic.

Key words: Thyrotropin receptor, Antagonist, Inverse agonist, Agonist, Graves' disease

THE THYROTROPIN RECEPTOR (TSHR) has long attracted the interest of thyroidologists, since it has critical roles not only in thyroid growth and function but also in the pathogenesis of several thyroid diseases. In Graves' disease (GD), autoantibodies are elicited against TSHR, over-stimulating the thyroid glands and causing hyperthyroidism [called thyroid stimulating antibodies (TSAbs)] [1]. In rare cases, anti-TSHR autoantibodies block TSH binding to TSHR and lead to hypothyroidism [thyroid blocking antibodies (TBABs)]. TSHR is also expressed in some extrathyroidal tissues such as fat, fibroblasts and bone [2, 3]. Particularly, TSHR expressed in fibroblasts and adipocytes in the orbital cavity is thought to act as an autoantigen in Graves' ophthalmopathy (GO), where TSAbs stimulate cell proliferation and production of extracellular matrix such as hyaluronic acid (HA). Germline or somatic mutations of TSHR are responsible for congenital non-autoimmune hyperthyroidism and toxic adenoma in the case of gain-of-function mutations, and congenital hypothyroidism in the

case of loss-of-function mutations [4]. Furthermore, differentiated thyroid cancers preserve TSHR expression and their growth is, to some extent, dependent on TSH stimulation [3]. Thus, it is clear that TSHR is involved in the pathogenesis of many thyroid diseases; however, the development of therapies targeting TSHR in these TSHR-related pathologies has been slow.

The modulators of TSHR function, namely, receptor agonists, antagonists and inverse agonists, have recently attracted attention as therapeutic modalities for the above-mentioned ailments. These modulators include low molecular weight compounds (LMWCs) and monoclonal antibodies (mAbs). We here focus on TSHR antagonists and inverse agonists, summarize their characteristics, and then discuss their possible application to thyroid diseases. The purpose of this review is to outline the current status and future prospects of these inhibitors, and we leave discussion of their structural details and the role of individual amino acids in TSHR binding to these inhibitors.

Overview of TSH structure and function

The glycoprotein hormone receptors belong to the G protein-coupled receptor superfamily and include TSHR, luteinizing hormone receptor (LHR) and follicle stimulating hormone receptor (FSHR). Common to all

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members of this superfamily is the transmembrane region, which is comprised of seven transmembrane helices, three extracellular and intracellular loops, and a C-terminal tail. However, the difference between glycoprotein hormone receptors and the other members is the large extracellular domain in the former, which is the main binding site for the large, endogenous ligands of the respective glycoprotein hormones, TSH, LH and FSH (~30 kDa). Their extracellular domains contain 11 leucine-rich repeats (LRRs) with a scythe blade-like shape and a hinge region (Fig. 1). In contrast, the ligands for other members are small, thereby binding to the pocket formed by the seven transmembrane helices.

It is generally understood that in unliganded TSHR, its extracellular domain constrains the activity of the trans-

membrane region [5]. Upon TSH binding to the LRRs and the hinge region [containing an amino acid essential for TSH binding (Tyr385)], conformational changes are induced in these regions, which dissolves tethered inhibition of the transmembrane region by the extracellular domain and allows intracellular signal transduction, meaning TSH-induction of switching of the receptor extracellular domain from a tethered inverse agonist to an agonist. A recent study reported that the hinge region contains an “internal agonist sequence” (aa 405 to 414), which is liberated after TSH binding and stimulates the TSHR transmembrane region [6].

Discovery of TSHR antagonists and inverse agonists

Several TSHR antagonists and inverse agonists have been reported, and the characteristics of these agents are summarized in Table 1. Here, antagonists inhibit receptor activation by agonists, and inverse agonists inhibit not only agonist-activation of the receptor but also agonist-independent (constitutive) receptor activity. In general, agonists induce outward displacement of the 6th transmembrane helix and facilitate the coupling of G proteins, while inverse agonists induce its inward displacement and hinder it [7, 8]. These agents are largely divided into two categories: LMWCs and mAbs. The LMWCs have been reported by five groups, and the mAbs have been isolated by three groups [9].

