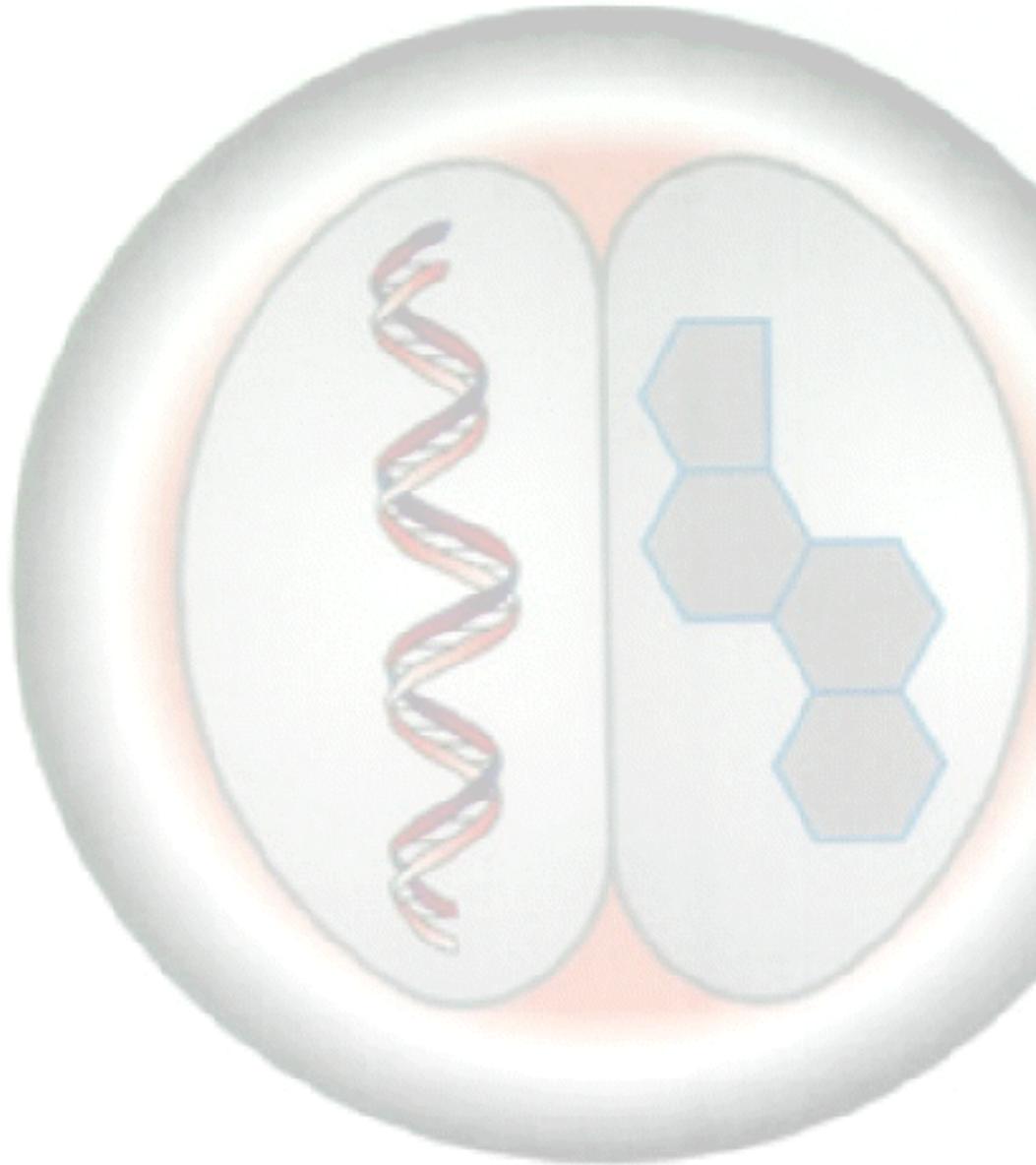# 2011

April 13, 2011

Fluno Center for
Executive Education
601 University Ave.
Madison, WI 53706

ANNUAL RESEARCH SYMPOSIUM

Endocrinology & Reproductive Physiology Program

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2010 – 2011 Symposium Committee Members
Dr. Manish Patankar, PhD
Dr. Ian Bird, PhD
Soraya (Silva) Arriaga
Katie Hackbart
Brian Kenealy
Yan Li
Meghan Maguire

