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A resistance to thyroid hormone syndrome mutant operates through the target gene repertoire of the wild-type thyroid hormone receptor

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ABSTRACT

Thyroid hormone receptors (TRs) play crucial roles in vertebrates. Wild-type (WT) TRs function primarily as hormone-regulated transcription factors. A human endocrine disease, Resistance to Thyroid Hormone (RTH)-Syndrome, is caused by inheritance of mutant TRs impaired in the proper regulation of target gene expression. To better understand the molecular basis of RTH we compared the target genes regulated by an RTH-TRβ1 mutant (R429Q) to those regulated by WT-TRβ1. With only a few potential exceptions, the vast majority of genes we were able to identify as regulated by the WT-TRβ1, positively or negatively, were also regulated by the RTH-TRβ1 mutant. We conclude that the actions of R429Q-TRβ1 in RTH-Syndrome most likely reflect the reduced hormone affinity observed for this mutant rather than an alteration in target gene repertoire. Our results highlight the importance of target gene specificity in defining the disease phenotype and improve our understanding of how clinical treatments impact RTH-Syndrome.

1. Introduction

Thyroid hormones (chiefly T3 tri-iodothyronine and T4 tetra-iodothyronine) play multiple crucial roles in vertebrate differentiation and homeostasis, including control of overall metabolic rate, body temperature, neural development, heart rate, color vision, and hearing (Forrest and Vennstrom, 2000; Larsen et al., 2003; Mullur et al., 2014; Yen, 2015). Thyroid hormones manifest most of these actions by binding to thyroid hormone receptors (TRs): members of the nuclear receptor family of ligand-regulated transcription factors (Brent, 2012; Cheng et al., 2010; Flamant et al., 2006). Three major TR isoforms, denoted TRα1, TRβ1, and TRβ2, are encoded from distinct genetic loci and by alternative splicing; they are expressed at different abundances in different tissues and play distinct biological roles (Chan and Privalsky, 2009a,b; Cheng, 2005a,b; Forrest and Vennstrom, 2000; Lazar, 1993; Lin et al., 2013).

TRs are thought to function primarily through their ability to bind to specific target genes and to regulate gene expression either up or down in response to binding of thyroid hormone (Cheng et al., 2010; Flamant et al., 2006; Viguerie and Langin, 2003; Zhang and Lazar, 2000). The best understood of these target genes display "positive regulation" by thyroid hormone. TRs bound to these genes recruit auxiliary proteins, denoted corepressors, in the absence of hormone and repress transcription (Astapova and Hollenberg, 2013; Moehren et al., 2004; Privalsky, 2004; Zhang and Lazar, 2000). Conversely in the presence of thyroid hormone the TRs release corepressors, bind a distinct set of coactivators, and induce gene expression (Brent, 2012; Feige and Auwerx, 2007; Stashi et al., 2014). However, additional, less well-elucidated modes of transcriptional regulation by TRs have also been observed, including the "negative regulation" of target genes that are induced in the absence of thyroid hormone yet repressed in its presence (e.g. (Abel et al., 2001; Chan and Privalsky, 2009a,b; Feng et al., 2000; Furumoto et al., 2005; Gloss et al., 2005; Kim et al., 2005; Lin et al., 2013; Miller et al., 2004; Moehren et al., 2004; Nakano et al., 2004; Ortiga-Carvalho et al., 2005; Viguerie and Langin, 2003; Wang et al., 2009; Weitzel et al., 2003; Yen et al., 2003)). Not only the directionality, but also the magnitude and hormone-sensitivity of the T3-mediated gene response differs at different target genes (e.g. (Chan and Privalsky, 2009a,b; Chatonnet et al., 2014; Cheng et al., 2010; Lin et al., 2013)). These gene-specific regulatory effects by TRs are likely due to recruitment of distinct sets of coregulatory proteins when the receptor is bound to different genes and/or interactions of the TRs with additional *trans*-acting factors co-assembled on each given target gene (e.g. (Ayers

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et al., 2014; Wang et al., 2010)).