LMWCs

Gershengorn's group has reported a diverse panel of LMWCs with TSHR antagonistic and inverse agonistic activities; NIDDK/CEB-52 [10], NCGC00242595 [11] and NCG00242364 (ANTAG3) [12] are antagonists and NCGC00161856 [13] and NCG00229600 (ANTAG2) [14] are inverse agonists, all of which were obtained by high-throughput screening or derived from the TSHR/LHR agonist Org41841 [15] and the TSHR agonist NCGC00161870 [16] by chemical modifications. Merck & Co. discovered Org-274179-0 [17, 18]. VAK-14, S37a and TP48/TPY1 are from groups represented by Davies, Krause and Shpakov, respectively [19-22]. The IC₅₀ for basal activities of wild-type (wt) TSHR and constitutively activating mutants (CAMs), and for TSH/TSAb-stimulation are at nanomolar levels in the inverse agonist Org-274179-0 [17, 18], but those for all other chemicals are at micromolar levels, *i.e.*, approximately 1,000-fold less potent than Org-274179-0.

All LMWCs with antagonistic or inverse agonistic activities, except S37a (see below), bind to the pocket formed by the seven transmembrane helices of the receptor, indicating that they are non-competitive, allosteric ligands (red in Fig. 1). This is reminiscent of the binding pattern seen in other members of the G protein-coupled

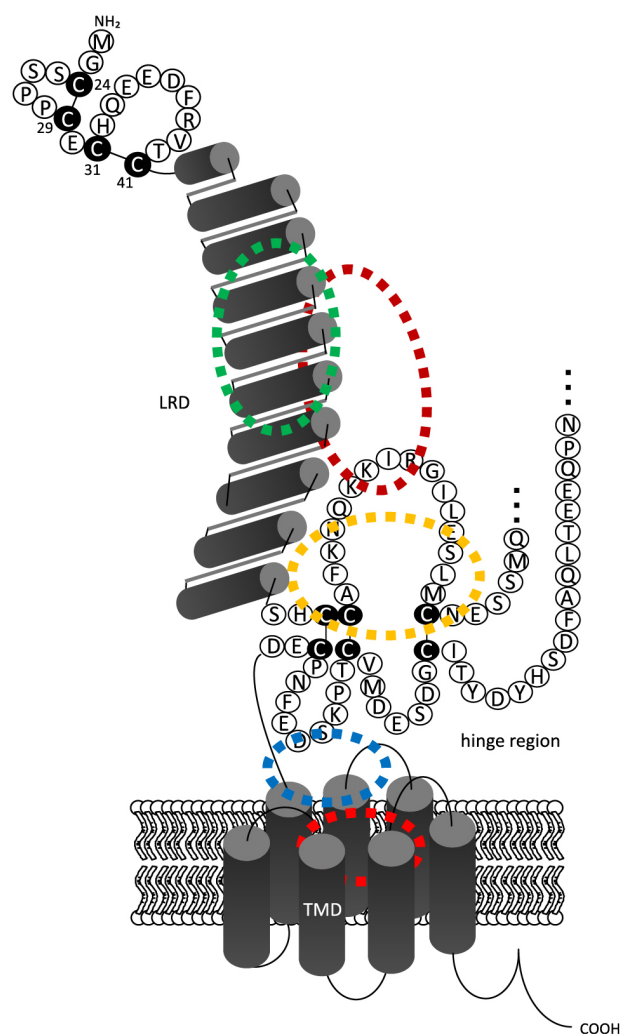


Fig. 1 Structure of TSH receptor. The binding sites for TAB-8/K1-70/5C9, Cs-17, Tab-4, most LMWCs and LMWC S37a are shown in green, brown, orange, red and blue, respectively. LRD, leucine rich domain; TMD, transmembrane domain. Reproduced and modified from ref. [55] with permission.

Table 1 Summary of the characteristics of TSHR antagonists and inverse agonists.