Program Administration
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Tiffany Bachmann, MA, Student Services Coordinator

Schedule of Events

Time	Activity
8AM-9AM	Registration and breakfast
9 AM-10:00 AM	**Dr. Jon Levine, PhD** **Center Director, Wisconsin National Primate Research Center, Professor, Department of Neuroscience** "Sex, Drives, and Body Weights: Functions of Non-classical ERalpha Signaling in Brain"
10 AM-10:20 AM	Ann Rozner –PhD T32 Trainee, Golos Laboratory "The effects of macrophages on early implantation in the rhesus monkey"
10:20 AM-10:40 AM	Gopika Nair – PhD Student, Odorico Laboratory "Modeling pancreas development in murine embryonic stem cells by ectopic expression of PTF1a"
10:40 AM-11 AM	Coffee break
11 AM-11:20 AM	Mayra Pastore – PhD Student, Magness Laboratory "Structural Base Analysis for Estrogen Receptor Alpha vs. Beta Interaction with Cav-1 of Uterine Endothelial Cells-derived from Late Pregnant Ewes."
11:20 AM-11:40 AM	Dr. J Igor Iruretagoyena, MD - Maternal Fetal Medicine Fellow "Human Fetal Heart/Brain Gene Expression during Late First and Early Second Trimester"
11:40 AM-12:40 PM	Dr. Francisco Pelegri, PhD Associate Professor, Genetics "Maternal genes required for pronuclear fusion and germ plasm recruitment in the zebrafish zygote"
12:40 PM-1:45 PM	Lunch and Poster viewing
1:45 PM-2:45 PM	**Keynote Speaker: Dr. Leslie Myatt, PhD** **University of Texas Health Sciences Center– San Antonio** "Obesity, Oxidative and Nitrative Stress and the Placenta: Functional Consequences"
2:45 PM-3:30 PM	Posters
3:30 PM-3:50 PM	Samantha Lewis: - PhD T32 Trainee, Jorgensen Laboratory Activated Steroidogenic Factor 1 Induces Estradiol Synthesis in Benign Prostate Hyperplasia Cell Line BPH-1
3:50 PM-4:10 PM	Dr. Mian M.K. Shahzad, MD – Gynecologic Oncology Fellow "Conjugated Linoleic Acid Inhibits Ovarian Cancer Cell Proliferation"
4:10 PM-4:30 PM	Closing remarks and prizes

Dr. Leslie Myatt is Professor of Obstetrics and Gynecology and Co-Director of the Center for Pregnancy and Newborn Research at the University of Texas Health Science Center San Antonio. He moved to Texas in Jan 2009 having been a faculty member at the University of Cincinnati for 22 years and Director of the NIH-funded Physician Scientist Training Program (MD/PhD) and the Women's Reproductive Health Research Scholars Program. Dr Myatt served as North American Editor of the journal Placenta (1997 to 2004), President of the Perinatal Research Society (1997), President of the International Federation of Placenta Associations (2002 to 2004) and President of the Society for Gynecologic Investigation (2009 to 2010). Dr Myatt's research interests are control of fetal placental vascular reactivity, the role of oxidative and nitrative stress in placental function and fetal programming and the regulation of prostaglandin synthesis and action in intrauterine tissues at parturition. He has published over 200 papers and 300 abstracts and has served on many review panels and study sections for NIH, CIHR and other international grant giving bodies.

