filopodia formation after co-culture with astrocytes. These results indicate that nuclear ERs and TRs play an essential role in isoflavones-induces neuritogenesis. Non-genomics signaling through membrane receptor and F-actin are necessary for the isoflavones-induces synaptogenesis. Astrocytes-neurons communication also increased isoflavones-induced neuritogenesis, but not synaptogenesis.

Steroid Hormones and Receptors
STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

A Steroid Receptor Coactivator Stimulator MCB-613 Attenuates Adverse Remodeling After Myocardial Infarction

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Previous work from ours and other laboratories have shown that steroid receptor coactivators (SRCs) are involved in heart development and in mitigating cardiac dysfunction in cardiac injury models. Members of the p160 SRC family, SRC-1 (NCOA1), SRC-2 (NCOA2/TIF2/GRIP1) and SRC-3 (NCOA3/AIB1/ACTR/pCIP), interact with nuclear receptors and other transcription factors to drive target gene expression by assembling transcriptional coactivator complexes to increase transcription. This indicates a potential for SRC targeting drugs pertinent to cell migration, proliferation and survival-promoting paracrine interactions in cardiac tissue injury responses. We have identified a small molecule activator of SRCs (MCB-613) that selectively and reversibly binds to SRCs as shown by surface plasmon resonance and is a potent SRC stimulator that acts to greatly enhance SRC transcriptional activity with no apparent toxicity in mice. We postulated that MCB-613 could enable wound repair and preservation of cardiac function after an acute MI by reducing the extent of injury-related fibrosis and the subsequent chronic loss of cardiac function associated with non-contracting scar tissue. We thus tested the effect of MCB-613 on the cardiac injury response by administering MCB-613 two hours after ischemic injury in a mouse model of MI. Along with measurements of functional cardiac output and damage, we sought to identify the cell-type specific responses responsible for MCB-613's cardio-protective effects by utilizing single cell transcriptomics of cardiac interstitial cells to characterize the effects of SRC stimulation on cardiac function post-MI. We show that MCB-613, a potent small molecule stimulator of steroid receptor coactivators (SRCs) attenuates pathological remodeling post-MI. MCB-613 decreases infarct size, apoptosis, hypertrophy, and fibrosis while maintaining significant cardiac function. MCB-613, when given within hours post-MI, induces lasting protection from adverse remodeling concomitant with: (i) inhibition of macrophage inflammatory signaling and IL-1 signaling which attenuates the acute inflammatory response, (ii) attenuation of fibroblast differentiation, and (iii) promotion of Tsc22d3 expressing macrophages - all of which may limit inflammatory damage. Our results indicate MCB-613 controls the cellular interstitial cardiac repair response to ischemia. Distinct molecular and cellular mechanisms related to stimulation of SRC-3 have been identified that pave the way for the further exploration of SRCs as drug targets that can be engaged to improve the management of myocardial injury response outcomes. SRC stimulation with MCB-613 (and derivatives) is a potential novel therapeutic approach for inhibiting cardiac dysfunction after MI.

Steroid Hormones and Receptors
STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Androgen Receptor Blocker Improves the Cardiometabolic Profile in a Rat Model of Polycystic Ovary Syndrome, but at What Cost?

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Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. PCOS is characterized by androgen excess and ovulatory dysfunction high prevalence of cardiovascular risk factors such as increased blood pressure (BP), insulin resistance (IR), and obesity. We have demonstrated previously that exposing prepubertal female rats to dihydrotestosterone (DHT) leads to increase in food intake (FI), body weight (BW), BP, and IR. We tested the hypothesis that administration of the AR blocker bicalutamide (BICA) would decrease BP, IR, and obesity in PCOS model. As there are previous reports of severe hepatotoxicity with the AR blocker flutamide, we also examined BICA effects in the liver. **Methods:** Four-week old female Sprague Dawley rats implanted with DHT pellets (7.5mg/90 days) or placebo (PBO) were randomized to standard chow diet with or without the AR blocker bicalutamide (BICA) at a dose of 250 mg/kg/day throughout the study (n=10/group). BW and FI were measured weekly. BP and heart rate (HR) were measured by radiotelemetry. Fasting plasma was collected for IR (Homeostatic model assessment for IR, HOMA-IR). At euthanasia, the liver was collected, as well as plasma for gamma glutamyl transferase (GGT), alanine transaminase (ALT), and aspartate transaminase (AST) quantification. **Results:** PCOS rats had increased BW, FI, IR, and BP compared to PBO. BICA treatment had no impact on BW (285.3 ± 7.0 vs 270 ± 8.2 g, P=0.2) as well as FI and HR in PCOS. However, in PCOS, BICA decreased HOMA-IR (5.10 ± 0.40 vs 3.33 ± 0.31, P<0.05) and BP (115.4 ± 0.7 vs 105.3 ± 0.2 mmHg, P<0.01). Compared to PBO, PCOS+BICA rats had similar IR (3.83 ± 0.28 vs 3.33 ± 0.31, P=0.7) and BP (107.4 ± 0.8 vs 105.3 ± 0.2 mmHg, P=0.9). In addition, the liver weight to tibia length ratio was drastically increased by BICA in PCOS (222.9 ± 9.5 vs 360.4 ± 16.9 mg/mm, P<0.0001) as well as GGT (0.88 ± 0.88 vs 11.67 ± 0.58 U/L, P<0.0001), though it decreased AST (60.2 ± 6.9 vs 42.4 ± 1.9 U/L, P<0.05) and had no impact