Names	Antagonist/ inverse agonist	Interaction with TSHR	Interaction with LH/FSHR	Refs.
LMWCs				
NIDDK/CEB-52 (compound 52)	Antagonist	IC ₅₀ = 4.2 µM for TSH-stimulation. Agonistic at the CAMs and a TSHR/LHR chimera M9.	IC ₅₀ >30 µM for binding to the LHR, partially agonistic at LHR and no activity at FSHR.	[10]
NCGC00242595	Antagonist	IC ₅₀ = 2.7 µM for TSH-stimulation.	Not examined.	[11]
NCGC00161856	Inverse agonist	IC ₅₀ = 3.0 µM for basal activity, and IC ₅₀ = 0.78 µM for TSH-stimulation, of the wt receptor. IC ₅₀ = 0.5~3.7 µM for basal activities of CAMs.	Not examined.	[13]
NCG00229600 (ANTAG2)	Inverse agonist	IC ₅₀ = ~30 µM for basal activity and TSH stimulation.	Antagonistic for LH and FSHR (the details not demonstrated) [37].	[14]
NCG00242364 (ANTAG3)	Antagonist	IC ₅₀ = 2.1 µM for TSH stimulation.	IC ₅₀ >30 µM for LH/FSH stimulation at LH/FSHRs.	[12]
Org-274179-0	Inverse agonist	IC ₅₀ = 22 nM for basal activity and IC ₅₀ = 2~56 nM for TSH/M22 stimulation at wt receptor, and IC ₅₀ = 2~74 nM for basal activities of CAMs.	Antagonistic at FSHR (IC ₅₀ = 17 nM), and partially agonistic at LHR (IC ₅₀ = 1.1 µM).	[17]
VA-K-14	Antagonist	IC ₅₀ = 12.3 µM for TSH stimulation.	Weakly antagonistic for LH/FSHRs.	[19]
S37a	Antagonist	IC ₅₀ = ~50 µM for TSH/M22 stimulation. Inverse agonistic at the CAMs with amino acid changes in the hinge region or the extracellular region.	No effect on LH/FSHR.	[20]
TP48	Antagonist	~60 and 85% inhibition with 1 and 100 µM.	No effect on LHR.	[21]
TPY1	Antagonist	~75% inhibition with 30 µM.	Not examined.	[22]
mAbs				
TAbs-4, TAb-8	Antagonists	10% inhibition of TSH binding, and 43% inhibition of TSH stimulation at 5 µg/mL TAb-4. 91% inhibition of TSH binding, and 81% inhibition of TSH stimulation at 5 µg/mL TAb-8.	No effect on LH/FSHR.	[28]
CS-17	Inverse agonist	IC ₅₀ = ~7 nM for basal activities of wt receptor and CAMs.	No effect on LH/FSHR.	[29]
K1-70	Antagonist	IC ₅₀ = ~0.2 nM for TSH stimulation.	No effect on LH/FSHR.	[31]
5C9	Inverse agonist	IC ₅₀ = ~0.3 nM for TSH stimulation of wt receptor, and IC ₅₀ = 0.1~10 nM for basal activities of CAMs.	No effect on LH/FSHR.	[30]

CAM, constitutively activating mutants; LMWC, low molecular weight compound; mAb, monoclonal antibody.

receptor superfamily with small ligands. Also, this is reasonable because it is very difficult to inhibit protein-protein interactions with LMWCs [14]. Thus, these LMWCs block signal transduction from the extracellular domain to the transmembrane region of the receptor without affecting TSH/TSAb-binding, and restrain the conformational changes required for TSHR activation. Interestingly, studies on the relationship between LMWCs and TSHR have shown that amino acids in the receptor transmembrane helices that participate in LMWC binding to the receptor are closely related to activating and inactivating TSHR mutations [23, 24], indicating that the LMWC binding sites are in the signaling-sensitive domain in TSHR [25]. The authors have also proposed that CAMs induce a shift from the basal state toward the active conformation of the recep-

tor, either by disrupting an interaction network critical for receptor stabilization or creating new interactions. Thus, by their binding to these critical amino acids, antagonists appear to prevent this shift, and inverse agonists facilitate an oppositely directed shift from the ligand-independent, basal state to the inactive state. Although inverse agonists also inhibit TSHR CAMs [13], it is reported that the efficacies are dependent on the locations of amino acid substitutions. The CAMs in the receptor extracellular domain can be effectively suppressed, but less so for those in the transmembrane region. The active conformation induced by CAMs (see above) is likely to be resistant to inverse agonists.