Key Note Talk:

"Obesity, Oxidative and Nitrative Stress and the Placenta: Functional Consequences"

As the interface between mother and fetus the placenta fulfills nutrient transport and hormonal signaling functions to support optimal fetal growth and development. Alterations in these functions are found in several placental pathologies that ultimately lead to adverse outcome of pregnancy. Pregnancy per se is a state of oxidative stress which is heightened in pregnancies complicated by diabetes, and the inflammation associated with preeclampsia and increasing maternal BMI. Both reactive oxygen and nitrogen species including superoxide and nitric oxide have roles in placental physiology including regulation of vascular reactivity and signaling. With inflammation and heightened oxidative stress increased production of these reactive species leads to their interaction to produce a more powerful pro-oxidant, peroxynitrite, that can covalently modify proteins by tyrosine nitration leading to either gain or loss of function. We have shown functional effects of protein nitration on vascular reactivity on the placenta and using a proteomic approach found p38MAP kinase activity to be reduced following nitration. The potential for rapid and reversible nitration of many proteins in the placenta implies a potential regulatory role. The increasing inflammatory and pro-oxidative milieu seen with increasing BMI is associated with a switch from protein carbonylaton reactions towards protein nitration suggesting again potential changes on covalent modification of proteins and their functions. There is also a sexual dimorphic effect on placental function and pregnancy outcomes potentially mediated via inflammatory pathways.

Keynote Speaker: Jon Levine, PhD

Dr. Jon E. Levine, Ph.D. completed his B.A at Oberlin College in Ohio, and his Ph.D. from the University of Illinois, Champaign-Urbana. Dr. Levine completed postdoctoral training at the Oregon National Primate Research Center & Oregon Health Sciences University. Before coming to Madison he was a Professor of Neurobiology and Physiology at Northwestern University. He is currently the Director of the Wisconsin National Primate Research Center and Professor in the Department of Neuroscience at the University of Wisconsin—Madison. For the past 30 years Dr. Levine has studied the neuroendocrine regulation of gonadotropin releasing hormone (GnRH) neurons, in part by developing and utilizing physiological methods to monitor and analyze GnRH neurosecretion in awake animals. Dr. Levine's research has also focused on the mechanisms by which ovarian steroids exert their effects in the brain, the latter including the negative feedback mechanisms that maintain homeostatic control within the reproductive axis, as well as the positive feedback actions of steroids that culminate in release of preovulatory gonadotropin surges. His recent work has made use of newly developed mutant mice to analyze the cell signaling mechanisms that mediate negative and positive feedback actions of estradiol, as well as the effects of estrogens on energy homeostasis and body weight. Dr. Levine is currently Editor-in-Chief of the journal Frontiers in Neuroendocrinology, and is a member of the Steering Council for the Office of Research on Women's Health at the NIH. He is an active member of numerous professional societies including the Endocrine Society, Society for Neuroscience, Society for the Study of Reproduction, American Neuroendocrine Society, and the Society for Behavioral Neuroendocrinology.

Faculty Talk:
"Sex, Drives, and Body Weights: Functions of Non-classical ERalpha Signaling in Brain"

Ovarian estrogens exert critically important actions in hypothalamic neurons to regulate ovulatory cyclicity, reproductive behaviors, and energy homeostasis. Estrogen receptor alpha (ERα) appears to mediate most of these effects, as disruption of ERα signaling leads to infertility and metabolic syndrome. ERα signaling mechanisms may include "classical genotropic" effects mediated by direct binding of receptor dimers to DNA, "non-classical genotropic" effects involving tethering of ERs to other transcription factors, and "non-classical non-genotropic" actions mediated by cytoplasmic ERs coupled to membrane-initiated signal transduction pathways. Our studies are making use of novel ERα mutant mouse models to ascertain the cellular mechanisms by which ERα mediates estradiol (E_2) effects on these physiological and behavioral processes. We have utilized a novel mutant ERα knock-in mouse model, which confers non-classical genotropic and non-genotropic signaling in the absence of classical signaling, to determine that non-classical ERα signaling can convey E_2 effects integral to homeostatic feedback control of reproductive hormone secretions, as well as E_2 actions governing paracopulatory behavior and body weight regulation.

Francisco Pelegri, PhD

Dr. Francisco Pelegri, PhD completed his B.Sc. (highest honors) at the University of California, Berkeley, in Genetics, and his PhD at the Massachusetts Institute of Technology, Cambridge MA, in Cellular and Molecular Biology. Subsequently, Dr. Pelegri completed postdoctoral training in Developmental Genetics at the Max Planck Institute for Developmental Biology in Tübingen, Germany. Dr. Pelegri joined the faculty of the Laboratory of Genetics at the University of Wisconsin Madison in 1999, where he has been since. Research in Dr. Pelegri's laboratory focuses on the role of maternal genes on early embryonic development.

