REVIEW

Role of thyroglobulin on negative feedback autoregulation of thyroid follicular function and growth

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Abstract

Thyroid function is tightly regulated by TSH. Although individual follicles are exposed to the same blood supply of TSH and express relatively homogenous levels of the TSH receptor, the function of individual follicles is variable. It was shown that thyroglobulin (Tg), stored in the follicular lumen, is a potent negative feedback regulator of follicular function. Thus, physiological concentrations of Tg significantly suppress thyroid-specific gene expression and antagonize the TSH-mediated stimulation that induces expression of thyroid-specific genes. Tg coordinately regulates both basal and apical iodide transporters in thyroid follicular cells. Recently, it was also reported that Tg could induce thyroid cell growth in the absence of TSH. These results indicate that Tg is an essential autocrine regulator of physiological thyroid follicular function that counteracts the effects of TSH.

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Introduction

Serum levels of thyroid hormone are tightly regulated by the levels of TSH in serum. However, TSH is not the only factor regulating thyroid function; it is also regulated by various other factors, including iodine and autoantibodies. Here, we show that the thyroglobulin (Tg) stored in thyroid follicles (the most abundant product of thyroid cells) functions as a potent negative feedback autocrine regulator of endocrine function and cell growth in individual follicles. We also discuss potential pathological conditions that may occur in human patients due to irregularities in Tg-mediated regulation of thyroid function.

Follicular function and heterogeneity of the thyroid

The thyroid follicle is the most unique structure of the thyroid gland; the thyroid follicle is where iodine is trapped, Tg is stored, and thyroid hormones are formed and stored. The function of the entire thyroid gland reflects the sum of the function of each follicle (Dunn & Dunn 2000). In some marine fish, such as lamprey, Tg and thyroid hormones are synthesized and secreted by specialized cells in the endostyle, and there is no follicular structure (Wright *et al.* 1978, Fredriksson *et al.* 1988). Therefore, the large follicular space found in many other animals, including all mammals, may be required to efficiently utilize dietary iodide and store the thyroid hormone and its precursors until needed.

TSH stimulates iodide uptake from the blood stream and the synthesis of Tg, thyroid peroxidase (TPO), and the sodium/iodide symporter (NIS), in order to iodinate Tg to form thyroid hormones. After Tg is synthesized, it is transported to the apical membrane and released into the follicular lumen (Dunn & Dunn 2000). TPO and H_2O_2 at the apical membrane catalyze the iodination of tyrosine residues on Tg and then couple two iodotyrosine residues to form thyroxine (T_4) and triiodothyronine (T_3). TSH stimulates the reabsorption and hydrolysis of follicular Tg and the subsequent secretion of thyroid hormones in the blood. Iodinated Tg is reabsorbed back into follicular cells through the apical membrane and degraded to form T_3/T_4 in lysosomes. The T_3/T_4 is then secreted through the basal membrane (Dunn & Dunn 2000).

TSH is supplied to thyrocytes by a fine capillary network that surrounds each follicle. Serum TSH levels are fairly

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constant, and the expression of the TSH receptor (TSHR) along the basolateral membrane of follicular cells is largely homogeneous. Despite the fact that each follicle has a uniform TSH supply and reasonably uniform TSHR expression, the function of individual follicles is not uniform, but rather heterogeneous. Follicles are quite heterogeneous in their morphology, i.e. shape and size (or colloid density) and in their function, i.e. iodide uptake, diffusion of iodinated Tg within the follicular lumen, and accumulation of Tg and thyroid hormones (Gerber *et al.* 1985, 1986, Suzuki *et al.* 1999*a*,*b*,*c*).

There are several possibilities that could explain the nature of follicular heterogeneity. Some evidence suggests that individual thyrocytes within the follicle are not functionally uniform. Using chimeric mice, it has been shown that epithelial cells surrounding a follicle do not originate from a single cell, but are mosaic (Feder 1976, Thomas *et al.* 1989). Using a three-dimensional collagen-gel culture method, it was shown that cell aggregates formed follicular structures (Toda *et al.* 1993). Primary cultured thyrocytes showed quite heterogeneous behaviors (Baptist *et al.* 1991), and it is known that cloned thyroid cells show significant variability in growth and function in culture (Huber *et al.* 1990). These findings suggest that thyrocytes surrounding a single follicle are heterogeneous in origin and that the function of each follicle is also heterogeneous, such that the heterogeneity cannot be explained solely by differences in the composition of follicular cells within the follicle. Until recently, the true nature of such follicular heterogeneity was unclear.