Due to the high homology in transmembrane regions among TSHR, LHR and FSHR, cross-binding of these LMWCs to LHR and/or FSHR is often observed as an

off-target effect; some act as agonists [10, 18] and others as antagonists [14, 18, 19] in LHR and/or FSHR. In this regard, it is of interest that no cross-reactivity is reported in S37a and TP48 [20, 21]. The binding site of S37a is reported to be mainly at the interface between the hinge region and the extracellular loops (blue in Fig. 1), and the amino acids in the hinge region show low homology among TSHR, LHR and FSHR [26]. In contrast, the binding site for other LMWCs is the allosteric binding pocket deeper in the transmembrane bundle, and the majority of amino acids involved in binding are common among TSHR, LHR and FSHR [15, 27]. These differences clearly explain the lack of cross-reactivity of S37a to LHR/FSHR. Thus, the binding site for S37a is different from those of other LMWCs, and S37a can block both TSH- and LMW agonist-induced receptor activation. It is also of interest that S37a is antagonistic for wtTSHR, and is an inverse agonist for CAMs having amino acid alterations in the hinge region or the extracellular loops, but not in the transmembrane region.

Therefore, in terms of high affinity and inverse agonistic activity, Org-74179-0 may be the most promising; however, when considering specificity, S37a seems to be the most interesting TSHR inhibitor for future development. At present, there are no reports of products derived from Org-74179-0 or S37a.

Anti-TSHR mAbs

Davies and colleagues isolated several mAbs from hamsters immunized with adenovirus expressing human TSHR, and two of them, TAB-4 and TAB-8, have blocking activity [28]. Rapoport and McLachlan were the first to isolate a TSHR inverse agonist, CS-17, from experimentally hyperthyroid mice [29]. Rees Smith also isolated a series of anti-TSHR mAbs with thyroid stimulating or blocking activities from peripheral lymphocytes of patients with autoimmune thyroid disease; two, M22 and K1-18, were stimulating, and the other two, K1-70 and 5C9, were blocking [30-32] (Table 1).

TAB-8, K1-70 and 5C9 bind to the concave side of the LRRs, not the hinge region, on the extracellular domain (green in Fig. 1), thereby directly inhibiting TSH-binding. Thus, they work competitively with TSH. Their binding affinity to TSHR is much higher than those of LMWCs; this is because of their highly complex binding pattern with several tens of amino acids in each involved in the binding [32]. For example, the binding affinities of K1-70 and 5C9 to TSHR are 4×10^{10} mol/L, similar to those of TBABs in patient sera. In contrast, the main binding site for TAB-4 is the hinge region (orange in Fig. 1), and thus the competition of TAB-4 binding to TSHR with TSH is weak [28]. CS-17 binds to the convex surface of the LRRs and the hinge region (brown in Fig. 1)

with a somewhat weak TRAb activity [33]. CS-17 binding site may overlap with the TSH binding site or there is a steric hindrance between CS-17 and TSH.

Because of their different binding sites on TSHR, the mechanism for the inverse agonistic activity of CS-17 and 5C9 must be quite different from that of LMWCs. The TSHR extracellular domain itself is thought to be a tethered inverse agonist, as mentioned above, and 5C9 and CS-17 seem to enhance this suppressive activity [29].

Preclinical [34] and phase I clinical trials of K1-70 in GD patients [35] have shown that treatment with K1-70 is safe and well tolerated, and can effectively suppress thyroid hormone and increase TSH levels. Also, the beneficial effects of K1-70 on a patient with GD, GO and thyroid cancer has been reported in a case report, showing that cancer progression was arrested, although temporarily, and eye disease improved dramatically [36].

