

Invited critical review

Thyroglobulin and human thyroid cancer

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Abstract

Thyroglobulin (Tg) is a large molecule containing 2750 amino acids with a molecular weight of 330 kD and twenty putative *N*-linked glycosylation sites. Tg gene expression is regulated by thyroid transcription factor 1 (TTF-1) and human paired box 8 (Pax-8). Iodinated Tg is stored in the lumen of the thyroid follicles and is released in response to specific hormonal stimulation by thyroid stimulating hormone (TSH). Following Tg reabsorption by thyrocytes and subsequent degradation, thyroid hormones triiodothyronine (T₃) and thyroxine (T₄) are secreted in the bloodstream. Mutations within the Tg gene cause defective thyroid hormone synthesis, resulting in congenital hypothyroidism. Thyroid carcinoma may develop from dysmorphonogenic goiters due to Tg mutation. Post-thyroidectomy Tg levels are apparently associated with prognosis of papillary and follicular thyroid carcinomas and may predict tumor recurrence and metastatic potential. The detection of Tg by biochemical and molecular means has important diagnostic significance due to its pleiotropic roles in identification of tissue of thyroid origin, differentiation, and post-operative follow-up. © 2007 Elsevier B.V. All rights reserved.

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1. Introduction

Thyroglobulin (Tg) is the matrix protein within the thyroid gland that provides the physical backbone for thyroid hormone synthesis. In addition to this important function, Tg

also serves as a specific biomarker of papillary and follicular thyroid cancer and is one the main antigens in autoimmune thyroid disease. A variety of methods have been used to detect Tg expression including immunohistochemical [1,2], cytologic [3], molecular [4,5] and biochemical [6,7]. Quantitative evaluation of Tg expression is highly dependent on detection method, sample type and stage of malignant transformation.

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2. Structure and expression of Tg

Human Tg is composed of 2750 amino acids with molecular weight of 330 kD [8]. Although 20 putative *N*-linked glycosylation sites have been proposed [9], only 16 are glycosylated in the mature protein. The Tg gene is encoded by human chromosome 8q24.2–8q24.3 in a 8.5 kb coding sequence that is distributed over 48 exons [10]. The full-length 8.7 kb mRNA transcript is the most highly expressed mRNA transcript in normal thyrocytes with an expression level of 2.7% (Fig. 1).

Iodinated Tg is stored in the lumen of thyroid follicles. Upon stimulation by thyroid stimulating hormone (TSH), Tg is reabsorbed into thyrocytes and degraded to end product T₃ and T₄ (thyroid hormones) that are subsequently secreted into the bloodstream. Thyroid peroxidase (TPO), the TSH receptor and the sodium/iodide symporter are other important proteins associated with thyroid hormone synthesis. These thyroid-specific proteins also serve as differentiation markers for thyroid follicular cells and play a critical role in the concentration of iodide in thyrocytes. Tissue-specific expression of Tg genes is regulated at the transcriptional level by thyroid transcription factor 1 [TTF-1, also termed thyroid-specific enhancer-binding protein (T/EBP) and NKX 2.1] and human paired box-containing 8 (Pax-8). TTF-1 is a homeodomain-containing protein that binds to and activates all known regulatory sequences of the thyroid-specific genes whereas Pax-8 interacts with the promoter C-site [11].

Simultaneous expression of Pax-8 and TTF-1 within thyrocytes triggers synergistic activation of the thyroid differentiation marker genes. Thyroid hormonogenesis involves iodide uptake by the sodium iodide symporter and the iodination of Tg

catalyzed by thyroid peroxidase in the presence of hydrogen peroxide with subsequent coupling of Tg iodotyrosine residues [12]. Thyroid-specific gene expression is predominantly regulated by TSH and involves TTF-1 (Titf1, NKX 2.1 or T/EBP), Pax-8 as well as another transcription factor TTF-2 (FOXE1) [13,14]. Although present in several tissues [15–18], these transcription factors are only co-expressed in developing and adult thyroid due to their role in the early commitment, differentiation and maintenance of the differentiated thyroid state [13,14]. The Tg promoter responsive to TSH comprises a 207-bp fragment extending from –168 to +39 relative to the transcription initiation site of the gene. The elements necessary for cell type-specific transcription are contained in a smaller fragment extending from –168 to –42. In this region, three TTF-1 binding sites, one TTF-2 binding site and one for Pax-8 binding site overlap with the 3'-sequence recognized by TTF-1, ie, the C-site [19–21].

