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Objective: Thyroid hormones have various effects on cardiac and circulatory systems, leading to arrhythmias and heart failure. In Europe and the United States, it has been reported that elevated thyroid hormones within the normal range have been reported to be associated with a risk of atrial fibrillation, however, there was no report on Japanese cases, a country that differs in iodine intake and ethnicity from the West. Therefore, we evaluated the abnormality of thyroid function in a large number of cases of atrial fibrillation (AF) who received catheter ablation (RFCA) in Japan. **Methods:** We evaluated 2,937 cases of atrial fibrillation (2,084 males, mean age 64.1±10.7 years and 853 females, 69.0±8.5 years) who underwent RFCA at the Gunma Prefectural Cardiovascular Center between 2012 and 2018. As a control we used a total of 15,660 participants for health check-up (9,176 males, mean age 49.7±9.8 years and 6,484 females, 48.9±10.3 years) from 2006 to 2013, and we evaluated thyroid function after adjusting for gender-specific age. **Results:** The prevalence of overt hyperthyroidism was significantly higher in the RFCA-treated male group (0.43%) than in the control group (0.07%), even after adjusting for age ($p<0.01$). Similarly, the prevalence of subclinical hyperthyroidism was also significantly higher in the RFCA-treated male group (3.12%) than in the control group (0.94%) after adjusting for age ($p<0.01$). On the other hand, subclinical hypothyroidism was significantly lower in the RFCA-treated group after adjusting for age (2.97% in the RFCA-treated group and 3.93% in the control group, $p<0.01$). Females showed the same results as males. **Conclusions:** In an iodine rich country Japan, not only overt hyperthyroidism but also subclinical hyperthyroidism is an obvious risk factor for severe atrial fibrillation in Japan. Intriguingly, subclinical hypothyroidism might contribute to the prevention of atrial fibrillation, suggesting that slightly higher serum TSH levels might be better for elderlies.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

Thyroid Hormone Action in Liver: A Coregulator Shift Rather Than the Canonical Switch

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Thyroid hormone receptors (TR) are transcription factors that mediate the effects of thyroid hormones (TH) in development, physiology, and metabolism. TR canonically activates gene expression via a "switch" whereby TH converts chromatin-bound TR from a transcriptional repressor to an activator. In this model, the unliganded repressed state is mediated by binding of the nuclear receptor corepressor (NCoR), while the TH-activated state is caused by dismissal of NCoR and stabilization of binding of coactivators including CREB-binding protein (CBP). TH also negatively regulates gene expression, although the mechanism is controversial. Elucidation of the TR transcriptional mechanism *in vivo* has been hampered by the low concentration of endogenous TRs and the unavailability of high quality antibodies. To address this, we generated a mouse line in which endogenous TRβ1 was epitope-tagged to allow precise analysis at physiological levels, and explored TR function in liver where the actions of TR regulate body weight, cholesterol, and liver fat. ChIP-seq analysis revealed TRβ binding at genomic sites with epigenomic characteristics of enhancers, at sequences enriched for the canonical DR4 motif bound by TR with its RXR partner, at both positively- as well as negatively-regulated genes. The NCoR/HDAC3 corepressor complex was reduced but not completely dismissed by TH at positive enhancers and, surprisingly, at enhancers associated with negatively. CBP binding was also not "all or none" but, rather, shifted toward increased binding at enhancers in their active state, i.e., in the presence of TH for activated genes, but in the absence of TH for repressed genes. Thus, TH action is due to a shift, not an on/off switch, in coregulator association with TRβ-regulated enhancers determines their activity and transcriptional outcomes.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

Transcriptional and Genomic Regulation of Pituitary Function by Thyroid Hormone Receptor Beta

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Background: The pituitary is a key target for thyroid hormone but underlying transcriptional mechanisms are poorly understood. Thyroid hormone modifies expression of hormones, including growth hormone (GH) and thyroid-stimulating hormone (TSH, thyrotropin). Wider transcriptome responses are undefined. Thyroid hormone receptor beta (TRb) encoded by *THRB* are expressed in the anterior pituitary and *THRB* mutations cause human resistance to thyroid hormone. **Method:** To investigate genomic genomic regulation by TRb, we derived *Thrb*-HAB knockin mice that express TRb protein with a tag that is biotinylated *in vivo* in presence of an *R26*-BirA allele. Specific, sensitive streptavidin pull-down facilitated Chromatin-Affinity-Purification-sequencing (ChAPseq) to identify genomic TRβ binding sites in pituitary of male mice. Hypo- and hyperthyroidism were produced using methimazole (MMI) in drinking water for 4 weeks with/without added thyroid hormone (T3) for the 4th week. Pituitaries from wild type and *Thrb*-KO mice were also isolated for RNA-sequencing (RNA-seq). Selected expression changes were confirmed by quantitative PCR. Epigenetic changes were determined by ChIPseq for histone acetylation and methylation and open chromatin analysis (ATAC-seq). **Results:** Transcriptome analysis revealed genes with

statistically different expression induced by T3, including known response genes such as *Tshb*, *Hr* and *Gh*. Responses were impaired in *Thrb*-KO mice. T3 induced recruitment of TRb binding, chromatin opening and specific histone acetylation marks. **Conclusion:** Most T3 response genes in pituitary depend to some extent upon TRb. T3-dependent chromatin modifications indicate properties of TRb-dependent enhancer regions and a critical role for TRb in transcriptional regulation of pituitary function.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

