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Research paper

Interdependence of thyroglobulin processing and thyroid hormone export in the mouse thyroid gland

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ABSTRACT

Thyroid hormone (TH) target cells need to adopt mechanisms to maintain sufficient levels of TH to ensure regular functions. This includes thyroid epithelial cells, which generate TH in addition to being TH-responsive. However, the cellular and molecular pathways underlying thyroid auto-regulation are insufficiently understood. In order to investigate whether thyroglobulin processing and TH export are sensed by thyrocytes, we inactivated thyroglobulin-processing cathepsins and TH-exporting monocarboxylate transporters (Mct) in the mouse. The states of thyroglobulin storage and its protease-mediated processing and degradation were related to the levels of TH transporter molecules by immunoblotting and immunofluorescence microscopy. Thyroid epithelial cells of cathepsin-deficient mice showed increased Mct8 protein levels at the basolateral plasma membrane domains when compared to wild type controls. While the protein amounts of the thyroglobulin-degrading cathepsin D remained largely unaffected by Mct8 or Mct10 single-deficiencies, a significant increase in the amounts of the thyroglobulin-processing cathepsins B and L was detectable in particular in Mct8/Mct10 double deficiency. In addition, it was observed that larger endo-lysosomes containing cathepsins B, D, and L were typical for Mct8- and/or Mct10-deficient mouse thyroid epithelial cells. These data support the notion of a crosstalk between TH transporters and thyroglobulin-processing proteases in thyroid epithelial cells. We conclude that a defect in exporting thyroxine from thyroid follicles feeds back positively on its cathepsin-mediated proteolytic liberation from the precursor thyroglobulin, thereby adding to the development of auto-thyrotoxic states in Mct8 and/or Mct10 deficiencies. The data suggest TH sensing molecules within thyrocytes that contribute to thyroid auto-regulation.

1. Introduction

Thyroid dysfunctions caused by altered thyroid hormone (TH)[2] generation or disturbed TH action are considered to be among the most frequent endocrine disorders (Brix et al., 2011; Führer et al., 2015). TH are of crucial importance for the proper functioning of nearly every organ, including the thyroid gland itself. Hence, all TH target cells need to adopt mechanisms to maintain sufficient levels of TH to ensure regular functions. Since the thyroid gland itself not only produces TH but also responds to TH levels in a self-sustaining manner, auto-protection of the thyroid gland is vital not only for thyrocytes but also for all other TH-dependent cells. Proper thyroid states of an organism are centrally regulated by the hypothalamus-pituitary-thyroid (HPT)–axis, whereby low serum TH levels trigger hypothalamic thyrotropin-releasing hormone (TRH) release which then promotes release of thyroid stimulating hormone (TSH) from the pituitary (Fliers et al., 2014), thereby triggering TH generation in the thyroid gland, and TH release into the blood.

To fulfill and maintain its tasks, the thyroid gland is composed of functional units, the so-called thyroid follicles, that are built by

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[2] Abbreviations: CMF-PBS, calcium- and magnesium-free phosphate buffered saline; Cy3, indocarbocyanine; HPT, hypothalamus-pituitary-thyroid; Mct, monocarboxylate transporters; PTC, papillary thyroid carcinoma; Tg, thyroglobulin; TH, thyroid hormones; TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; WT, wild type.

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Table 1
Specific functions of different cathepsins expressed in the thyroid regarding their processing of Tg.

	extracellular Tg solubilisation from cross-linked storage forms	extracellular Tg processing	intracellular limited Tg processing (endosomal)	intracellular Tg degradation (lysosomal)	direct T_4 liberation from Tg	exhaustive Tg degradation for Tg utilization (endo-lysosomal TH liberation)	References
Cathepsin B	x	x	x	x		involved to major extent	Brix et al., 1996; Dunn et al., 1996; Tepel et al., 2000; Friedrichs et al., 2003; Jordans et al., 2009
Cathepsin D				x		involved to minor extent	Brix et al., 1996; Dunn et al., 1996; Friedrichs et al., 2003
Cathepsin K		x	x		x		Tepel et al., 2000; Friedrichs et al., 2003; Jordans et al., 2009
Cathepsin L	x	x	x	x		involved to major extent	Brix et al., 1996; Dunn et al., 1996; Tepel et al., 2000; Friedrichs et al., 2003; Jordans et al., 2009