on ALT. **Conclusion:** In summary, in a model of PCOS, BICA treatment abolished IR and BP, independent of FI, BW and HR. Prompt treatment with an AR blocker can normalize increased IR and BP triggered by androgen excess in females. Further studies need to be done to fully understand the effect of BICA in the liver in PCOS. The beneficial effect of AR blockers as a therapeutic option to improve the cardiometabolic profile in PCOS may be hampered by its liver toxicity.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Androgen Receptor Highjacks ErbB-2 Nuclear Function to Induce Triple Negative Breast Cancer Growth

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Triple negative breast cancer (TNBC) has poor prognosis and neither established biomarkers nor therapeutic targets. On the one hand the androgen receptor (AR), a steroid hormone receptor (SR) which is expressed in 10-53% of TNBC and proved to be critical for BC proliferation, has been proposed as a new target in TNBC. On the other hand, we and others have shown that membrane ErbB-2 migrates to the nucleus (nuclear ErbB-2, NErbB-2) where it binds DNA at HER-2 associated sequences (HAS) to regulate BC proliferation and migration. Since we have previously shown a functional interplay between growth factors and SR signaling pathways in BC, we propose the existence of an interaction between AR and ErbB-2 which is involved in NErbB-2+/AR+ BC growth. The experimental model used was the human TNBC cell line MDA-MB-453 which displays high expression levels of AR and NErbB-2. By Western Blot (WB) we found that dihydrotestosterone (DHT) treatment for short times (minutes) did not regulate ErbB-2 phosphorylation status at residues Tyr1221/1222 and 1248 which were constitutively activated. However, DHT led to an increase in ErbB-2 phosphorylation at residue Tyr877 which we have proved to be required for ErbB-2 nuclear migration. The latter effect was blocked by the AR antagonist enzalutamide (enza). Blockage of Src activity with dasatinib inhibited DHT-induced ErbB-2 phosphorylation at Tyr877. By Immunofluorescence and confocal microscopy analyses and subcellular fractionation studies we demonstrated that DHT induced ErbB-2 nuclear migration which was inhibited by enza. By chIP we found that DHT induced ErbB-2 recruitment to a HAS site in ERK5, a gene involved in BC proliferation, and to a HAS site in FKBP5, a classical AR responsive gene. By WB we demonstrated that transfection with an ErbB-2 mutant which is unable to translocate to the nucleus and functions as a dominant negative inhibitor of ErbB-2 nuclear migration (hErbB-2ΔNLS), inhibited FKBP51 up-regulation by DHT. Finally, by microarray and bioinformatics analysis we identified 315 differentially expressed genes (DEGs) in the presence of DHT and NErbB-2 eviction. Enrichment analyses showed that the DEGs belonged to the immune response and interferon pathways. Kaplan-Meier analysis revealed that the expression of 6 genes was significantly associated with overall survival in TNBC patients from the METABRIC cohort: CXCL10, TAP1, STAT1, NMI, HLA-A and NLRC5. Multivariate Cox regression analysis identified the combined expression of the 6 genes as an independent predictor of better clinical outcome in TNBC (HR: 0.56, 95% CI 0.38-0.82, P = 0.003). In conclusion, our findings evidence that DHT-activated AR induces Src-mediated ErbB-2 rapid activation and its migration to the nucleus where it binds to HAS sites in the DNA. Moreover, based on the DEGs of NErbB-2 eviction in presence of DHT we identified a gene signature associated with favorable outcome in TNBC.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

AR Is Not an Independent Marker for TNBC: The Lesson We Learn From Two PDX Models

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Extensive efforts, through cell line-based models, have been made to characterize the androgen receptor (AR) signaling pathway in triple-negative breast cancer (TNBC). However, these efforts have not yet reached a consensus with regards to the mechanism of AR in TNBC. On the other hand, patient-derived xenografts (PDXs) are generally considered more appropriate than cell line-based models for recapitulating the structural and molecular features of a patient's tumor, but only a few have been reported to be AR-positive TNBC. In our study, we identified and molecularly characterized two new, AR-positive TNBC PDX models and assessed the impacts of AR agonist (DHT) and antagonist (enzalutamide) on tumor growth and gene expression profiles by utilizing immunohistochemistry (IHC), western blots, and RNA-Seq and TNBC subtyping analyses. Two PDX models, termed TN1 and TN2, were derived from two grade 3 TNBC tumors, each containing 1~5% of AR positive tumor cells. DHT activated AR in both PDX tumors by increasing AR nuclear localization and protein levels. However, the endpoint tumor volume of DHT-treated TN1 was 3-folds smaller than that of non-treated TN1 tumors. Conversely, the endpoint tumor volume of DHT-treated TN2 was 2-folds larger than that of non-treated TN2. Moreover, enzalutamide failed to antagonize DHT-induced tumor growth in TN2. The RNA-Seq analyses revealed that DHT suppressed gene expression in TN1 (961 down-regulated genes versus 149 up-regulated genes), while the