An apparent discrepancy between Tg mRNA and protein levels after lipopolysaccharide (LPS), ie, endotoxin, treatment was recently observed [22]. TSH-stimulated Tg mRNA level was decreased whereas the Tg protein level was increased by endotoxin. This inverse relationship suggested that the action of LPS on TSH-induced Tg expression was complex and may involve post-transcriptional modifications that could affect Tg mRNA stability as well as Tg translation efficiency, degradation and trafficking. A similar mechanism could be proposed for the apparent lack of parallel changes in Pax-8 and TTF-1 mRNA and protein levels following LPS treatment. Endotoxin decreased TSH-stimulated Pax-8 and TTF-1 mRNA levels, but simultaneously increased Pax-8 and TTF-1 protein levels. These apparent contradictions may be partially explained by increased protein half-life of Tg, Pax-8 and TTF-1. These observations

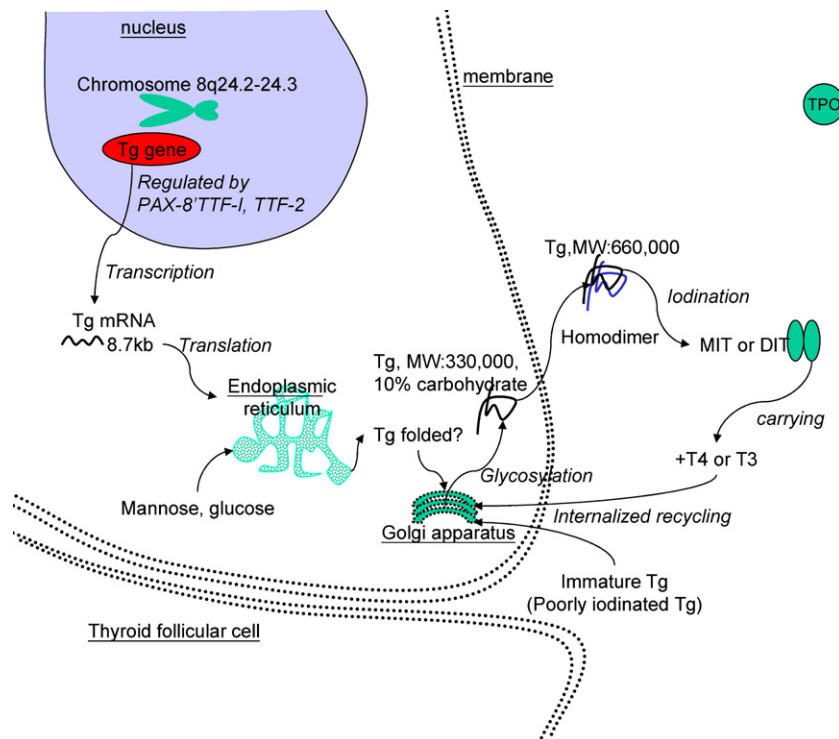


Fig. 1. Gene regulation and synthesis of thyroglobulin (Tg) in normal thyroid follicular epithelial cell.

suggest that LPS may alter post-translational regulation and/or intracellular processing thus leading to increased stability. Similarly, increased TSH-dependent promoter activity and decreased TSH-dependent mRNA accumulation by LPS. Together these observations strongly support a multi-level action for LPS on Tg, Pax-8 and TTF-1 gene expression [22].

3. Tg gene abnormalities

Tg gene mutations cause defective thyroid hormone synthesis resulting in congenital hypothyroidism. Mild hypothyroidism or euthyroidism with goiter are commonly detected within the Tg gene mutation family [23–25]. In fact, a recent review article about animal and human Tg gene mutation was illustrated in Afrikaner cattle (p.R697X), Dutch goats (p.Y296X), *cog/cog* mouse (p.L2263P), rdw rats (p.G2300R) and human thyroglobulin genes associated with congenital goiter or endemic, non-endemic simple goiter and 35 inactivating mutations [26].