Others

The interaction between TSH and insulin-like growth factor 1 (IGF1) signaling has long been well documented, and is now considered to be important in the pathogenesis of GO [37]. TSHR and the IGF1 receptor (IGF1R) are overexpressed in the orbital tissues from GO patients compared to normal controls [38-40]. TSHR expression levels are low in *in vitro* cultured orbital fibroblasts, and increase when fibroblasts are differentiated to adipocytes [17, 39, 41]. TSHR antagonists/inverse agonists, and IGF1R blocking antibodies (1H7 and teprotumumab) and an IGF1R tyrosine kinase inhibitor (linsitinib) inhibit basal levels and/or TSH/TsAb and IGF1, respectively, stimulated cell proliferation and HA production in fibroblasts/adipocytes (see Tables 2 and 3). According to the recent works by Gershengorn and colleagues [42], TSHR, IGF1R and arrestin constitute the signalosomes (where two receptors are closely proximate to each other within 40 nm), which play a significant role in cross-talk between two receptors. Arrestin is an important component of signalosomes as a scaffold, as knock-down of arrestin reduces co-localization of the two receptors. They have clearly demonstrated that, although TSH does not bind to IGF1R and IGF1 does not bind to TSHR, TSHR agonists stimulate TSHR-IGF1R cross-talk (IGF1R-dependent TSHR signaling) at lower concentrations, and stimulate not only TSHR-IGF1R cross-talk but also IGF1R-independent TSHR signaling at higher concentrations. Thus, IGF1R antagonistic mAbs or an IGF1R kinase inhibitor can block the stimulation by lower, but not higher, concentrations of TSHR agonists (Table 2), while TSHR antagonists can inhibit both IGF1R-dependent and -independent TSHR signaling [43, 44].

Table 2 Summary of the characteristics of IGF1R inhibitors.

Names	Antagonist/inverse agonist	Interaction with TSHR	Interaction with LHR and FSHR	Refs.
LMWC				
Linsitinib	Antagonist	IC ₅₀ = 151 nM for HA production in fibroblasts stimulated with a moderate (not high) conc. of M22.	Not examined.	[43]
mAbs				
1H7	Antagonist	IC ₅₀ = 14.3 nM for HA production in fibroblasts stimulated with a moderate (not high) conc. of M22.	Not examined.	[43]
Teprotumumab	Antagonist	Inhibition of TSH-stimulated cytokine secretions.	Not examined.	[47]

Table 3 Potential application of TSH receptor antagonists/inverse agonists.

Target illness	Related articles
Antagonists	
Graves' hyperthyroidism	Inhibition of TSAb-mediated cAMP production in TSHR-expressing cells by NIDDK/CED-52 [10], ANT2 [14], Org274279-0 [18], ANT3 [12], VA-K-14 [19], K1-70 [30] and 5C9 [31]. Decline of T ₄ in experimental mouse hyperthyroidism induced with TRH or M22 by ANT3 [12].
Graves' ophthalmopathy	Inhibition of the basal and/or TSH/M22 or IGF1-mediated cAMP/HA productions by the TSHR inhibitors [Org274179-0 [17], ANT2 [12, 56], NCGC00242595 [56] and ANT3 [43] and IGF1R inhibitors [linsitinib and 1H7 [57] and teprotumumab [58]], respectively, in orbital fibroblasts. Clinical use of K1-70 for a patient with FTC, GD, GO [36].
Thyroid cancer	[3, 13, 18, 25]
Thyroid hormone resistance	
Inverse agonists	
Non-autoimmune hyperthyroidism	Inhibition of the basal activity of the CAMs by CS-17 [29], NCGC00161856 [13] and Org274179-0 [18].
As agents targeting TSHR	
Thyroid cancer Scintigraphy Drug delivery	[3]

Potential clinical applications of these agents to thyroid diseases

Graves' hyperthyroidism and ophthalmopathy

There has been little progress in GD therapeutics over the last several decades, which include anti-thyroid drugs (ATDs) that inhibit thyroid hormone synthesis, thyroid ablation by surgical thyroidectomy or radioiodine, and inorganic iodine. However, these therapeutics are not pathogenesis based, and have limited efficacy and non-negligible drawbacks (adverse effects). For example, although ATDs have been used relatively safely for a long time, they have rare but serious adverse effects such as agranulocytosis and liver dysfunction; high relapse rates are observed after ATD cessation; and hypothyroidism can occur following thyroid ablation by surgery and radioiodine therapy. Further, GO is frequently treated with high-dose glucocorticoids, often resulting in dissatisfaction with poor outcomes and adverse effects. Given that TSABs are greatly responsible for the pathogenesis of GD and GO, many agree that Graves' hyper-

thyroidism and ophthalmopathy are good candidates for treatment with TSHR antagonists.