"Maternal Genes Required for Pronuclear Fusion and Germ Plasm Recruitment in the Zebrafish Zygote"

Developmental processes that occur prior to the activation of the zygotic genome necessarily depend on maternal products produced during oogenesis and deposited in the egg. My laboratory studies a number of such maternally-driven processes, which range from fertilization to patterning and morphogenesis during embryogenesis. In this talk, I will focus on two sets of genes involved in some of the earliest developmental events that occur after fertilization. We have found that maternal function for the gene *futile cycle* is essential for nucleus-centrosome attachment in the early embryo, and this defect eventually leads to the inability to undergo pronuclear fusion after fertilization. Another set of identified genes are important for the coordination of cell division and the segregation of the germ plasm, a specialized cytoplasm containing specific RNA and protein products which confers the germ cell fate. We discuss the function of these genes and their products, and the role of posttranscriptional regulatory mechanisms in these processes.

Ann E. Rozner, Maureen Durning, and Thaddeus G. Golos

At the time of implantation and throughout pregnancy, the primate uterus contains numerous leukocytes, with a significant majority of natural killer (NK) cells and macrophages. These cells and the cytokine balance at the maternal-fetal interface are thought to play an important role in the maternal fetal immune response. We have established a co-culture system with rhesus monkey embryos to model implantation. Rhesus blastocyst stage embryos were derived by in vitro fertilization of oocytes. Embryos were cultured to the peri-implantation blastocyst stage, then co-cultured with macrophages collected from day 36 decidual tissue. Culture media were collected to determine cytokine and chorionic gonadotropin (CG) secretion, embryo growth was monitored, and apoptosis and proliferation of trophoblasts were evaluated. Trophoblast outgrowths expanding from the embryo were noticeable after 4 to 6 days of culture and multiple cytokines were consistently detectable in the media of macrophage co-cultured embryos. Overall trophoblast outgrowth was decreased when blastocysts were cultured with macrophages, however, the percentage of apoptotic cells also decreased. Preliminary data suggests no changes in proliferation or CG secretion of co-cultured embryos from controls. These data suggest the implanting blastocyst interacts with the local macrophages, evoking a differential trophoblast response by decreasing growth and apoptosis of trophoblasts when macrophages were present.
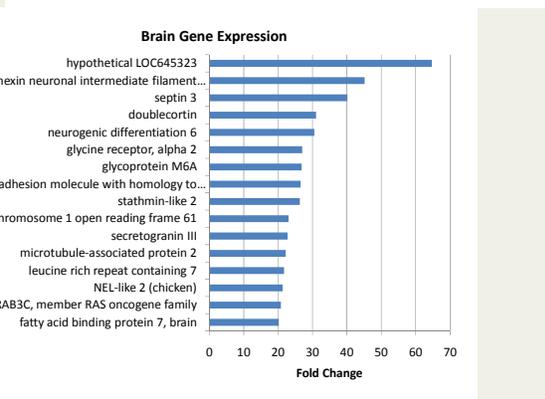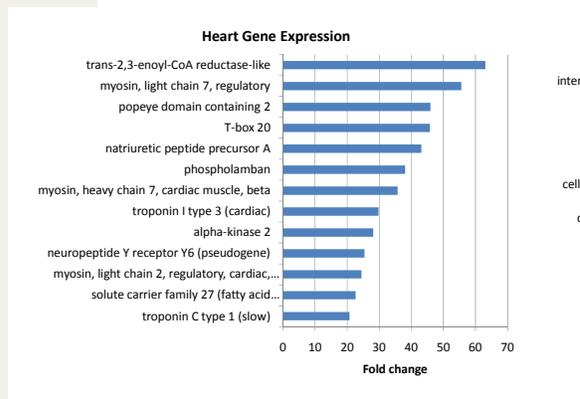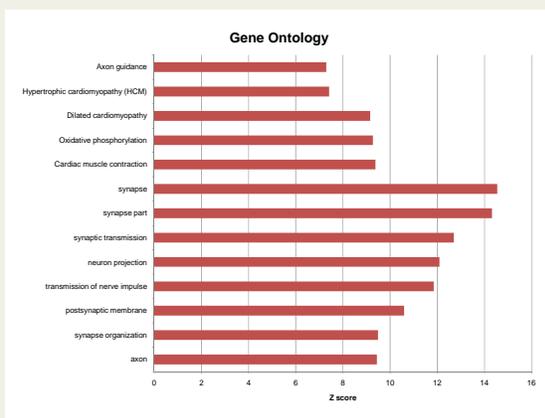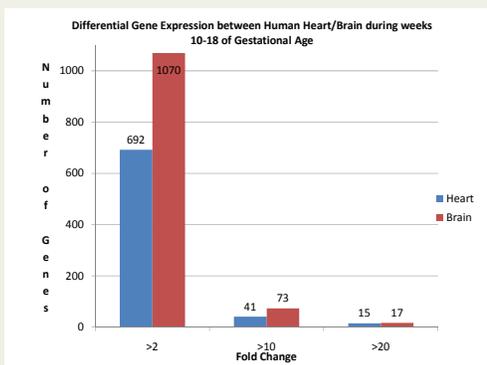
Oral - Modeling pancreas development in murine embryonic stem cells by ectopic expression of PTF1a

