DHT promoted gene expression in TN2 (673 up-regulated genes versus192 down-regulated genes). TNBC subtyping analyses based on RNA-Seq data predicted distinct molecular subtypes of TN1 and TN2: TN1 correlated to a basal-like 1 (BL1) subtype, and TN2 correlated to a basal-like 2 (BL2) subtype. These analyses suggest that TN1 and TN2, which both express functional AR, are two molecularly distinct PDX models that expand our current knowledge of AR-positive TNBC. Our results do not support that AR is a suitable therapeutic target in TNBC. To our best knowledge, the molecular mechanisms of AR in TNBC are equivocal and should be evaluated using clinically relevant models, considering both the heterogeneous expression of AR in TNBC and the general complexities of AR signaling.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Association of Maternal-Neonatal Steroids With Early Pregnancy Endocrine Disrupting Chemicals and Pregnancy Outcomes

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Steroids are important for fetal development and parturition, and aberrant exposure during gestation can lead to abnormal fetal outcome. Gestational exposures to endocrine disrupting chemicals (EDCs) have the potential to alter pregnancy steroidal milieu. Most studies to date have focused on individual EDCs, when in real life humans are exposed to a host of EDCs in parallel, emphasizing the need to consider cumulative impact. To meet this goal, 121 pregnant women (18-42 years of age) were recruited between 8 and 14 weeks of gestation from Southeastern Michigan, and maternal samples at recruitment and delivery were collected, as well as neonatal cord blood.

Maternal and neonatal steroids were measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS) in blood samples collected from 121 mother-infant dyads of spontaneously conceived singleton pregnancies. A total of 41 EDCs encompassing commonly encountered environmental EDCs (phenols and phthalates), metals and metalloids were quantified in early pregnancy maternal urine from a subset (56 dyads) via LC-MS/MS.

Maternal and neonatal steroid levels from all 121 subjects were related to pregnancy outcomes and, in the subset, individual and uniquely weighted EDC mixtures were related to steroid milieu. Additionally, the influence of BMI, maternal age, and offspring sex in modulating the EDC associations with steroids were determined. To determine the association of steroids at each time point with pregnancy outcomes or individual EDCs, multiple linear regression was used. Correlations between the steroids and potential confounding variables were analyzed using Spearman correlation. The cumulative effect of EDC mixtures generated by Principal Component Analysis on steroid measures was determined using Principal Component regression. The Benjamini-Hochberg False Discovery Rate procedure was employed to account for the multiple outcomes in each analysis.

The findings showed 1) steroid-specific positive or negative associations with pregnancy measures; (2) many maternal first trimester EDCs were negatively associated with estrogens

and positively with androgen/estrogen ratios; (3) EDC-steroid associations were influenced by maternal age, pre-pregnancy BMI, and fetal sex and (4) EDCs individually and as mixtures showed direct and inverse fetal sex-dependent associations with maternal and neonatal steroids. These findings indicate that maternal and neonatal steroids influence pregnancy outcomes depending on maternal age, pre-pregnancy BMI, and fetal sex and that the effects of EDCs on steroids differs when considered individually or as mixtures. Our results suggest that steroid measures might serve as biomarkers for the impact of EDC exposures on fetal outcomes during pregnancy, but these measures must be corrected for maternal factors. (Supported by P01 ES022844/RD 83543601, 1U2C ES026553, P30 ES017885)

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Calcineurin-NFATc4 Pathway Is Activated Upon K^+-stimulation of Adrenal Aldosterone Production

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The mineralocorticoid aldosterone secreted by the adrenal zona glomerulosa (ZG) cells promote renal K^+ secretion and Na^+ reabsorption; thereby it is critical for the control of ion homeostasis and blood pressure. While the Ca^{2+}/calmodulin-dependent protein kinase (CAMK) pathway regulating K^+ stimulated aldosterone production is well studied, little is known about the potentially involved phosphatases. Interestingly, immunosuppression therapy of transplanted patients with protein phosphatase 3 (calcineurin) inhibitors often results in rather low plasma aldosterone levels despite a concomitant hyperkalemia and hyperreninemia. Calcineurin (Cn) is a calcium and calmodulin-dependent protein phosphatase expressed in the adrenal cortex. We tested the hypothesis that Cn participates in the signal transduction pathway mediating the K^+-dependent stimulation of aldosterone production. To address this question, we used the adrenocortical cell model NCI-H295R, mouse and human ex vivo adrenal preparations and a ZG-specific and inducible Cn knockout mouse model (ZG-CnB1-KO). Inhibition of Cn with tacrolimus abolished the K^+-stimulated expression of CYP11B2 in NCI-H295R cell line as well as mouse and human adrenal pieces, *ex vivo*. Using a phosphoproteomics analysis, we identified *n*uclear *f*actor of *a*ctivated *T*-cells, *c*ytoplasmic 4 (NFATc4) as a critical downstream factor mediating Cn function. In support of