Negative feedback autoregulation of thyroid-specific gene expression and follicular function by Tg

Using cultured rat thyroid FRTL-5 cells, we have shown that mRNA levels of Tg, TPO, and NIS were significantly suppressed by physiological concentrations of follicular Tg added to the culture medium (Suzuki *et al.* 1998, 1999*d*). The Tg action was concentration-dependent, and the same concentrations of BSA, immunoglobulin, or mannitol had no effect. Inorganic iodide, T_3, or T_4 did not affect Tg mRNA levels either. In contrast to the suppression of thyroid-specific gene expression, the mRNA levels of the Pendred syndrome gene (PDS) and major histocompatibility complex (MHC) class I gene were increased by low concentrations of Tg (Suzuki *et al.* 1998, Royaux *et al.* 2000). Actually, Tg is the only factor that can induce PDS gene expression in the thyroid (Royaux *et al.* 2000).

Tg suppresses radioiodine uptake in FRTL-5 cells in a dose- and time-dependent manner (Suzuki *et al.* 1999*d*). Although iodide accumulation in rat thyroid follicles is quite heterogeneous (Wollman & Loewenstein 1973, Suzuki *et al.* 1999*a*,*c*), *in vivo* studies further indicate that follicular Tg is a negative feedback regulator of iodide uptake in each follicle. For example, in a study of propylthiouracil-treated rats, we showed that a rim of Tg that bound to the apical membrane in the follicular lumen was associated with the suppression of thyroid transcription factor (TTF-1) mRNA and Tg biosynthesis (Suzuki *et al.* 1999*b*). It was further demonstrated that there was a clear inverse correlation between Tg accumulation and radioiodine uptake in each follicle (Suzuki *et al.* 1999*d*).

The range of Tg concentrations required for these effects was 1–10 mg/ml, which is within the range of normal follicular Tg concentrations, 0·1 up to 250 mg/ml, as measured by aspiration biopsy or micropuncture of a single follicle (Hayden *et al.* 1970, Smeds 1972, Salabe *et al.* 1996). The relatively large range of the estimates of follicular Tg concentration may be the result of significant functional heterogeneity among follicles. Tg concentrations of 0·1 mg/ml, which is still much higher than serum Tg concentration, had a minimal effect. Thus, we believe that Tg acts through the apical, not the basal membrane, of the follicular epithelium.

Changes in mRNA levels seemed to be regulated transcriptionally because the promoter activity of Tg, TPO, and NIS decreased with the decreases in mRNA levels (Suzuki *et al.* 1998, 1999*d*). In fact, follicular Tg significantly decreased mRNA levels of three thyroid-specific transcription factors, TTF-1, TTF-2, and Pax-8, but not ubiquitous factors, Sox-4, TSHR suppressor element-binding protein-1 (TSEP-1), and single-strand binding protein-1 (SSBP-1). Consistent with the suppression of mRNA levels of thyroid-specific transcription factors, Tg also decreased TTF-1, TTF-2, and Pax-8 binding to their specific DNA-binding sites on the Tg and TSHR promoters (Suzuki *et al.* 1998). Additionally, the promoter activity of the 5′-flanking region of a TTF-1-luciferase reporter gene chimera was decreased by Tg in a concentration-dependent manner (Suzuki *et al.* 1998, Nakazato *et al.* 2000). These results indicate that Tg protein suppresses expression of Tg, TPO, and NIS by suppressing multiple thyroid-specific transcription factors. Therefore, we suggest that Tg protein, accumulated within the follicle, acts as a negative feedback regulator and contributes to the follicular heterogeneity.

