liquid chromatography/equilibrium dialysis. Sex hormone binding globulin (SHBG) was measured by chemiluminescence. mRNA was prepared from MNC. Quantitative RT-PCR was conducted using commercially available, predesigned TaqMan primers and probes targeting ACE2. The ACE2 relative level was calculated after normalization to beta Actin and GAPDH, with lowest ACE2 level being set to 1.

Results: Subjects' age ranged from 20 to 65 years. Type 2 diabetes was present in 74% of the men and the mean HbA1 was 7.2±1.6% (mean ± S.D.). Fifteen subjects had subnormal free testosterone (<50 pg/ml). Compared to the 27 subjects with normal free testosterone, they were older (49±12 vs 40±13 years, p=0.03) but had similar BMI (36±10, 35±10 kg/m^2, p=0.71). As expected, they had lower total testosterone (Median [25^{th}, 75^{th} percentile]; 222 [171-266] vs 431 [335, 618] ng/dl, p<0.001) and free testosterone concentrations (39 [21, 44] vs 72 [59, 92], p<0.001). Total estradiol was also lower in this group (19±1 vs 29±13 pg/ml, p=0.03) but free estradiol (0.44±0.33 vs 0.56±0.34 pg/ml, p=0.44) and SHBG (27 [19, 33] vs 30 [21, 39] nmol/L, p=0.52) were similar. Quantitative PCR data showed there was a large inter-individual variation of ACE2 expression level, up to 15-fold difference. Average ACE2 level did not differ between subnormal and normal testosterone groups (3.4 [2.7, 5.0] vs 3.9 [2.5, 5.7] arbitrary units). ACE2 expression was not related to free testosterone (r=0.11), free estradiol (r=0.18) or sex hormone binding globulin (r=0.15) on linear regression analyses (p>0.30 for all). ACE2 expression was also not related to age (r= -0.15, p=0.34), BMI (r= -0.2, p=0.23) or presence of diabetes. We were not able to detect a significant expression of TMPRSS2 in MNC.

Conclusion: Our results do not support a role for testosterone or estradiol as regulators of ACE2 expression in peripheral blood MNC in males.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Leptin Secreted in the Testicular Microenvironment Promotes the Endogenous Function of Leydig Cells Through Hedgehog Signaling Pathway

HIMANSHU ARORA, PhD[1], Madhumita Parmar, MS[1], kajal khodamoradi, PhD[1], REHANA QURESHI, PhD[1], Joshua M. Hare, MD[1], Ranjith Ramasamy, MD[2].
[1]UNIVERSITY OF MIAMI, MIAMI, FL, USA, [2]University of Miami, Miami, FL, USA.

Objective: Testosterone deficiency (TD) is a common health concern, affecting around 1 in 5 men globally. However, the factors responsible for TD remain largely unknown. Leydig cells produce testosterone in the testes under the pulsatile control of luteinizing hormone (LH) from the pituitary gland. Leydig stem cells (LSC) have the potential to differentiate into adult Leydig cells, which can increase testosterone levels; however, the factors promoting differentiation are unknown. In the present study we evaluated the paracrine factors released from the testicular microenvironment (TME) (comprised of Sertoli and peritubular myoid cells) that modulate the differentiation of Leydig stem cells to adult Leydig cells. Additionally, we explored the underlying mechanism of action of these paracrine factors.

Methods: Tissue samples were obtained from a total of 13 men with testicular failure, who underwent testis biopsies for sperm retrieval. Using an IRB approved protocol, about 10mg of testicular tissue from each sample were processed for LSC isolation, culturing, and characterization. Cytokine antibody array was performed to identify the paracrine factors released by Sertoli and peritubular myoid cells using unsorted and $CD146^{+ve}$ sorted cells. The cells were treated with hedgehog signaling agonist and antagonist to validate the specificity of paracrine factors identified. Immunostaining was performed to evaluate changes at the protein level. Flow cytometry was performed to study the shift in the population of cells post leptin treatment. GraphPad Prism (GraphPad Software) was used for statistical analysis.

