A Commentary on a Thyroid Hormone Receptor Mutation Mediated Disease State

My initial interest in the thyroid hormone began before I was aware of the field of endocrinology. It was from a series of attempts to understand more about how exactly hormonal interactions can lead to a homeothermy. It lead me to a review article (Silva, JE. *Annals of Internal Medicine*, 2003) on the stimulating effect of this thyroid hormone on thermogenesis. The hormone does so by reducing the thermodynamic efficiency of ATP synthesis by acting at its metabolic pathways that alter the ionic gradients that play a role in ATP generation through ion pumps.

So, to this end, I thought it would be interesting to look at the interactions of the hormone with its receptors and I immediately found that, due to the ubiquitous roles of the multiple variants of the thyroid hormone and its multiple receptor sites, there was a wide range of things that the thyroid hormones were involved in. And thereby a wide range of points in the pathways that could go wrong. Many of the papers that I initially found that pertained to thermogenesis were very clinical. And those that seemed biochemically interesting, focused on postulating models that explained how the thyroid hormone receptor was interacting at its variety of promoters (Ramados, P et al. *Journal of Biological Chemistry*, 2015).

But, to focus on a disease related dysfunction associated with the thyroid hormone, I found a paper (see attached page with reference and abstract) that discusses mutations in the LBD (ligand binding domain) of TRβ (thyroid hormone receptor beta) that result the disease state: ‘resistance to thyroid hormone’ (RTH or TRH). RTH is explained as a loss of sensitivity to thyroid hormone levels at its stimulation center. That is, increased thyroid receptors not effectively having a negative-feedback response. The paper develops a model to explain that LBD mutations fail to properly release nuclear corepressor and thereby inhibit associated downstream transcriptional machinery to be activated. I found this very interesting from a biochemistry point of view in terms of understanding the different mutation states of corepressors vs coactivators. As, you would expect transcriptional downregulation to happen as a result of mutations that don’t allow for coactivator binding rather than mutations that enhance (increase protein-protein interactions) corepressor binding!

The corepressor in question is Nuclear Corepressor 1 (Ncor1). With the hypothesis above, it follows from what we have learned in 135A, that if we have the mutant form present of Ncor1 whereby it cannot interact with the receptor, then we can simply confirm the theory with a co-immunoprecipitation analysis. The Co-IP performed in two a sustainable tissue culture cell line (a cancer cell line) through transduction and viral transfection demonstrates this inability of Ncor1-mut to be detected when the mutant TRβ is pulled down. But it is evident that the wild-type Ncor1 can be detected when the mutant TRβ is pulled down in a similar fashion. It would have been interesting to see, through these western blots, if there is a quantifiable difference in how much more
effectively the mutant TRb is able to bind and pull down with it the wild type Ncor1. This could be done by initially pulling down with an antibody for the mutant TRb or wt-TRb (in two cell lines with the individual constructs expressed) and then do the western with the antibodies for the wt-Ncor1. However, given the background levels and difficulty in quantifying the difference when the variation could come from how much of each is initially pulled down, this might be difficult to do. [And would be largely inconclusive because you’d have to normalize from a different western blot (which are not entirely analytically comparable)].

In the next series of experiments, they use a functionality assay. This assay is quite simply a measurement of the hormonal levels. What I initially also found different about the in vivo experiment conducted here was the fact that it was done in mice with knock-outs of the genes and subsequent genotypes (knock-in or simultaneous knock-outs) were created by breeding. This was not a factor of experimental study that we considered in class because of the variability in the different model organisms that can be employed. What is profound about their analysis from this assay is the ability to “cure” the disease phenotype (arising from the mutant TRb genotype) by essentially removing the co-repressor. That is, because the increased thyroid hormonal levels come about due to an increased interaction with the co-repressor. The knock-out of the co-repressor in the mouse, restores the thyroid levels to wild-type levels!

This analysis of hormonal levels does create a very compelling argument for how exactly the disease state can be reduced in its effects. And I think it is appreciable that they have been able to see these organismal scale results from a hypothesis based on the molecular interactions. What follows are more such phenotypic analyses (size of the thyroid gland, etc. that confirms the hormonal analysis above) that I do not find that intriguing because of an interest in the actual molecular dynamics.

Which is why the quantitative transcriptional level measurements are more convincing to me in terms of validating that the observed effects are really coming from the direct interaction of the receptor with its co-repressor. The removal of the co-repressor interaction allows for the transcription of genes that wt-TRb positively regulates and decreases the transcription of genes that wt-TRb negatively regulate (or, in some cases, does not). The loss of the interaction effectively restores the transcriptional levels to the wild-type levels in the case of the mutant TRb! This essentially confirms but more thoroughly and more biochemically, the significant role of the interaction.

What I found most interesting from the paper’s general conclusion and approach is the variability in how the thyroid hormone is interacting with the genes it regulates. It is a lot more dynamic and variable compared to the relatively straightforward transcriptional regulation discussed in class. Therefore, there is clearly a lot of merit in conclusively understanding how exactly this activated receptor is involved in positive and negative regulation. It relates to the Ramados et al. paper mentioned in the introduction! There have been more recent developments in the field (especially from the same lab) and while it seems to be more established that the interaction of the co-repressor Ncor1 is pivotal, actual clinical treatments to the disease state might not be all that simple considering the
Mutations in the ligand-binding domain of the thyroid hormone receptor β (TRβ) lead to resistance to thyroid hormone (RTH). These TRβ mutants function in a dominant-negative fashion to interfere with the transcription activity of wild-type thyroid hormone receptors (TRs), leading to dysregulation of the pituitary-thyroid axis and resistance in peripheral tissues. The molecular mechanism by which TRβ mutants cause RTH has been postulated to be an inability of the mutants to properly release the nuclear corepressors (NCORs), thereby inhibiting thyroid hormone (TH)-mediated transcription activity. To test this hypothesis in vivo, we crossed Thrb(PV) mice (a model of RTH) expressing a human TRβ mutant (PV) with mice expressing a mutant Ncor1 allele (Ncor1(ΔID) mice) that cannot recruit a TR or a PV mutant. Remarkably, in the presence of NCOR1ΔID, the abnormally elevated thyroid-stimulating hormone and TH levels found in Thrb(PV) mice were modestly but significantly corrected. Furthermore, thyroid hyperplasia, weight loss, and other hallmarks of RTH were also partially reverted in mice expressing NCOR1ΔID. Taken together, these data suggest that the aberrant recruitment of NCOR1 by RTH TRβ mutants leads to clinical RTH in humans. The present study suggests that therapies aimed at the TR-NCOR1 interaction or its downstream actions could be tested as potential targets in treating RTH.