Gopika G Nair, Robert K Vincent, Victoria Browning, Nathan Treff, and Jon S Odorico

Pancreas-specific transcription factor 1a (ptf1a) is known be to be involved in pancreas specification in the foregut endoderm 1 and transactivation of the PDX1 promoter. A tetracycline-inducible murine ES cell line with temporally-controlled induction of ptf1a (tet-ptf1a) was established, which we utilized as a new tool to interrogate the role of PTF1a in directing differentiation of ES cells down the pancreatic lineage. Induction of ptf1a expression by doxycycline (Dox) treatment in EB derived cultures resulted in extensive expression of PDX1. In particular, the EBs gave rise to PTF1a+PDX1+ bud-like structures reminiscent of in vivo pancreatic organogenesis, in that cells of the differentiating PDX1+ epithelium expressed other pancreatic markers, such as carboxypeptidase A1 (CPA1) and neurogenin 3 (NGN3), and became progressively post-mitotic with time. The expression of amylase and islet endocrine hormones at later time points indicated that these cells were capable of further differentiation into mature pancreatic cell types. To understand the dosage dependent effects of PTF1a in directing differentiation into endocrine cells, we induced the tet-ptf1a cell line with different concentrations of Dox. At low levels of PTF1a we observed increased numbers of Ngn3+ Pdx1+ endocrine progenitor cells compared to the number of like cells at high levels of PTF1a. On further differentiation, low dose PTF1a cultures exhibited the highest number of insulin and PDX1 co-expressing cells. We then interrogated the transcript levels of Rbpj and Rbpj-l, mammalian Suppressor of Hairless paralogs, whose gene products are stage-specific partners of PTF1a in the PTF1 complex. Immediately after induction, Rbpj-l levels increased with the dose of PTF1a, while Rbpj levels was relatively constant. That Ngn3+ and insulin+ endocrine cells were more prevalent at low levels of PTF1a, and amylase and Rbpj-l expression was greater at high doses of PTF1a indicates that the intracellular level of PTF1a controls the switch between exocrine and endocrine fates in our ESC-derived pancreatic progenitors similar to the results from studies on embryonic pancreas in zebra-fish 3.Furthermore, this is the first study to show branching morphogenesis with both acinar and islet formation similar to in vivo pancreatic development in ES cell derived cultures.