Many *in vitro* experiments show inhibition of TSAb-mediated cAMP production in thyroid cells or TSHR-expressing non-thyroidal cells, and of HA production in orbital fibroblasts or adipocytes by TSHR antagonists and inverse agonists (Table 3). In *in vivo* experiments with hyperthyroid mice, induced by administration of TRH or M22 stimulating mAbs, serum T₄ declined by approximately 50% with a large amount of ANT3, suggesting efficient but insufficient inhibition for clinical application [45]. As mentioned above, K1-70 is already used in preclinical and Phase I clinical trials [34, 35]. mAbs are likely more effective than LMWCs because of their much higher affinity for TSHR. However, no study has been conducted on the effect of antagonists on experimental GD in mice.

The advantages of mAbs include higher affinity and longer half-life (11 to 22 days, depending on dose administered) [35] than LMWCs, while the disadvantages

include parenteral administration, higher antigenicity (may induce allergic-like responses), difficulty in generation/purification/quality control and are thereby costly.

As mentioned above, anti-IGF1R antagonists can effectively inhibit the proliferation and HA production of orbital fibroblasts, and an anti-IGF1R mAb, teprotumumab, was approved for GO in 2020. Since teprotumumab was originally developed as an anti-neoplastic agent and has already been administered to hundreds of patients in Phase I and II studies, it was approved for GO in a relatively short period of time [46]. Two clinical studies and post-approval case reports have shown a rapid and significant resolution of symptoms [47]. However, Krieger *et al.* stated that “drugs that inhibit TSHR activation, such as K1-70 or small molecule TSHR antagonists, may be as effective as teprotumumab in treating thyroid eye disease, and possibly more, because they inhibit both IGF1R-dependent and IGF1R-independent pathways” [44]. As they have further mentioned, combination therapy with TSHR and IGF1R antagonists at lower doses may be desirable to reduce adverse effects.

IGF1R is expressed in many tissues, and the high degree of homology with insulin receptor (IR) may cause inhibition of IR by IGF1R inhibitors as an off-target effect. Moreover, other adverse effects such as muscle spasm and hearing impairment have also been reported [47]. The same concerns can also be applied, albeit to a lesser degree, to TSHR mAbs, because of extrathyroidal expression of the receptor.

Thyroid cancer

Except for the recent introduction of tyrosine kinase inhibitors, treatment modalities for thyroid cancer have remained unchanged for the last decades—surgical resection, radioiodine ablation therapy and TSH suppressive therapy by excess LT_4 . Regarding TSH suppressive therapy, TSHR expression is well preserved in the majority of differentiated thyroid cancers, despite being highly variable, because serum Tg increases in response to TSH induced by LT_4 withdrawal or recombinant human (rh) TSH administration before radioiodine ablation in post-surgical thyroid cancer patients. Also, the growth of differentiated thyroid cancers is, at least in part, dependent on TSH signaling. For example, constitutively activating TSH mutations are one of the causes of thyroid tumor development, and increased levels of serum TSH are associated with increased risk of thyroid cancer, increased pathological stage and increased incidence of nodal metastasis [48]. Thus, TSH suppression by excess LT_4 is now widely and effectively used to reduce progression of post-surgical residual thyroid cancers. In fact, TSH-suppression has a clear benefit in high-risk thyroid cancer patients. However, there is a lack of consensus if long-term TSH-suppressive therapy is truly necessary for

thyroid cancer patients with a lower risk of recurrence because of the adverse effects of excess LT_4 , such as osteoporosis and atrial fibrillation [3]. Therefore, theoretically, TSHR antagonists may be safer than TSH-suppressive therapy, and TSHR inverse agonists may be more efficacious than TSH-suppression and TSHR antagonists, since the former also suppresses the basal, constitutive activity of TSHR.

