Review

Thyroid Transcription Factor-1 (TTF-1)

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Thyroid transcription factor 1 (TTF-1), as noted earlier, is a “thyroid specific” transcription factor that is a homeodomain-containing protein responsible for transcriptional activation of thyroid-specific genes, thyroglobulin (Tg), thyroperoxidase (TPO), and thyrotropin receptor (TSHr) genes, in thyroid follicular cells. Little is known about the histological localization or distribution of TTF-1 protein and mRNA, even though many papers on TTF-1 have been published in the past ten years. Therefore, we decided to examine the expression of TTF-1mRNA in

normal and neoplastic thyroid tissues using immunohistochemistry and *in situ* hybridization as well as molecular techniques such as Northern blot and RT-PCR. In addition, extrathyroidal expression of TTF-1 was also investigated in some organs. In this paper, we reviewed literature and discussed the role of TTF-1 in thyroid hormonogenesis, and presented its precise localization or distribution in normal and neoplastic thyroid tissues. In addition, extrathyroidal expression of TTF-1 is briefly discussed.

Key words: Thyroid transcription factor 1, Review, TTF-1, Thyroid, *In situ* hybridization

I. General Considerations

Synthesis and secretion of thyroglobulin (Tg), thyroperoxidase (TPO) and thyrotropin receptor (TSHr) are characteristics of the thyroid; nevertheless, the regulation and role of each are different. The thyroid-specific gene expression of these proteins is regulated at the transcriptional level and the promoters of all three genes have been studied [4]. Thyroid specific transcription factors, thyroid transcription factor (TTF)-1, TTF-2, Pax-8, have been identified from this analysis. TTF-1 is a homeodomain-containing transcription protein able to bind specific DNA sequences [7]. It binds all three thyroid-specific promoters, Tg, TPO and TSHr gene promoters, and is able to activate their transcriptional activity [2, 5, 14]. Therefore, TTF-1 plays a pivotal role in maintenance of the thyroid differentiated phenotype. In addition,

recently we found that major histocompatibility complex (MHC) class I gene expression is also regulated by TTF-1 and Pax 8 in the thyroid [17].

TTF-1 protein has been detected very early during thyroid development; 5 days before the appearance of Tg and TPO mRNAs [12]. It has therefore, been proposed that TTF-1 may also have a role in thyroid cell commitment [12]. A knockout study established that TTF-1 is essential for the organogenesis of the lung, ventral forebrain, and pituitary, as well as the thyroid [11]. TTF-1 is, therefore, not thyroid-specific but thyroid-restricted. In the lung, TTF-1 is a transcription factor important in the expression of surfactants [6, 8]; the role of TTF-1 as a transcription factor regulating neural function is unknown.

II. Structure of Thyroid-Specific Promoters

The TTF-1 site in the Tg, TPO and TSHr genes is important for their expression (Fig. 1). The sequence extending 170 bp upstream from the rat TG gene has

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Fig. 1. Structure of thyroglobulin (Tg), thyroperoxidase (TPO) and thyrotropin receptor (TSHr) promoters. Symbols refer to binding sites mapped by DANse 1 footprinting. The structure of these promoters is drawn from data of reference no.1.

three TTF-1 binding sites, two of which are important for promoter activity [3, 5, 19]. TTF-1 also interacts with three binding sites on the rat TPO promoter, which are located in positions similar to those in the TG promoter [5, 19]; multiple TTF-1 sites are also needed for expression of the TPO gene. Additionally, 5.5 kb upstream from the transcription start site, the human TPO gene has a thyroid-specific enhancer region that contains at least two additional TTF-1 binding sites [10, 13]. Pax-8, a paired-domain-containing protein whose binding site overlaps with a TTF-1 binding site, is involved in both TG and TPO gene regulation [19]. A role for the TSH receptor

(TSHr)/TTF-1 binding element in thyroid-specific expression of the TSHr gene as well as TPO and TG was evidenced [15]. However, its role is very different in TSHr expression than in expression of the TG and TPO gene.