Patients may also present with goiter and overt or compensated hypothyroidism, occasionally with high serum triiodothyronine levels [27]. Goiters are often remarkably large and display continuous growth. In most instances, affected individuals have parents who are homozygous for inactivating mutations in the Tg gene. Less common, compound heterozygous mutations lead to a loss of function in both alleles. Molecular analyses indicate that at least some of these alterations result in a secretory defect and an endoplasmic reticulum storage disease [28].

An increasing number of euthyroid to mildly hypothyroid patients with Tg mutations have been identified. A recent study in Japan illustrated 26 different mutations of the Tg gene in 52 patients from 41 families [29]. Thirty-five of these patients had homozygous mutations whereas the others were compound heterozygotes. The occurrence of Tg mutation within the general Japanese population is approximately one in 67,000. The frequently observed mutations, ie, C1058R and C1977S, result from founder effect thus providing a genetic basis for familial euthyroid goiter. A recent study demonstrated congenital goiter and defective Tg synthesis in members of a Brazilian family who were either compound heterozygotes for R277X/IVS34-1G>C or R277X/R1511X [30]. Mutation of Tg gene may display significant intra-allelic heterogeneity.

Thyroid carcinoma developing from dys hormonogenic goiters due to Tg mutation have been reported [24]. It has been suggested that constant and prolonged stimulation by TSH might result in the appearance of thyroid carcinoma. In fact, an early experimental animal study demonstrated that malignant thyroid tumor could develop in mice following prolonged thyroid hormone imbalance due to thiouracil ingestion [31].

4. Clinical applications of Tg detection

Fine needle aspiration (FNA) of thyroid nodules during cytologic examination results in release of Tg into the circulation that can persist for up to 15 days [32]. As such, Tg measurement of serum Tg concentration for detection of local or distant metastases should be performed at least 15 days following this invasive procedure.

4.1. Detection methods

Thyrotropin (TSH)-stimulated Tg levels for thyroidectomized patients without clinical evidence of residual tumor and Tg below 1 µg/L during TSH suppression have been suggested in a surveillance guideline for well-differentiated thyroid cancer (American and European Consensus Conference). Analytical sensitivity is critical for detecting small amounts of Tg and/or observing minimal changes in Tg concentration in the management of papillary and follicular thyroid carcinoma patients. Thus methods are required to provide the greatest discrimination between the lower limit of euthyroid reference range (approximately 3.0 µg/L) and the functional assay sensitivity (at least 1 µg/L) in order to detect residual, but functional thyroid tissue in the TSH-suppressed state. In the last 30 years, analytical sensitivity of Tg assays has greatly improved. Current methods are accurate at extremely low Tg concentration (0.1–1 µg/L). Additionally, with the introduction of fully automated assays, results are readily available to clinicians. Despite this progress, significant limitations exist in Tg analysis including lack of standardization, heterogeneity of circulating Tg, interference from auto-antibodies and differences in epitope recognition by antibodies used in the various assays [33].

5. Use of Tg in thyroid nodules and cancer

5.1. Serum Tg level

An early prospective study that evaluated the role of Tg in thyroid nodules demonstrated that Tg level was significantly increased in thyroid cancer patients vs patients with benign disorders [34]. The predictive value of the test was, however, inadequate. An European survey of diagnosis and treatment of solitary thyroid nodules concluded that the favored diagnostic strategy in the workup of patients with a solitary thyroid nodule included determination of serum TSH combined with serum T₄ and/or free T₄ followed by FNA and ultrasound together with scintigraphy. A majority of survey respondents supported a non-surgical strategy using T₄. In cases where clinical factors increased the likelihood of malignancy, the majority of respondents recommended that diagnostic thyroidectomy be performed even if the results of the FNA suggested a benign condition [35]. Before surgery, Tg did not have an important role in differential diagnosis of benign and malignant lesions. Furthermore, Tl-201 scintigraphy has proven more useful than measurement of Tg for predicting malignant thyroid follicular lesions [36]. However, in other studies, Tg level in thyroid cystic fluid and very high serum Tg level were deemed useful in the diagnosis of thyroid malignancy [37,38].