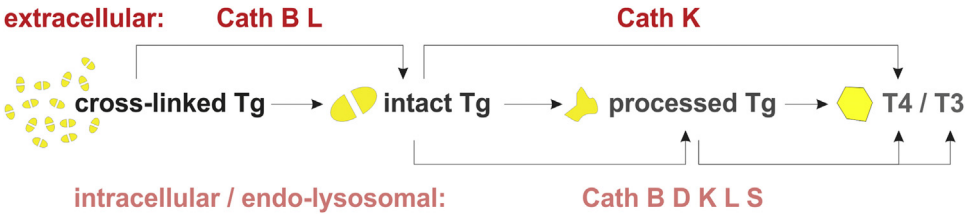

Fig. 1. Cathepsin-mediated solubilization, processing, and degradation of Tg.
Schematic drawing summarizing the sequential steps of Tg processing and degradation by endo-lysosomal cathepsins (Brix et al., 1996; Tepel et al., 2000; Brix et al., 2001; Linke et al., 2002a; Friedrichs et al., 2003; Jordans et al., 2009; Dauth et al., 2011). Cathepsins B and L involve in extracellular Tg solubilization from its covalently cross-linked storage forms, while cathepsin K is instrumental in Tg utilization for T4 liberation. Upon re-internalization of intact and partially degraded Tg, it is further processed and degraded by aspartic and cysteine cathepsins within endo-lysosomes of thyrocytes.

a monolayer of thyroid epithelial cells (Fujita, 1988; Nilsson and Fagman, 2013). The macromolecular TH precursor thyroglobulin (Tg) is stored in covalently cross-linked form in the extracellular thyroid follicle lumen (Herzog et al., 1992; Berndorfer et al., 1996; Klein et al., 2000; Saber-Lichtenberg et al., 2000). Covalently cross-linked Tg is processed for solubilization and degradation by proteases, which are secreted in a TSH-regulated fashion into the extra-cellular follicle lumen upon a demand of TH in the body periphery (for reviews, see Brix et al., 2001; Dauth et al., 2011). Thus, solubilization of Tg from its covalently cross-linked storage forms is enabled through limited, extracellular protein processing facilitated by cathepsins B and L, while TH liberation from Tg is mediated by cathepsins K and L, and also initiated in the thyroid follicle lumen (Brix et al., 1996; Linke et al., 2002a; Friedrichs et al., 2003; Tepel et al., 2000). Subsequently, partially degraded Tg is internalized and reaches endo-lysosomal compartments, where T_3 and T_4 liberation is completed for Tg utilization through exhaustive proteolysis mediated by a variety of hydrolytic enzymes including aspartic and cysteine cathepsins B, D, K, L, and S (Brix et al., 1996; Tepel et al., 2000; Linke et al., 2002a; Friedrichs et al., 2003; Jordans et al., 2009). Cathepsin K is special among the Tg-processing proteases as it is able to liberate T_4 directly from Tg (Tepel et al., 2000). In brief summary, thyroid functions are enabled by sequential proteolytic processing of Tg (Table 1, Fig. 1), resulting in the liberation of T_4 and, to a lower extent, T_3.

Considering that thyroid epithelial cells need to release TH through transmembrane translocation into the blood circulation, and keeping in mind that thyrocytes are TH target cells themselves, a regulatory mechanism must exist that determines how much of the T_4 liberated from Tg is either retained in the thyrocytes or transported back into the same. We propose that intra-thyroidal TH transporters like the monocarboxylate transporter molecules Mct8 and Mct10 may act as such sensors of (i) the extent of proteolytic TH liberation, (ii) the amounts of TH released from thyroid follicles, and (iii) the levels of re-uptake of circulating TH back into thyroid follicles.

Mice with a global Mct8-deficiency (Mct8 knock-out mice) exhibit central TH-resistance, *i.e.* TRH levels are dramatically elevated despite highly increased serum T_3 levels, whereas serum T_4 levels are decreased, and TSH is slightly elevated (Dumitrescu et al., 2006; Trajkovic et al., 2007; Wirth et al., 2011). This very unusual thyroid status (Table 2) is in part explained by the fact that Mct8 is localised at the basolateral plasma membrane domain of thyroid epithelial cells, and thus, its functional absence in mice leads to diminished T_4 export from thyroid follicles (Trajkovic-Arsic et al., 2010a,b; Di Cosmo et al., 2010). Moreover, intra-thyroidal levels of non-Tg-bound and Tg-bound TH are 2- to 3-fold elevated over WT in Mct8-deficient mice (Di Cosmo et al., 2010; Trajkovic-Arsic et al., 2010b; Liao et al., 2011; Muller et al., 2014), which also feature enlarged thyroid follicles, enhanced thyroid epithelial extensions, and develop pathological abnormalities like papillary structures characterized by nuclear alterations, in particular, at older age (Wirth et al., 2011). Consequently, the Mct8-deficient mouse thyroid gland is considered to be characterized by autothyrotoxic as well as thyrotoxic states affecting peripheral TH target organs (for review, see Müller and Heuer, 2014; Heuer and Visser, 2013). The transporter Mct10 (Slc16a10/Tat1) is expressed to much lower amounts in the thyroid gland but in a pattern similar to Mct8; however, a lack of Mct10 alone did not alter the serum TH states in comparison to wild type mice (Mariotta et al., 2012; Muller et al.,