Mayra B. Pastore, Benjamin C. Hofeld, Dong-bao Chen, and Ronald R. Magness

BACKGROUND: Estrogen receptors (ERs) are predominantly nuclear; however, a small pool of ERs localize to the plasma membrane of endothelial cells and are responsible for nongenomic vasodilatory responses. ER-a and ER-b have previously been demonstrated to induce very rapid activation of ERK signaling cascade and eNOS to induce nitric oxide (NO) production. NO production is partly responsible for the maintenance of vasodilatation during physiologic states of high circulating estrogen levels such as pregnancy. ER-a and ER-b localize within specialized plasma membrane domains termed caveolae and are responsible for diverse estrogen-mediated endothelial adaptations. It has been reported that ER-a interacts directly with the main scaffolding protein in the caveolae, Cav-1, however, it is unknown if there exits a similar protein-protein Cav-1 interaction with ER-b. **HYPOTHESIS:** We hypothesize that ER-a and ER-b maintain similar protein-protein interaction with Cav-1 in an "unstimulated" state in uterine artery endothelial cells (UAECs) and that structural analysis of these ERs will provide evidence for ERs' association with the plasma membrane. **METHOD:** Bioinformatic database approaches using SDSC Biology WorkBench web-base tool for analysis and modeling and UCSF Chimera molecular modeling system were employed to obtain structural information on hydrophobicity, transmembrane domain and crystal structure-based analyses on ER-a, ER-b and Cav-1 domains. Based on these bioinformatic parameters, the structural differences and similarities between receptor subtypes were overlaid with computer imaging to directly compare and to draw conclusions regarding 3D orientation and interaction within the plasma membrane. Proof of principle was established using immunoisolation column assays from passage 4 whole UAECs using Cav-1 antibody followed by Western blotting to identify protein-protein interaction. **RESULTS:** Database bioinformatic analyses on ER-a and ER-b and Cav-1 secondary structure showed that these protein do not possess any transmembrane domains. UCSF Chimera software was used for crystal structure comparison between ER-a and ER-b and this comparison revealed identical configuration between these proteins suggesting that 3D structure alone cannot account for ER and Cav-1 interactions. These bioinformatic database analyses are consistent with Cav-1 immunoisolation "pull-down" column UAEC protein which demonstrated that ER-a is tightly bound to Cav-1. In contrast, Cav-1 did not appear to interact with ER-b with high affinity. Finally, protein database sequence analysis revealed the presence of a candidate sequence that may be specific to caveolin binding motif in both ER-a and ER-b that we are evaluating further. **CONCLUSIONS:** These data collectively support the thesis that unlike ER-a, ER-b maybe at least loosely associated with caveolar lipid raft, but not via transmembrane loop. The direct/indirect association between ER-b and Cav-1 needs further study. NIH GM083252, HL49210, HD38843, HL87144, HL70562.

J.Igor Iruretagoyena, Wayne Davis, Christina Kenziorski, Cynthia Bird, Rebecca Radue, Aimee Teo Bromar, Sandra Splinter BonDurant, Thaddeus Golos, Ian Bird, Dinesh Shah

Objective: To describe the changes in gene expression (mRNA) occurring during fetal development in the heart and the brain. This information will be used to uncover pathways involved in fetal pathology. In this abstract we describe the initial methods development necessary to achieve that goal. **Study Design:** 12 fetal hearts and 12 fetal brains from 10-18 weeks of gestational age were prospectively collected at the time of termination of pregnancy. Total RNA was isolated and then hybridized into the Affymetrix ST 1.0 human array. The Affymetrix probe level data was processed using Robust Multi-Array Analysis (RMA) as implemented in the affy package to obtain normalized summary scores of expression for each probe set on each array. EBarrays was used to identify genes differentially expressed in any two conditions. Cut offs for sensitivity that ensured a reliable positive detection result were 0.95. Cutoffs for a significant change in expression were initially set at 2 fold change. **Results:** Comparison of the data sets for brain and heart showed that with a cut off of 2 fold there were 1070 and 692 differences respectively, while at a cut off of 10 there were 73 and 41, at cut off of 20 there were 17 and 15 genes. In brain there was abundant expression of Internexin, Septin, Doublecortin, stathmin and NEL2. In heart there was abundant expression of Myosin light and heavy chain 7, Myosin light chain 2, Popeye domain, T-box 20 and Phospholamban. Gene ontology confirmed tissue specificity of the highly expressed genes for the brain and the heart.