It is well known that during malignant transformation, epithelial cells modulate the glycosylation profile of their secretion products and that these post-translational changes may represent exploitable diagnostic/prognostic markers, which may provide important tools for elucidating molecular mechanisms responsible for the progression of these tumors. Previous studies of thyroid neoplasms have identified an abnormality in papillary thyroid carcinoma that consisted of poly-*N*-acetylactosamines

glycans carrying 6-*O*-sulfations on some galactose or *N*-acetylglucosamine units and sialic and/or fucosyl groups at non-reducing termini [39,40]. Preliminary studies used *Lens culinaris* agglutinin-reactive Tg ratios in serum produced promising results in the ability to detect normal thyroid, thyroid cancer with and thyroid cancer without lymph node metastases [41]. In cases of thyroid carcinoma with lymph node metastasis, lens culinaris agglutinin-reactive Tg ratios were significantly decreased in patients with thyroid carcinomas without metastasis vs patients with benign tumor regardless of serum Tg concentration.

Post-operative Tg levels have been associated with prognosis of papillary and follicular thyroid carcinomas [7,42]. Tumor recurrence and prediction of distant metastases have also been examined [43–45]. As can be expected, remnant thyroid tissue, cancer cell differentiation and other factors may influence serum Tg concentration. Table 1 shows the main factors influencing the use of Tg as a predictor of tumor recurrence and metastases. Meta-analysis illustrated that Tg-guided follow-up in patients treated for papillary and follicular thyroid carcinomas was most accurate if treatment included remnant ablation and if Tg testing was performed when the patient was off exogenous thyroxine treatment.

Different methods were used to correct for remnant tissue influencing Tg levels [46,47]. The ratio of Tg to ^{131}I uptake measured before the first ^{131}I treatment was used as initial dose of ^{131}I [45]. In that study, using threshold ratio 7 ng/mL/% as the upper limit of normal value provided useful information with higher sensitivity and specificity in identifying patients with metastatic disease. The added sensitivity allowed selective use of higher initial doses in iodine therapy. After total thyroidectomy and ^{131}I for remnant ablation, increased rate of serum Tg concentration obtained after thyroid hormone withdrawal appeared to provide the highest positive predictive value [47]. Sequential follow-up of Tg levels after total thyroidectomy were, however, considered to be more informative for thyroid cancer recurrence [48]. Positive predictive values of 62.5% and 16.6% were associated with Tg serum changes of $>10\ \mu\text{g/L}$ and $<5\ \mu\text{g/L}$, respectively. Decreased Tg may be associated with rapid deterioration of disease as well as tumor dedifferentiation. In this study, most patients with detectable stimulated Tg during the first year after operation exhibited no metastases. Evaluation of the rate of Tg change using sequential assessment discriminated cases with apparent disease.

Table 1
Factors influence thyroglobulin (Tg) used in papillary and follicular thyroid carcinomas

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1. Thyroid tissue remnant
 2. Thyroxine use or not
 3. rhTSH stimulation
 4. Low-risk or high risk patients
 5. Histological patterns
 6. Combination with other examination tools: ultrasound, PET, ^{131}I scans
 7. Cancer cell differentiation
 8. Tg assay methods
 9. Presence of anti-thyroglobulin antibody
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A multicenter international study demonstrated that serum Tg measurements alone were not as sensitive in identifying patients with persistent or recurrent tumor vs recombinant human TSH (rhTSH)-stimulated serum Tg determinations [49]. A single recombinant human thyrotropin-stimulated serum Tg measurement was shown to accurately predict differentiated thyroid carcinoma metastases. A serum Tg concentration $<0.5\ \text{ng/mL}$ without Tg antibody has an approximate 98% likelihood of identifying patients who were completely tumor-free [43].

Hypothyroid status after thyroxine withdrawal exhibited a longer stimulation time for cancer cells in distant metastases compared with rhTSH. In a small group of patients with well-differentiated thyroid cancer and metastatic disease, the effectiveness of radioiodine therapy following rhTSH was anticipated to be less than that in hypothyroid patients after levothyroxine withdrawal [50].