Human Resistance to Thyroid Hormone (RTH)-Syndrome is a genetic disorder most often resulting from inherited mutations in TRβ1 (Brent, 2012; Chatterjee, 2008; Cheng, 2005a,b; Dumitrescu and Refetoff, 2013; Jameson, 1994; Weiss et al., 1997). RTH-TRβ1 mutants typically have sustained base substitutions within their hormone binding and/or transcriptional regulatory domains that render them defective in the ability to bind and/or respond to hormone. As a result RTH-TRs typically bind corepressors in the absence of T3, yet fail to release these corepressors and/or acquire coactivators properly on addition of T3 (Privalsky, 2008; Safer et al., 1998; Yen, 2003; Yoh et al., 1997). When coexpressed with WT-TR, RTH-TRs can act as dominant negatives and attenuate WT-TR function in response to T3 (Collingwood et al., 1994; Hayashi et al., 1995; Jameson, 1994; Matsushita et al., 2000; Miller et al., 2004; Wondisford, 2003). Two broad categories of clinical RTH have been proposed: generalized (GRTH) and pituitary-specific (PRTH) (Chatterjee, 2008; Cheng, 2005a,b; Jameson, 1994; Olateju and Vanderpump, 2006; Refetoff and Dumitrescu, 2007; Wondisford, 2003; Yen, 2003). GRTH manifests as a broad failure to respond to circulating T3, resulting in a loss of both negative feedback on T3 synthesis in the hypothalamus/pituitary/thyroid axis (HPT) and of T3 sensing in the peripheral tissue; GRTH mimics several of the symptoms of hypothyroidism despite the presence of elevated T3, TSH, and TRH levels. In contrast, PRTH reflects a selective loss of negative feedback sensing of T3 in the HPT axis (leading to large increases in circulating T3) but with significant retention of T3 responsiveness in the periphery; PRTH therefore mimics certain symptoms of hyperthyroidism. The divergent PRTH versus GRTH phenotypes likely reflect differences in the ability of a given RTH-TRβ mutant to interact with coactivators and corepressors when expressed as the TRβ2 isoform (primarily in the hypothalamus and pituitary) versus as the TRβ1 isoform (primarily in peripheral tissues) (Lee et al., 2011; Machado et al., 2009; Wan et al., 2005).

Significantly, several outwardly similar dominant-negative TRβ1 (and TRα1) mutants are associated not with endocrine disorders but with two forms of human neoplasia: hepatocellular carcinoma (HCC) and renal clear cell carcinoma (RCCC) (Kamiya et al., 2002; Lin et al., 2001). Most of these neoplasia-associated TRα1 and TRβ1 mutants have lesions not only in their hormone-binding/transcriptional regulatory regions, but also in regions able to alter their DNA recognition specificity (Chan and Privalsky, 2006; Rosen et al., 2011). Apparently as a result, the HCC and RCCC TR mutants display target gene repertoires distinct from those of the corresponding WT-TR isoforms (Chan and Privalsky, 2006, 2009, Chan and Privalsky, 2009a,b; Chan and Privalsky, 2006; Rosen et al., 2011). We proposed that the HCCe and RCCC-TR mutations alter both the transcriptional properties and target gene recognition of the encoded TRs, resulting in novel gene repertoires that lead to an aberrant regulation of oncogenes and/or tumor suppressors not normally regulated by the WT-TRs and thereby contributing to the neoplastic outcomes associated with these particular mutants. In contrast we suggested that the RTH-TR mutations change the transcriptional properties/hormone binding of the encoded receptor, but not its DNA recognition or gene repertoire, leading to the endocrine disruptions characteristic of RTH-Syndrome.