Tg induces growth of thyrocytes in the absence of TSH

The growth of thyroid cells is tightly regulated by the coordinated action of TSH and insulin/insulin-like growth factor 1 (IGF1) signaling (Takahashi *et al.* 1991, Nedachi *et al.* 2000). However, we have shown that Tg alone can stimulate cell growth and ^{3}H-TdR uptake in FRTL-5 cells as effectively as the combination of TSH, insulin, and serum (Noguchi *et al.* 2010). Although Tg stimulated cell growth, it did not increase cAMP levels in FRTL-5 cells, and an inhibitor of cAMP-dependent protein kinase, H-89, had no effect on Tg-induced cell growth. Moreover, TSH/cAMP counteracted the ability

of Tg to induce cell growth. Therefore, the intracellular signaling utilized by Tg appears to be quite different from that used by TSH.

Both Tg and insulin/IGF1 activated AKT, a key downstream molecule that mediates insulin/IGF1 action, and a phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002, significantly suppressed Tg-induced ^{3}H-TdR incorporation (Noguchi *et al.* 2010). The effect of Tg and insulin in stimulating cell growth was additive, which suggests that Tg may utilize PI3K/AKT to induce cell growth, but possibly by a mechanism that involves a signaling cascade that is not yet known to function in this process.

Lower concentrations of Tg (1–5 mg/ml) induce more cell growth than higher concentrations (>10 mg/ml). All these Tg concentrations are within the lower range of follicular Tg in different follicles of the normal thyroid (Hayden *et al.* 1970, Smeds 1972, Salabe *et al.* 1996). Tg-mediated suppression of thyroid-specific gene expression is dose-dependent (Suzuki *et al.* 1998, 1999*d*, Suzuki & Kohn 2006, Noguchi *et al.* 2010); however, Tg induced expression of at least two genes, MHC class I and PDS (Suzuki *et al.* 1998, Royaux *et al.* 2000, Suzuki & Kohn 2006). Thus, mRNA levels of MHC class I and PDS are highest at lower concentrations of Tg and decrease with higher Tg concentrations (Suzuki *et al.* 1998, Royaux *et al.* 2000, Suzuki & Kohn 2006, Noguchi *et al.* 2010). Therefore, optimal Tg concentrations that induce growth promotion and suppression of gene expression showed biphasic pattern.

It is interesting that the 'stimulation' of thyroid cells, i.e. the induction of cell growth and gene expression of MHC class I and PDS – is higher with lower concentrations of Tg, while the 'suppression' of gene expression and follicular function is induced by higher concentrations of Tg in a dose-dependent manner. These results suggest that there are at least two distinct mechanisms of Tg action that have different kinetics. Both effects, 'stimulation' and 'suppression', increase in the order of 27S>19S>12S Tg. Thus, using gel-fractionated Tg moieties, it was demonstrated that 27S Tg has the strongest effect on induction of cell growth (Noguchi *et al.* 2010) and suppression of TTF-1 expression (Suzuki *et al.* 1999*a*,*c*, Ulianich *et al.* 1999). Therefore, it is possible that different recognition systems, rather than the two different domains of Tg protein, are responsible for the biphasic action.

An important question is how such a large molecule could exert its regulatory action under physiological conditions at the transcriptional level. Several Tg-binding proteins have been described and are thought to be involved in the post-translational modification of Tg or in a cyclical micropinocytotic process: an *N*-acetylglucosamine receptor (Miquelis *et al.* 1987), an asialoglycoprotein receptor (ASGPR; Consiglio *et al.* 1979), and gp330/megalin (Zheng *et al.* 1998). Unfortunately, the Tg-recognition system and the detailed signaling pathways downstream of Tg remain largely unknown.

Physiological cycle of follicular activity regulated by Tg

TSH primarily affects endocytosis, hydrolysis, and secretion of hormones rather than activating gene expression or initiating synthesis and storage of Tg. Resorption of follicular colloid occurs within a short time after TSH stimulation, whereas transcription, synthesis, and secretion of newly formed Tg are quantitatively limited; therefore, the storage space, i.e. the follicular lumen – refills over a longer time frame (Yi *et al.* 1997, Suzuki *et al.* 1999*a*,*c*). This delay is evident in morphological observations after TSH stimulation in which follicles have less colloid and collapsed follicular lumen, while follicles with low TSH stimulation are filled with dense colloid, rich with Tg and thyroid hormones (Wollman & Loewenstein 1973, Yi *et al.* 1997, Suzuki *et al.* 1999*b*). These differences can be readily understood, as resorption and degradation are relatively simple physical and chemical reactions, whereas transcription, translation, and post-translational modification require many other proteins and steps, and therefore, considerably more time and energy. These observations indicate that, although TSH stimulates gene expression of Tg, TPO, and NIS, this TSH-mediated stimulation of gene expression is not enough to refill the follicular stores that were absorbed by follicular cells following TSH action. As a result, the volume of reabsorbed follicular colloid is much larger than the volume resulting from synthesis and secretion through transcription, translation, and protein modification.