Results: This study revealed that the TME plays an instrumental role in Leydig stem cell differentiation and testosterone production through regulation of the desert hedgehog (DHH) signaling pathway. TME-secreted leptin induces LSC differentiation and increases testosterone production. However, these effects are inversely concentration-dependent: positive at low leptin doses and negative at higher leptin doses. Mechanistically, leptin acts on LSCs upstream of DHH in a unidirectional fashion, as DHH gain or loss of function was shown to have no effects on Leptin levels.

Conclusions: These findings identify leptin as a key paracrine factor released by cells within the TME that modulate LSC differentiation and testosterone production from adult Leydig cells, a finding that is key to developing new niche therapies for TD.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Metabolic PCOS Features Are Ameliorated by Mitochondrial Uncoupler BAM15 in a PCOS Mouse Model

Valentina Rodriguez Paris, PhD, Stephanie J. Alexopoulos, BSc (Hons), Ying Hu, MD, Divya P. Shah, BSc (Hons), Ali Aflatounian, MD, Melissa C. Edwards, BSc (Hons), Michael Bertoldo, PhD, Hoehn L. Kyle, PhD, Kirsty A. Walters, PhD.
University of New South Wales, Sydney, Australia.

Polycystic ovary syndrome (PCOS) is a prevalent endocrine condition characterized by endocrine, reproductive and metabolic dysfunction. At present, there is no cure for PCOS and current treatments are suboptimal. Obesity and adverse metabolic features are prevalent in women with PCOS, with weight loss having a beneficial effect on PCOS features. The use of dietary interventions aimed at weight loss have low long-term compliance in women suffering from PCOS. Recent data from animal studies has shown that a small molecule mitochondrial uncoupler, BAM15, is an effective method to pharmacologically treat obesity and metabolic diseases. Therefore, the aim of this study was to investigate the efficacy of BAM15 to ameliorate PCOS-traits in a hyperandrogenic PCOS mouse model. As expected, exposure of female mice to dihydrotestosterone

(DHT) induced the PCOS metabolic features of increased body weight (P<0.05), lean mass (P<0.001), increased parametrial and mesenteric fat pad weights (both P<0.05) and adipocyte hypertrophy (P<0.05). Additionally, DHT-induced PCOS mice exhibited insulin resistance measured by HOMA-IR, increased cholesterol and fasting triglyceride levels and hepatic steatosis (all P<0.05). In contrast, DHT-induced PCOS females treated with BAM15 displayed body weights which were comparable with controls, a significant decrease in parametrial and mesenteric fat depot weights (P<0.05) and reduced adipocyte hypertrophy. Furthermore, BAM15 treatment decreased insulin resistance, cholesterol and fasting triglyceride levels, as well as the degree of hepatic steatosis observed in PCOS females, to levels comparable with controls. PCOS mice presented the reproductive PCOS traits of irregular cycles and ovulatory dysfunction, however BAM15 did not improve these PCOS traits. These findings demonstrate that the pharmacologic mitochondrial uncoupler BAM15 is able to ameliorate metabolic PCOS features in a hyperandrogenic PCOS mouse model. These data provide compelling evidence to support BAM15 as a potential innovative and viable therapeutic approach to manage metabolic traits associated with PCOS.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Molecular Background of Estrogen-Dependent and -Independent Gene Expressions in Endometriotic Lesions

Masao Izawa, PhD, Yukihiro Azuma, MD, PhD, Fuminori Taniguchi, MD, PhD, Tasuku Harada, MD, PhD.
Tottori University Faculty of Medicine, Yonago, Japan.

