beneficial effects of exercise that is recommended in treatment of both depression and T2DM. Here we compared the effects of ucOCN or exercise in female C57-BL/6J mice under two different metabolic conditions. Mice were fed either a high-fat diet (60% calories from fat) to induce T2DM or a control diet (10% calories from fat). Groups of mice were either sedentary or exercised daily by 30 min treadmill running for two months, with or without daily administration of ucOCN (30 ng/g/day). Mice with T2DM displayed depressive behaviours marked by a higher immobile time in tail suspension tests compared to control mice (97±25 n=11 vs 207±9.0 s n=12; $t_{21}$=4.21, P=0.0004). Exercise and osteocalcin both improved depressive behaviour (137±8 n=12; $t_{22}$=5.85, P<0.0001 & 127±15 s n=12; $t_{22}$=4.46, P=0.0002). Anxiety, measured by the elevated-plus maze revealed the mice with T2DM displayed increased anxiety spending less time in the open arms and had a lower ratio of open to closed arm entries than the control group (0.37±0.03 n=10 vs 0.21±0.032 n=11; $t_{19}$=3.56, P=0.0021). Neither exercise nor osteocalcin improved anxiety in the T2DM mice. The puzzle box test revealed the negative effects of the high-fat diet in problem solving and memory, where the sedentary mice displayed greater latencies to solve each task compared to control mice. Exercised and mice receiving osteocalcin displayed performances comparable to that of the control group. Under normal metabolic conditions (low fat diet), neither osteocalcin nor exercise altered responses in any of the behavioural tests. Together, these results: 1. The effects of osteocalcin on behaviour and cognition are comparable to that of the effects of exercise in female mice with T2DM; 2. Behaviour and cognition did not improve from exercise or osteocalcin in female mice on a low-fat diet.

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# Adrenal

## ADRENAL PHYSIOLOGY AND DISEASE

### *The Role of Filamin A (FLNA) in the Regulation of Insulin-Like Growth Factor System in Adrenocortical Carcinomas*

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**SUN-213**

Adrenocortical carcinomas (ACCs) are rare endocrine tumors with poor prognosis. They overexpress insulin-like growth factor 2 (IGF2), that drives a proliferative autocrine loop by binding to IGF1R and IR, with molecular dynamics still poorly identified. Although promising, IGF1R/IR-targeted therapies have demonstrated a limited efficacy in clinical trials in ACC patients. The cytoskeleton actin-binding protein filamin A (FLNA) was shown to impair IR and IGF1R signalling in melanoma and neural progenitor cells, respectively. The aims of this study were to test in ACC cells: 1) FLNA involvement in regulating IGF1R and IR expression and signalling; 2) FLNA role in modulating responsiveness to IGF1R and IGFR/IR inhibitors; 3) FLNA expression in ACCs and correlation with IGF system. In ACC cells we found by immunoprecipitation that both IGF1R and IR interacted with FLNA in basal condition, with an increased or decreased FLNA recruitment to IGF1R and to IR, respectively, after IGF2 stimulation. Genetic silencing of FLNA in ACC cell lines H295R and SW13 induced a significant increase of IGF1R expression (1.4- and 2.3-fold, respectively) and a reduction of IR (-85.5±9.1%, p<0.001 and -32±19.1%, respectively, p<0.05), with a downstream effect of increased cell proliferation (130±13.4%, p<0.01 in H295R and 144.3±23.3%, p<0.01 in SW13 cells) accompanied by an enhanced ERK phosphorylation. Accordingly, in ACC primary cultured cells FLNA silencing increased IGF1R levels (2.9-fold) and enhanced IGF2 effects on ERK phosphorylation by 2.2-fold. In addition, FLNA knockdown potentiated the antiproliferative effects of IGF1R/IR inhibitor Linsitinib and IGF1R specific inhibitor NVP-ADW742 in H295R cells and SW13. This key role of FLNA was even more evident in A7/M2 melanoma cell model, since IGF2 and Linsitinib exerted the expected effects on ERK phosphorylation in M2 cells, lacking FLNA, but not in FLNA-expressing counterpart (A7 cells). Finally, western blot analysis showed significantly lower, although variable, FLNA expression in ACCs (n=10) than in adrenocortical adenomas (ACAs) (n=10) (FLNA/GAPDH ratio 0.37±0.38 and 0.90±0.63, respectively, p<0.05). Interestingly, FLNA/IGF1R ratio inversely correlated with ERK phosphorylation status in ACCs (p<0.05) but not in ACA. In conclusion, we demonstrated that low levels of FLNA enhance both IGF2 proliferative effects and IGF1R/IR inhibitors efficacy in ACC cells, suggesting FLNA as a new factor possibly influencing tumor clinical behavior and the response to the therapy with IGF1R/IR-targeted drugs.