One study suggested that any rise in rhTSH stimulating Tg (rhTSH Tg), even at a low level, should raise suspicion of persistent or recurrent differentiated thyroid cancer [51]. Patients with rhTSH Tg at high levels should be carefully evaluated because persistent differentiated thyroid cancer is highly probable. The TSH-WBS provided little adjunctive information in 104 patients who had previously undergone near-total thyroidectomy and ^{131}I ablation for differentiated thyroid carcinoma. rhTSH Tg test results were considered negative ($<0.9\ \text{ng/mL}$), low positive (1–5 ng/mL) and high positive ($>5\ \text{ng/mL}$). Testing for rhTSH Tg was negative in 70 patients, one of whom had a lymph node metastasis, but no ^{131}I uptake. Seven patients had low positive rhTSH Tg tests and no ^{131}I uptake, but two of these patients had cervical lymph node metastases. Twenty-seven patients had high positive rhTSH Tg tests and ^{131}I uptake was detected in lung, bone or mediastinum in eleven of these [51]. In total thyroidectomy cases, increased Tg in the rhTSH Tg test was easier to interpret. In the lobectomy cases, subtotal thyroidectomy cases and patients not receiving thyroid remnant ^{131}I ablation, the significance of elevated Tg levels was unclear. Investigations in follow-up post-operative well-differentiated thyroid carcinoma patients were reported by using a high-sensitive Tg assay. Tg assay with improved sensitivity could eliminate the need for rhTSH stimulation when baseline Tg was below $0.1\ \text{ng/mL}$ [52]. Tg assay under thyroxine treatment by a high-sensitivity method combined with neck ultrasound may avoid the need for rhTSH stimulation in low-risk papillary and follicular thyroid carcinoma patients after surgery and radioiodine thyroid ablation [53].

Papillary microcarcinomas that have not undergone radioiodine therapy neck ultrasound need to be used with Tg levels in order to better predict tumor recurrence [54]. Venous gradient in Tg concentration by venipuncture has been performed simultaneously from the internal jugular vein adjacent to the tumor and the ipsilateral antecubital vein [55]. Venous sampling for Tg has demonstrated localization in some papillary and follicular thyroid cancer patients with high or increased serum Tg concentration but negative radioiodine scans or imaging studies.

Localization cases with elevated Tg and negative ^{131}I whole body scanning presents another difficult situation in clinical practice. Test results for (18)F-FDG PET/CT were true-negative

in 19 patients and false-negative in 12 patients [56]. Overall sensitivity, specificity and accuracy using (18)F-FDG PET/CT were 68.4%, 82.4%, and 73.8%, respectively. The sensitivity of (18)F-FDG PET/CT at serum Tg concentration <5 ng/mL, 5–10 ng/mL, and >10 ng/mL were 60%, 63% and 72%, respectively.

5.2. Tissue Tg expression

Examination of Tg expression in tissues by biochemical or molecular analysis is widely used for thyroid cancer differentiation. Because of its importance as a differentiation marker in thyroid follicular cells, papillary thyroid carcinomas and follicular thyroid carcinomas, presence of Tg in cancer cells indicates thyroidal origin. Protein expression from genetic level to mRNA and Tg immunochemical staining vary widely in different thyroid cancer tissues. Studies from lymph node aspiration washout fluid, cytology or tissues obtained from distant metastases indicate Tg expression may be diagnosed as thyroid origin [57–59]. Cases of thyroid microcarcinoma have been reported where tumor size was <5 mm and otherwise difficult to diagnose pre-operatively [60]. Cystic degeneration of papillary thyroid carcinoma metastases to lymph node was not unusual [61]. Obtaining sufficient cells through FNA from the cystic lymph node is often difficult. Detection of Tg in the aspirated fluid with negative cytology may confirm a diagnosis of papillary thyroid cancer [62].

According to previous studies, Tg mutation may occur during thyroid tumorigenesis [25]. Morphologic or functional changes that alter Tg concentration may decrease effectiveness of immunohistochemical staining with anti-Tg antibodies. As such, Tg mRNA expression combined with immunochemical staining have been used for prognosis of well-differentiated thyroid cancers [63–65]. Compared to sodium iodide symporter, most papillary and follicular thyroid cancers lose iodide-trapping ability prior to changes in Tg gene expression. Furthermore, unusual cases of thyroid cancer lacking Tg expression with concentrated radioiodine have been reported [65]. Enhanced Tg expression has been applied in *in vitro* cancer cell studies as a modality of differentiated therapy [66,67].

6. Conclusions

The detection of Tg by biochemical and molecular means has important diagnostic significance due to its pleiotropic roles in identification of tissue of thyroid origin, differentiation, and post-operative follow-up. Tg may also serve as a potential prognostic factor. Further research should focus on the identification of specific mutant Tg forms and the development of highly specific and sensitive assay methods.

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