Differential Gene Expression between Human Heart/Brain during weeks 10-18 of Gestational Age

Gene Ontology

Heart Gene Expression

Brain Gene Expression

Conclusions: The methodology has been validated as being both achievable and capable of accurately identifying the unique genes expressed in brain and heart respectively. The genes found with the greatest differential expression are involved in tissue-specific differentiation for further developmental requirements.

Oral - Activated Steroidogenic Factor 1 Induces Estradiol Synthesis in Benign Prostate Hyperplasia Cell Line BPH-1

Samantha R. Lewis, Joan S. Jorgensen

Prostate growth in development and disease depends on androgens synthesized by the testis. Thus, treatment of prostate cancer targets androgen production and activity at several fronts. Initially, prostate tumors subject to androgen deprivation therapy regress, but inevitably, rogue cancer cells prevail and an aggressive cancer returns that is unresponsive to this therapy. There are several theories to explain this aggressive resurgence, one of which suggests that prostate adenocarcinoma cells acquire machinery for de novo steroidogenesis. It has long been established that Steroidogenic Factor 1 (NR5A1, ADBP4, SF1) is essential for regulating steroidogenesis in normal endocrine tissues. While normal prostate tissue lacks SF1 expression and is nonsteroidogenic, the presence of SF1 in diseased tissue of the prostate has not been documented, presenting an unexplored potential driver of aberrant steroidogenesis in prostate cancer. We hypothesize then that SF1 is present in prostate cancer and that SF1 is necessary for steroidogenesis to occur in prostate epithelial cells. To test the first part of our hypothesis, we examined SF1 expression in a panel of both benign and cancerous prostate cell lines. All prostate cancer cell lines examined expressed SF1, while a benign prostate hyperplasia cell line did not. We then performed immunohistochemistry on prostate biopsy samples from human patients with benign prostate hyperplasia or recurrent prostate cancer following androgen deprivation therapy. Similar to our results from prostate cell lines, we detected positive nuclear staining for SF1 in prostate adenocarcinoma but not benign prostate hyperplasia specimens. To functionally link SF1 to steroidogenic production in prostate epithelial cells, we tested whether ectopic expression of SF1 was sufficient to induce steroid production in a benign prostate cell line (BPH-1). BPH-1 cells were transfected with expression vectors encoding the mCherry fluorescent protein (negative control), wild type SF1, or a constitutively active mutant of SF1 (SF1ΔLBD). After 48 hours in culture, media was collected and subject to steroid measurement by ELISA. Whereas accumulation of progesterone and testosterone were not detected, estradiol concentrations were increased in BPH-1 cells transfected with wild type SF1 and even more so with transfected SF1ΔLBD. Together, these data indicate that SF1 is induced both in prostate cancer cell lines and in human prostate adenocarcinoma biopsy specimens, and suggest that activated SF1 is sufficient for steroidogenesis in a benign prostate cell line. Our findings present a potential mechanism and target for therapy of androgen deprivation resistant prostate cancers. Future experiments are planned to elucidate the stimulatory signals that induce SF1 within these aggressive prostate tumor cells leading to aberrant steroid production that fuels growth of deadly prostate cancer. This work was supported by The University of Wisconsin-Madison and NIH 5T32-HD041921-07.

Mian M.K. Shahzad, Arvinder Kapur, Hannah R. Van Galder, Mildred A.R. Felder, Nick Claussen, and Manish S. Patankar.