Urine Proteomic Analysis of Differences Between Patients With Hyperthyroidism Before and After Carbimazole Treatment

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Background: Hyperthyroidism, characterised by increased circulating thyroid hormone (TH) levels, alters the body's metabolic and systemic haemodynamic balance and directly influences renal function However, the underlying mechanisms and metabolic implications of these changes are not well understood. **Objective:** In the present study we aimed to study the changes occurring in the urinary proteome of patients with hyperthyroidism before and after treatment. The levels of the excreted proteins in the urine were studied using an untargeted 2D DIGE MALDI TOF proteomic approach with network analysis. **Methods:** The study included 9 age matched patients with mean age 38.6 ± 12.1 years with newly diagnosed hyperthyroidism. The patients were evaluated at baseline and after receiving treatment with carbimazole. Urine samples were obtained from the same patient at baseline (hyperthyroid state) with serum FT4 levels of 35.4 + 9.9 pmol/L and TSH 0.014 + 0.014 mIU/L (mean + SD), and post treatment with anti-thyroid drugs (euthyroid state) with levels of FT4 17.0 + 2.8 pmol/L and TSH 0.6 + 0.5 mIU/L (mean + SD). **Results:** Alterations in the abundance of urinary proteins, analyzed by Progenesis software, revealed statistically significant differential abundance in a total of 40 spots corresponding to 33 proteins, 26 up and 7 down (≥1.5-fold change, ANOVA, $p \leq 0.05$). The proteins identified in the study are known to regulate processes related to cellular metabolism, transport, acute phase response. The urinary proteins upregulated with hyperthyroidism included serotransferrin, transthyretin, serum albumin, ceruloplasmin, α1B glycoprotein, syntenin-1, nesprin, and glutamilnyl peptide cyclotransferase while the 3 notable down regulated proteins were plasma kallikrein, protein glutamine gamma-glutamyl transferase and serpin B3. Bioinformatic analysis using Ingenuity Pathway Analysis (IPA) identified dysregulation of pathways related to cellular compromise, inflammatory response, cellular assembly and organization and identified the involvement of the APP and AKT signaling pathways via their interactions with interleukins as the central nodes. **Conclusion:** The urine proteomic profiling between the hyperthyroid and euthyroid states demonstrates alteration in the protein levels involved in acute phase response and in maintaining an individual's haemodynamic state.

Tumor Biology

EMERGING MECHANISMS AND THERAPIES IN ENDOCRINE-RELATED TUMOR BIOLOGY

ERa-Dependent Lethal Hyperactivation of the Anticipatory Unfolded Protein Response Induces Complete Regression Without Recurrence of Advanced Breast Cancer

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Metastatic estrogen receptor α (ERα) positive breast cancer is presently incurable and most patients die within 7 years. From a medicinal chemistry program, we identified a novel small molecule that acts through ERα to kill breast cancer cells and often induces complete regression without recurrence of large, therapy-resistant primary breast tumors and of lung, bone, and liver metastases. We exploited our finding that estrogen-ERα activates an extranuclear tumor-protective, signaling pathway, the anticipatory unfolded protein response (UPR). We repurposed this tumor protective pathway by targeting it with the small molecule, ErSO. ErSO kills cancer cells by acting non-competitively through ERα to induce lethal hyperactivation of the anticipatory UPR (a-UPR), triggering rapid necrotic cell death. Using luciferase to image primary tumors and metastases containing lethal ERαD538G and ERαY537S mutations seen in metastatic breast cancer, oral and injected ErSO exhibited unprecedented antitumor activity. In mouse xenografts bearing large breast tumors, oral and injected ErSO induced complete regression (>115,000 fold mean regression) in about 45% of mice (18/39). Although durable response without treatment for 4-6 months was common, tumors that did recur remained fully sensitive to ErSO re-treatment. Consistent with the essential nature of the a-UPR pathway targeted by ErSO, in more than 100 tumor-bearing mice, we have never seen an ErSO-resistant tumor. In just 7 days, oral ErSO induced complete regression of most lung, bone, and liver metastases. ErSO is well-tolerated in mice and blood-brain-barrier penetrant. Injected ErSO induced profound regression of challenging brain tumors. On average, ErSO-treated tumors were >180-fold smaller than vehicle-treated tumors. Moreover, use of ErSO is not limited to breast cancer. With its unique mechanism of action through the a-UPR, ErSO eradicated orthotoptic ERα positive ovarian tumors that do not require estrogen for growth. These xenograft studies used human cancer cells in immune compromised mice and therefore did not exploit the known ability of inducers of necrotic cell death to activate immune cells and induce immunogenic cell death. Notably, medium from breast cancer cells killed by ErSO contained high levels of the established immune cell activators, HMGB1 and ATP, robustly activated mouse and human macrophages and increased macrophage migration. ErSO's potent activity against advanced primary and metastatic ERα-positive breast cancers represents a paradigm shift in leveraging ERα for anticancer efficacy.