Importantly the target gene repertoires of the RTH-TRs compared to the WT-TRs were not previously investigated comprehensively, and our prior, very limited qRT-PCR analysis raised the possibility that even the RTH-mutants might differ in certain of their target gene preferences from those of the WT-receptors (Rosen et al., 2011). Given that such gene-specific aberrations would have profound consequences for understanding and treating the RTH-Syndrome phenotype, we returned to this question by using RNA-seq to more broadly survey target gene regulation by an RTH-TRβ1 mutant versus by WT-TRβ1 (Fig. 1A). We focused our initial analysis on R429Q-TRβ1, a mutant associated with PRTH and possessing several altered molecular properties that potentially might impact on its target gene repertoire (Adams et al., 1994; Clifton-Bligh et al., 1998; Collingwood et al., 1994; Kong et al., 2005; Machado et al., 2009; Safer et al., 1997). We report here that the WT-TRβ1 displayed a variety of gene-specific regulatory properties, both repressive and activating, by this analysis and that within the statistical limitations of our study the R429Q-TRβ1 mutant displayed virtually the same regulatory properties and target gene repertoire as did the WT-TRβ1 on the genes surveyed here. This was observed both in the absence of added T3 and in the presence of saturating hormone. We propose that the R429Q-TRβ1 mutant retains the inherent potential to regulate most of the same genes as does WT-TRβ1 at very high T3 levels, but contributes to RTH-Syndrome *in vivo* primarily due to its impaired ability to bind and respond to the lower T3 concentrations found physiologically. These results are consistent with the proposal that TRβ1 mutants displaying a primarily WT-TR target gene repertoire produce primarily endocrine disorders, whereas TRβ1 mutants displaying altered target gene repertoires, such as those associated with hepatocellular and renal clear cell carcinomas, contribute to the neoplastic phenotypes of these diseases by regulating genes not associated with normal thyroid endocrinology.

2. Materials and methods

2.1. Cell culture

HepG2 cells and HEK293T cells were maintained in Dulbecco's Modified Eagles Medium (DMEM) containing glutamine and 4.5 g/L glucose (Gibco ThermoFisher, Waltham MA), supplemented with 10% fetal bovine serum (FBS, Gibco/ThermoFisher low-endotoxin "Performance-Grade") at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Creation of the adenoviral vectors

Adenovirus vectors lacking an insert (AdEmpty), containing the

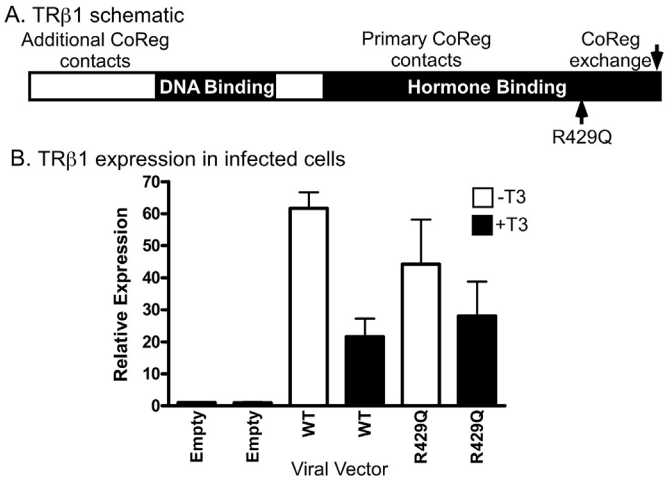

Fig. 1. Schematic of TRβ1 protein and its expression in vector-infected cells. A. The TRβ1 protein is presented schematically with the DNA and hormone binding domains indicated. Domains that mediate interaction with or exchange of coregulators ("CoReg") are depicted, as is the position of the R429Q mutation. **B.** The expression of TRβ1 mRNA is shown for HepG2 cells infected by the empty, WT, or R429Q adenoviral vectors in the absence or presence of added T3. The mean and standard deviation is shown (n = 3).