Non-autoimmune hyperthyroidism

Non-autoimmune hyperthyroidism, caused by constitutively activating TSHR mutants, includes familial non-autosomal dominant hyperthyroidism and persistent sporadic congenital non-autoimmune hyperthyroidism. Although there are at present no guidelines for the management of these conditions, the European Thyroid Association, for example, strongly recommends the complete ablation of the thyroid tissue by total thyroidectomy and radioiodine administration [49]. TSHR inverse agonists may also be suitable for the treatment of non-autoimmune hyperthyroidism by suppressing the constitutive activation of mutant TSHRs. Indeed, as shown in Table 1, suppression of the constitutive activities of TSHR mutants has been demonstrated with NCGC00161856, Org274179-0 [13, 18], and CS-17 [29].

Resistance to thyroid hormone (RTH)

RTH, defined by impaired sensitivity to thyroid hormone, is caused by mutations in the thyroid hormone receptor β gene, and is characterized biochemically by elevated thyroid hormones in the absence of TSH suppression. Variable phenotypes are observed, from no symptoms/signs to goiter, attention deficit disorder, *etc.* No specific therapy to fully correct thyroid hormone receptor β defect is currently available [50]. Among several symptoms and signs occasionally observed in RTH patients, large symptomatic goiters, for which one of the treatment options is administration of supraphysiologic doses of T_3 , may be a good candidate for treatment with TSHR antagonists. Furthermore, the Information Center for Specific Pediatric Chronic Diseases, Japan (https://www.shouman.jp/disease/details/05_12_024/, in Japanese) states that “if the TSHR antagonists become available, they are likely to be effective in treatment of pituitary-selective RTH, and thus their development is desirable”.

Other applications

Molecular imaging plays an important role in the evaluation and management of different thyroid cancer types. Scintigraphy with radioactive iodine (^{125}I) and, albeit to a lesser degree, PET/CT with [^{18}F] F-fluorodeoxyglucose are frequently used [51]. Ablative doses of radioactive iodine (^{131}I) can also be used for treatment of thyroid cancers that actively incorporate iodine. However, some residual thyroid cancers post-surgery and/or

-radiotherapy become resistant to radioiodine, called radioiodine-refractory differentiated thyroid cancers. As TSHR is more persistently expressed than other thyroid-specific, differentiation markers such as sodium-iodine symporter (mediates iodine uptake) and thyroglobulin [3], TSHR-targeted drug delivery [anti-cancer drugs, radionuclides and/or cytotoxic T lymphocytes (CAR-T therapy)] using TSHR ligands (irrespective of antagonists, inverse agonists or agonists) may be a useful theranostic approach for radioiodine-refractory differentiated thyroid cancers as a peptide receptor radionuclide scintigraphy/therapy. A recent article shows the therapeutic efficacy of CAR-T targeting TSHR with K1-70 scFv in the *in vitro* and *in vivo* experimental settings of thyroid cancer [52]. The usefulness of radio-labeled rhTSH for thyroid cancer scintigraphy has previously been shown [53, 54].

Conclusions

We here first summarized the characteristics of LMWCs and mAbs with TSHR antagonistic or inverse agonistic activities so far reported. Both are effective at inhibiting TSH/TSAb-stimulated cAMP and/or HA production in TSHR-expressing cells, but, at the same time, have their own unique advantages and disadvantages. For example, mAbs have higher binding affinities to

TSHR than LMWCs but should be administered parentally and are costly, while cross-reactivity to LHR and FSHR is unavoidable in most LMWCs. Although at present mAbs seem to be the most efficacious and promising approach and may appear on the market in the not-too-distant future, development of newer molecules with higher affinity and specificity by modifying current LMWCs is expected. The second half of this review article discussed thyroid diseases for which these inhibitors can be applicable. Graves' hyperthyroidism and ophthalmopathy are readily identified as therapeutic targets for these agents. Additionally, these agents may also be potentially useful for treatment of thyroid cancer, non-autoimmune hyperthyroidism and resistance to thyroid hormone, and can further be used for thyroid cancer radionuclide scintigraphy/therapy as a new theranostic.

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Disclosure

The authors declare no conflict of interest.

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