# Steroid Hormones and Receptors

## STEROID AND NUCLEAR RECEPTORS

### *When the Glucocorticoid Receptor Meets the Mineralocorticoid Receptor in the Nucleus of Human Cells*

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**OR12-02**

Adrenal corticosteroids, such as glucocorticoids and mineralocorticoids, are indispensable for mediating response to stress, development, limiting inflammation, and maintaining energy and fluid homeostasis. These hormones exert their actions via binding to two closely related nuclear receptors, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). The GR has low affinity for corticosteroids, but is expressed in nearly every cell. In contrast, the MR shows a higher affinity for corticosteroids and its expression is largely confined to those tissues where

electrolyte exchange and fluid balance are required. GR and MR act as ligand-activated transcription factors which, following interaction with co-regulators and DNA responsive elements, either promote or repress gene transcription. The affinity for the same ligands, structural homology, and binding to the same DNA regions suggest GR and MR can compensate for each other's actions. Yet, there are specific glucocorticoid and mineralocorticoid-mediated responses indicating GR-MR functional diversity. To investigate this interplay, we developed U-2 OS (human osteosarcoma) cell lines stably expressing GR, MR, and both GR and MR (GRMR). Immunofluorescence analysis showed that treatment of these cell lines with 1 nM of the synthetic glucocorticoid dexamethasone (Dex) induced nuclear traslocation of GR and MR. Conversely, treatment with 1 nM of aldosterone (Aldo) promoted nuclear translocation of the MR only. Moreover, Proximity Ligation Assay revealed that, in the absence of ligand, GR associated with MR in the cytoplasm and, upon 1 nM Dex exposure, GR-MR dimers were detected in the nucleus of GRMR cells. Surprisingly, nuclear GR-MR dimers were also detected in the presence of Aldo, suggesting that it is necessary to activate at least one receptor to induce nuclear traslocation of the heterocomplex. To decipher the functional contribution of GR-MR dimers in the transcriptional response of GR to Dex and MR to Aldo, we performed RNA-seq in GR, MR, and GRMR cells treated with 1 nM of Dex or Aldo. Transcriptome analysis revealed that Dex-activated GR regulated the transcription of 6180 genes. Co-expression of MR resulted in a blunted Dex-mediated gene response which affected only 1608 genes, suggesting a functional antagonism of MR. Aldo-activated MR regulated the transcription of 1660 genes. However, co-expression of GR expanded the Aldo-mediated gene response to 3150 genes. Strikingly, 74% of these genes were also regulated by Dex via GR, suggesting that GR-MR dimers in the presence of aldosterone are able to mimic the glucocorticod transcriptional response. Our data suggest that the role of distinct GR and MR homo- and hetero-dimers is relevant for regulating gene expression. Dissecting the mechanism and investigating the cross-talk between GR and MR may be useful to understanding these two receptors in heath and disease.

# Neuroendocrinology and Pituitary

## PITUITARY TUMORS I

### *Beta-Arrestin 2 Is Required for Dopamine Receptor Type 2 Inhibitory Effects on Akt Phosphorylation and Cell Proliferation in Pituitary Tumors*

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**SAT-312**

β-Arrestin 2 is Required for Dopamine Receptor Type 2 Inhibitory Effects on AKT Phosphorylation and Cell Proliferation in Pituitary Tumors

Dopamine receptor type 2 (DRD2) agonists are the first-choice treatment for PRL-secreting pituitary tumors but are poorly effective in non-functioning pituitary neuroendocrine tumors (NF-PitNETs). DRD2 reduces AKT phosphorylation in lactotrophs, but no data are available in NF-PitNETs. DRD2 effects on AKT are mediated by a β-arrestin 2-dependent mechanism in mouse striatum.
The aim of this study was to investigate DRD2 effects on AKT phosphorylation and cell proliferation in human primary cultured NF-PitNET cells and in rat tumoral lactotroph cells MMQ, and to test β-arrestin 2 involvement.
We found that DRD2 agonist BIM53097 induced a reduction of p- AKT /total-AKT ratio in MMQ (-32.8±17.6%, p<0.001 vs basal) and in a subset (n=15/41,36.6%) of NF-PitNETs (subgroup 1). In the remaining NF-PitNETs (subgroup 2), BIM53097 induced an increase of p- AKT. The ability of BIM53097 to reduce p-AKT correlated to its antimitotic effect, since the majority of subgroup 1 NF-PitNETs was responsive to BIM53097 and nearly all subgroup 2 NF-PitNETs were resistant. β-arrestin2 was expressed in MMQ and in 80% of subgroup 1 NF-PitNETs, whereas it was undetectable in 77% of subgroup 2 NF-PitNETs.
In MMQ, β-arrestin 2 silencing prevented DRD2 inhibitory effects on p-AKT and cell proliferation. Accordingly, β-arrestin 2 transfection in subgroup 2 NF-PitNETs conferred to BIM53097 the ability to inhibit both p-AKT and cell growth.
In conclusion, we demonstrated that β-arrestin 2 is required for DRD2 inhibitory effects on AKT phosphorylation and cell proliferation in MMQ and NF-PitNETs, paving the way for a potential role of β-arrestin 2 as a biomarker predicting NF-PitNETs responsiveness to treatment with dopamine agonists.

# Pediatric Endocrinology

## UNDERSTANDING AND TREATING PEDIATRIC GROWTH DISORDERS

### *Interpretation of Insulin-like Growth Factor-1 (IGF-1) Levels Following Administration of Somatrogon (a Long-acting Human Growth Hormone - hGH-CTP)*

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**OR10-04**

IGF-1 is often used as a biomarker to evaluate the efficacy and safety of hGH replacement therapy. Typically, the mean IGF-1 SDS level during the dosing interval, rather than the peak value, guides clinical decision-making: sustained mean values > +2 may require hGH dose modifications. With long-acting formulations (administered weekly), the IGF-1 evaluation paradigm needs to take into account when the sample was obtained relative to the last administered dose. Previous studies with OPKO's once weekly Somatrogon (hGH-CTP), demonstrated that IGF-1 SDS peaked ~ 48 hours post-dose and that values at ~ 96 hours best approximated the mean IGF-1 SDS throughout the dosing interval [1]. Data from the pivotal Phase 3 non-inferiority study comparing treatment with Somatrogon to