

confirm that in both KGN and primary mouse granulosa cells, similarly to the prostate, ligand-bound AR is primarily localized in the nucleus. Based on this and previous studies, we propose that paxillin enhances AR mRNA translation through interaction with PABP, and ligand binding further increases AR protein level by nuclear retention, protecting from degradation in the cytoplasm. Our findings highlight a previously unrecognized role of paxillin in granulosa cells, where it may be an important target for modulating androgen activity in androgen-related disorders of female reproduction.

## Steroid Hormones and Receptors

### STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

#### *Persistent COUP-TFII Expression Underlies the Myopathy and Impaired Muscle Regeneration Observed in Resistance to Thyroid Hormone-Alpha*

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Myopathic changes, including muscular dystrophy and weakness, are commonly described in hypothyroid and hyperthyroid patients. Thyroid hormone signaling, via activation of thyroid nuclear receptor alpha (THRA), plays an essential role in maintaining muscle mass, function, and regeneration. A mouse model of resistance to thyroid hormone carrying a frameshift mutation in the THRA gene (THRA-PV) is associated with accelerated skeletal muscle loss with aging and impaired regeneration after injury<sup>(1,2)</sup>. We previously demonstrated that the expression of nuclear orphan receptor chicken ovalbumin upstream promoter-factor II (COUP-TFII, or Nr2f2) persists during myogenic differentiation in THRA-PV myoblasts and skeletal muscle of aged THRA-PV mice. COUP-TFII is known to regulate myogenesis negatively and has a role in Duchenne-like Muscular Dystrophies<sup>(3)</sup>. COUP-TFII physically and functionally interacts with THRA in primary myoblasts isolated from WT and THRA-PV mice, as demonstrated via co-immunoprecipitation and chromatin-immunoprecipitation. We observed that satellite cells from THRA-PV mice display impaired myoblast proliferation and *in vitro* myogenic differentiation compared to WT cells. However, the silencing of COUP-TFII expression using siRNA probes restores *in vitro* myogenic potential of THRA-PV myoblasts and shifts the mRNA expression profile closer to WT myoblasts, with a higher proliferation of myoblasts and a higher number of fully differentiated myotubes after 5 days of myogenic induction. Moreover, RNAseq analysis on myoblasts from THRA-PV mice after COUP-TFII knockdown shows that COUP-TFII silencing reverses the transcriptomic profile of THRA-PV myoblasts and results in reactivation of pathways involved in muscle

function and extracellular matrix remodeling/deposition. These findings indicate that the persistent COUP-TFII expression in THRA-PV mice is responsible for the abnormal muscle phenotype. In conclusion, COUP-TFII and THRA cooperate during murine post-natal myogenesis, and COUP-TFII is critical for the accelerated skeletal muscle loss with aging and impaired muscle regeneration after injury in THRA-PV mice. These studies can help increase our knowledge of the mechanisms involved in thyroid hormone signaling during skeletal muscle regeneration, ultimately increasing the possibility of designing more specific treatments for patients with thyroid hormone-induced myopathies. **References:** 1. Milanesi, A., et al., *Endocrinology* 2016; 2. Kaneshige, M. et al., *Proc Natl Acad Sci U S A* 2001; 3. Lee HJ, et al, *Sci Rep.* 2017.

## Steroid Hormones and Receptors

### STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

#### *Production of 11-Oxygenated Androgens by Testicular Adrenal Rest Tumors*

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Testicular adrenal rest tumors (TART) are a common complication in male patients with classic 21-hydroxylase deficiency (21OHD). TART are considered to have steroid-producing properties and may contribute to the androgen excess in 21OHD patients. This study aims to define the production of 11-oxygenated 19-carbon (11oxC19) steroids by TART. Steroids were measured in left (n=7) and right (n=4) spermatic vein- and simultaneously taken peripheral plasma (n=7) samples from seven men with 21OHD and TART using liquid chromatography-tandem mass spectrometry. In addition, steroids were quantified in TART cell- and adrenal cell-conditioned medium, with and without adrenocorticotrophic hormone (ACTH) stimulation. Compared to peripheral blood of 21OHD patients with TART, the spermatic vein samples displayed the highest gradient for 11-hydroxytestosterone (11OHT; 96-fold) of the 11oxC19 steroids, followed by 11-ketotestosterone (47-fold) and 11-hydroxyandrostenedione (11OHA4; 29-fold), suggesting production of these steroids in TART. TART cell-conditioned medium contained higher levels of testosterone, and lower levels of androstenedione and 11OHA4 after ACTH stimulation compared to adrenal cell-conditioned medium, indicating ACTH-induced production of testosterone in TART. TART cells also produced 11OHT after 48 h of ACTH stimulation. Thus, in patients with 21OHD, TART produce 11oxC19 steroids, but in different proportions than the adrenals. The very high ratio of 11OHT in spermatic vein- versus peripheral vein blood suggests the 11-hydroxylation of testosterone by TART, and the *in-vitro* results indicate that this metabolism is ACTH-sensitive.