III. Distribution of TTF-1 in Thyroid Tissue

Little is known about the localization and distribution of TTF-1 protein and mRNA, even though many papers on TTF-1 have been published in the past ten years. Our immunohistochemical study with antibody for TTF-1 (a kind gift from Dr. Kimura, NIH, USA) revealed nuclear

Fig. 2. **A:** Immunofluorescence staining shows nuclear localization of TTF-1 protein in rat thyroid follicular cells. $\times 200$. **B:** Expression of TTF-1 mRNA is demonstrated in human thyroid follicular cells by non-isotopic *in situ* hybridization. $\times 200$.

localization in most follicular cells of the rat thyroid (Fig. 2A). On *in situ* hybridization study in human thyroid tissue, the expression of TTF-1 mRNA was heterogeneous among follicles; there was a tendency of strong expression of TTF-1 mRNA in small follicles (Fig. 2B).

IV. Expression of TTF-1 in Thyroid Tumors

It is generally accepted that most thyroid tumors can produce Tg and some of them produce thyroxine (T_4) and triiodothyronine (T_3) [8]. In this respect, it could be worth examining the expression of the TTF-1 gene, together with the production of its target protein Tg and TPO, both in normal and neoplastic human thyroid tissues. Expression of TTF-1 mRNA in thyroid tumors has been studied by Northern blot analysis [6]. As TTF-1 mRNA is always well detectable in papillary carcinomas and, in contrast, always absent in anaplastic carcinomas, it is suggested that TTF-1 gene expression could be a molecular marker in order to distinguish these two types of thyroid neoplasms. We examined thyroid tumors by RT-PCR, Northern blot, *in situ* hybridization and immunohistochemistry to estimate the expression of TTF-1. *In situ* hybridization revealed the localization of TTF-1 mRNA in neoplastic cells of all histological type of thyroid tumors including follicular adenoma (Fig. 3A), follicular carcinoma, papillary carcinoma (Fig. 3B), and undifferentiated carcinoma. This result was supported by RT-PCR and Northern blot analysis. Interestingly, some thyroid tumors did not show the localization of thyroid-specific proteins such as Tg, T_4 , T_3 and TPO, but they showed the expression of TTF-1. Therefore, it could be reasonable that investigation of TTF-1 provides useful information on the function of thyroid tumors. This may lead to an increase in our understanding of the biological nature of the thyroid tumor and could be the most fundamental marker of thyroid origin.

V. TTF-1 Expression in Other Organs

TTF-1 is also expressed in the embryonic forebrain and in the respiratory epithelium of the rat, appearing in fetal lung buds of embryonic rat lung on postconceptional day 9 or 10 [12]. A potential role for TTF-1 in lung epithelial cell gene expression was recently demonstrated by the finding that TTF-1 activates the transcription of human surfactant protein B gene [1]. TTF-1 binds to two closely apposed TTF-1 DNA binding sites located -80 to -100 base pairs from the start of surfactant B gene transcription. We demonstrated the expression of TTF-1 mRNA in the lung and brain as well as thyroid by RT-PCR (Fig. 4).

In the course of *in situ* hybridization studies examining TTF-1 expression in adult rat tissues, we have identified TTF-1 mRNA not only in thyroid follicular cells, but also in thyroid C cells and parathyroid cells. *In situ* hybridization additionally identified TTF-1 mRNA in other rat tissues not hitherto recognized to express this

Fig. 4. RT-PCR analysis demonstrates the expression of TTF-1 mRNA in brain and lung as well as thyroid.

Fig. 3. A: Neoplastic cells of follicular adenoma express TTF-1 mRNA demonstrated by non-isotopic *in situ* hybridization. $\times 200$. B: Papillary carcinoma shows expression of TTF-1 mRNA in neoplastic cells. $\times 200$.

gene: basal layer cells of flattened squamous epithelium in skin and esophagus, in all three layers of the retina, i.e. pigmented epithelium, inner granular layer, and ganglion cell layer, and in two areas of the brain, the cerebellum and hippocampus [16]. Recently, we found the expression of TTF-1 mRNA in pituitary by RT-PCR and *in situ* hybridization (unpublished data).

These findings suggest the possibility that TTF-1 may have a role in the regulation of different genes in different tissues.

VI. References

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