Background: Endometriosis is an estrogen-dependent disease, and the role of estrogen is obvious because the symptoms associated with endometriosis often disappear after menopause, and GnRH agonists or progestin relieve the pelvic lesions and endometriosis-associated pain. However, there are limitations to these treatments that target the estrogen reduction in endometriotic lesions. As a possible background, we hypothesized a role of the local environment with high estrogen depending on aromatase upregulation in endometriotic lesions. **Objective:** To test our hypothesis, we re-evaluated the expression profile of estrogen receptor (ER), and then searched for the estrogen-dependent gene expressions in endometriotic cells. Finally, we approached the epigenetic background of gene expressions in endometriotic cells.
Patients: Institutional Review Boards approved this project. We obtained the informed consent from all patients. The chocolate cyst lining in ovaries of patients with endometriosis was the source of endometriotic tissue. As the control, the eutopic endometrial tissues were obtained from uteri of premenopausal women who had uterine leiomyoma. **Methods:** Stromal cells were prepared from endometriotic and endometrial tissues. Gene expression was evaluated using RT-PCR. Specific primer sets of unique 5'-UTR exons/exon 2 in *ESR1* and specific primer sets of unique 5'-UTR exons/exon1 in *ESR2* were used for the analysis of promoter usage. Primer sets of exon 7 and exon 8 in *ESR2* were used to evaluate the expression of ERβisoform. Using SERM(PPT and DPN), ER-dependent gene expression was estimated. The potential function of hypomethylated gene sequence as an active enhancer was evaluated by ChIP analysis and eRNA expression. **Results:** 1) Relative expression of ERα mRNA in endometriotic cells was estimated to be one tenth of that in endometrial cells. 2) Relative expression of ERβ1 mRNA was 40-fold higher than that in endometrial cells, which is almost at a comparable level of the ERα. 3) In addition to ERβ1 mRNA, a splice variant ERβ2 was expressed at a comparable level of the ERβ1. 4) Top ten genes, up- or down-regulated in response to SERM, were extracted in endometriotic cells. 5) *TGFA* expression was upregulated at a comparable level in response to PPT and DPN. 6) A stretch of hypomethylated sequence, which includes an ERE at 50kb upstream from the TSS, was suggested as active enhancer. 7) *ESR1 and ESR2*showed a marginal response to SERM. 8) *GATA6* and *CYP19* were highly expressed in endometriotic cells, and hypomethylated sequences in these genes were suggested as active enhancer. **Conclusion:** In the hope of overcoming the limitations of endocrine treatments in endometriosis, we examined ER-dependent and -independent gene expressions using endometriotic cells. The results suggest one aspect of gene expression in endometriosis lesions.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

P53 Gain-of-Function Mutants and Steroids in Ovarian Cancer Cell Metastasis

Nicholes Candelaria, PhD[1], Achuth Padmanabhan, PhD[2], Rainer Lanz, PhD[1], Kwong Wong, Ph.D.[3], JoAnne S. Richards, PhD[1].
[1]Baylor College of Medicine, Houston, TX, USA, [2]Univeristy of Maryland, Baltimore, MD, USA, [3]MD Anderson Cancer Center, Houston, TX, USA.

High-grade serous ovarian cancer (HGSOC) is a heterogeneous disease for which there currentlyis no cure. Because p53 is mutated in >90% of all ovarian cancer, we studied specific gain-of-function (GOF) p53 mutants and steroid hormones for tumor morphology and metastasis *in vivo*. For this, we analyzed ALST (WT p53), SKOV3 (p53 null), TYK-NU (p53-R175H), OVCAR3 (p53-R248Q) and OVCA420 (p53-R273H) cell line xenografts in *Foxn1*$^{-/-}$ mice. ALST cells failed tometastasize, likely due to the known apoptotic effects of WT p53. SKOV3 and the p53-GOF celllines metastasized to the omentum and exhibited distinct morphologies: SKOV3, epithelial-like;TYK-Nu, vascular-like; OVCAR3, epithelial/mesenchymal; and OVCA420 epithelial exclusive. Despite different morphologies and p53 status, each tumor type contained large, Polyploid GiantCancer Cells (PGCCs) that are stem-like cells undergoing endoreplication. A specificphosphorylated, active form of β−catenin (pCTNNB1-S31/S37/T41; pCTNNB1) co-localizedselectively with GOF p53 and the mitotic stress regulatory kinase pMSK1-T581 in mitotic cells andPGCCs, indicating that in addition to GOF p53 mutants, pCTNNB1 and pMSK1 play a role intumor progression. To determine if ALST cells could be rendered