Some follicles store a large amount of Tg and thyroid hormones, while others store much less (Suzuki *et al.* 1999*a*,*b*). Tg seems to be the main regulator of thyroid-specific gene expression in individual follicles under relatively constant serum TSH level; therefore, we hypothesize that each follicle has its own synthesis and secretion cycle, depending on the amount of Tg stored in the follicle. We suggest that this cycle is regulated in part by a balance between maximal Tg suppression of gene expression and TSH-regulated resorption of the colloid. TSH increases resorption of the follicular colloid, decreases Tg storage, and releases Tg suppression, thereby allowing TSH-mediated stimulation of gene expression to become dominant (Suzuki *et al.* 1999*a*,*b*,*c*,*d*, Nakazato *et al.* 2000, Kohn *et al.* 2001, Suzuki & Kohn 2006).

In a follicle where large amounts of Tg has accumulated, gene expression is suppressed (Suzuki *et al.* 1998, 1999*d*); therefore, new Tg biosynthesis is low (Fig. 1). In this situation, TSH will act predominantly on resorption of the colloid. On the other hand, in a follicle where Tg concentration is low, negative feedback regulation of Tg is weak, and suppression of gene expression is minimal (Fig. 1). Consequently, TSH can fully activate gene expression and Tg biosynthesis increases. Efficiency of resorption and degradation of follicular Tg will be low because of the low levels of stored Tg in such a follicle. When Tg concentrations

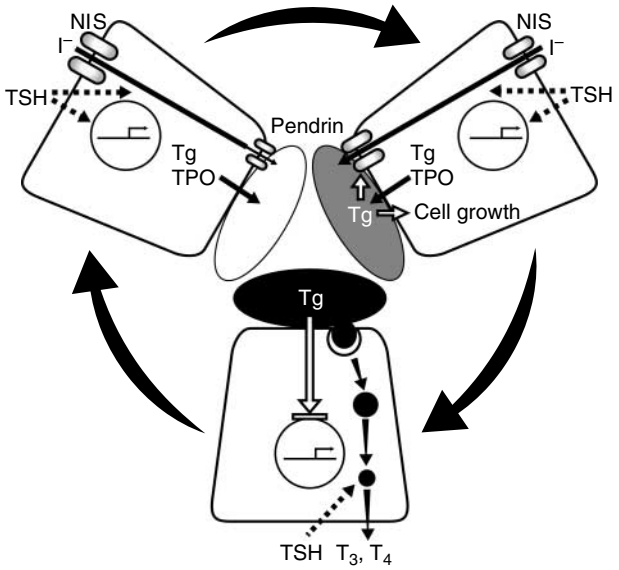

Figure 1 A revised model for the physiological regulation of gene expression, follicular function and cell growth by coordinate regulation of follicular Tg content and serum TSH. In a follicle where Tg accumulation is minimal, thyroid-specific gene expression is at a maximum (left). When a follicle accumulates a low concentration of Tg, maximal PDS gene expression is induced, and iodide efflux reaches a maximal (right). When sufficient Tg accumulates within a follicle (middle), all gene expression necessary for iodide transport and hormone biosynthesis is suppressed. When accumulated follicular Tg decreases to a certain level, Tg suppression of gene expression ceases, and the whole process could repeat.

in the follicular lumen gradually increase with new synthesis, cell growth and pendrin expression are maximally induced (Royaux *et al.* 2000, Suzuki & Kohn 2006, Noguchi *et al.* 2010). These conditions allow efficient transportation of iodide into the follicular lumen and expand the size of the follicle in order to synthesize and store thyroid hormone precursors. When enough Tg accumulates in the follicle, the processes of gene expression, iodide transportation, and cell growth are coordinately suppressed. The follicle with full Tg is ready to be reabsorbed and to secrete thyroid hormones. During the course of decreasing follicular Tg content due to reabsorption, the follicle is supposed to shrink. Although it will be difficult to assess this process *in vivo*, this mechanism will limit follicular size and, therefore, suppress the development of goiters under normal conditions.

One question that remains is whether Tg affects the processes of resorption and degradation of the follicular colloid. It is likely that the processes of resorption and degradation of follicular colloid occur differently in follicles with different levels of Tg. If colloid droplets are absorbed into thyrocytes from follicles with low Tg concentration, the efficiency of thyroid hormone generation is very low. Therefore, it would be reasonable to predict a mechanism in which follicular Tg stimulates the resorption/degradation pathway and the regulation of gene expression.

Pathologic consequences

We suggest the following pathologic consequences, which might be related to abnormalities of Tg-mediated regulatory mechanism and to failure of physiological regulation of follicular function. In cases where Tg suppression is weakened, either by an abnormality of Tg itself or aberrations in the mechanism by which Tg suppresses gene transcription, constant activation of gene expression may occur, resulting in persistent cell growth or accumulation of colloid and enlargement of follicles. This condition might be related to some form of goiter. In cases where Tg suppression is enhanced, gene expression is constantly reduced, which may result in hypothyroidisms and/or compensatory enlargement of follicles. When MHC class I expression is increased by Tg, inflammatory cell infiltration and immune reaction may be induced. Similarly, although the molecular mechanisms underlying Tg-induced growth stimulation are not known, an imbalance or a dysregulation of such a mechanism could relate to aberrant thyroid cell growth. Some of the thyroproliferative disorders with unknown etiology might be due to aberrations in Tg-regulated cell growth.

We have previously shown that Tg prepared from iodide-deficient goiters, colloid adenomas or congenital goiters suppresses thyroid-specific gene expression more effectively than does the Tg prepared from normal tissue (Suzuki *et al.* 1998, 1999*d*). Therefore, poorly iodinated and/or poorly sialylated Tg may induce thyroid cell growth more effectively than fully iodinated and/or sialylated Tg. This possibility is consistent with previous observations that inhibition of iodide coupling is influenced by the iodination and sialylation of the Tg molecule (Studer *et al.* 1986). It is also consistent with the observation that poorly iodinated Tg in nontoxic, multinodular sporadic goiters inhibits TSH-induced Tg utilization from the follicular lumen (Sinadinovic *et al.* 1978).

Tg has strong physiological effects that regulate follicular function and thyroid cell growth. Therefore, abnormality in Tg itself or in a Tg-mediated mechanism may result in pathological conditions in human patients. Although, it is not currently known whether any thyroid disorder is caused by one of these abnormalities, understanding of cellular recognition system of Tg and signaling cascades will lead to a further understanding of potential pathological consequences of these abnormalities.

Conclusion

Follicular Tg, a thyroid hormone precursor, is a potent suppressor of thyroid-specific gene transcription and stimulator of thyroid cell growth. Low levels of Tg in the follicular lumen stimulate cell growth and iodide transport to accelerate the iodide organification process, but elevated Tg levels in the follicle shut down all of these functions. This novel mechanism may be a major autocrine pathway within the

thyroid follicle and may serve as a compensatory function that maintains follicular homeostasis. Furthermore, this negative feedback mechanism may contribute to the heterogeneity of the size and function of individual follicles in the same gland, i.e. regulating follicular function, controlling the amount of colloid within the follicle, and limiting the size and cell growth of each follicle. Studies of the molecular mechanism of Tg action are needed to gain a better understanding of thyroid physiology and pathology.

Fundamental questions remain regarding which part of the Tg molecule is recognized by which mechanism (receptors) and which intracellular signals transduce the Tg signal and elicit these strong physiological effects. To address these questions, we are currently studying the physiological role of a short Tg variant (kTg) that we have recently cloned from kidney glomerulus (Wu *et al*. 2009). We are also analyzing changes in gene expression following Tg stimulation using DNA microarrays. Other efforts are also underway to address some of these concerns.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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