Background: Conjugated linoleic acid (CLA) has long been recognized to exert protective effects on carcinogenesis in breast, prostate and colon malignancies. Recent data suggests that CLA plays a key role in inhibiting several important survival pathways in breast cancer cell. However, data on effects of CLA in epithelial ovarian carcinoma are lacking and was the focus of this study. **Methods:** A2780 and SKOV3 ovarian cancer cell were treated with increasing concentrations of two different isomers of CLA (9:11, 10:12) and its effect on cell viability/proliferation was determined using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at different time points with and without different chemotherapeutic agents. Additionally, effect of CLA isomer 10:12 were evaluated on A2780 ovarian cancer cell apoptosis by flow (Annexin V/PE) and proliferation (MTT assay) while the molecular effect of CLA treatment were assessed using western blot. **Results:** CLA isomer-10:12 significantly affected the ovarian cancer cell viability in a time and dose dependent fashion. Seventy-two to 96h of CLA treatment (isomer 10:12, 7µ) resulted in ~50% reduction in cell viability compared to control ($p<0.01$). A 20 µM dose of the CLA isomer 10:12 resulted in >80% reduction in live cell compared to controls. However, CLA isomer 9:11 had no effect in all doses and time points tested (0.001 µM- 50mM). Additionally, at IC50 dose of oxaliplatin treatment, the addition of CLA, resulted in a substantial reduction (by ~50%, $p<0.05$) in percent live cell compared to oxaliplatin monotherapy. CLA isomer 10:12 significantly reduced ovarian cancer cell proliferation in a time and dose dependent fashion by modulating Src and MAPK pathways. **Conclusion:** Our preliminary data indicate that CLA may significantly affect the survival and reduce proliferation of ovarian cancer cells. These findings need further exploration and may have therapeutic implications for ovarian cancer.

Roxanne Alvarez, Fu-Xian Yi, Bikash Pattnaik, Ian Bird

Introduction: During pregnancy, uterine artery endothelial vasodilation is enhanced due to increased agonist-induced Ca2+ bursting leading to sustained nitric oxide production. Greater cell-cell communication and correspondingly enhanced Ca2+ influx via capacitative entry in response to agonists result in enhanced vasodilation. The Initial aims of this study are to characterize the contribution of potassium channel(s) to resting membrane potential as a means for pregnancy to alter Ca2+ influx via TRPC channels in pregnant uterine artery endothelial cells (P-UAEC). Previous studies from our lab have shown sensitivity to Ca2+ activated and delayed rectifier potassium channel drugs, suggesting their contribution in endothelial cell functional physiology. We began this study in order to understand the specific changes in contribution of these signaling ion-channels during pregnancy to coordinate cell-cell communication. Having established general electrical membrane properties in these cells, the current direction of research is to establish the correlation between the cells' membrane potential, TRPC3 activation, and enhanced calcium signaling. **Methods:** Whole-cell method of patch clamp electrophysiology was carried out on single voltage clamped passage 4 P-UAEC plated to 20% density. Cellular responses to a ramp protocol from -150 mV to +50 mV were recorded while using a –60 mV holding potential. The pipette solution contained K-gluconate (100mM), KCl (30mM), HEPES (5mM), EGTA-KOH (5.5mM), CaCl2 (0.5mM), and Mg-ATP (4mM) at pH 7.2. The bath solution contained KCl (5mM), NaCl (125 mM), HEPES (25mM), glucose (6mM), CaCl2 (2mM), KH2PO4 (1mM), and MgSO4 (1mM) at pH 7.4. Fluorescence imaging using MetaFluor was performed on P-UAEC grown to 100% density. Cells were treated with Fura-2 AM (10 uM) and subsequently with DIBAC4(3) (250 nM) to simultaneously monitor intracellular calcium concentration and cellular membrane potential. Cells were stimulated with the agonist ATP (100uM). Results: Preliminary data show that the resting membrane potential of low density P-UAEC is –20 ± 1.9 mV (n=57). The membrane capacitance was determined to be 14 ± 1.7 pF with a resistance of 4.0 ± 0.53 GΩ. In confluent P-UAEC, initial fluorescence data show a quick hyperpolarization upon ATP stimulation with a corresponding rise in intracellular calcium concentration. This is followed by depolarization of the membrane towards basal potential within approximately 5 minutes post-stimulation after which time the cells undergo a sustained hyperpolarization phase. The typical Ca2+ bursting pattern as determined by previous members in our lab is still occurring post-ATP stimulation in cells loaded with DiBAC4(3). **Conclusions:** Our data suggest that isolated P-UAEC have potassium channels that produce outward-rectifying current and hyperpolarize the membrane, and a smaller inward current at hyperpolarizing potential. Preliminary data from high density P-UAEC do indeed show membrane potential changes in response to ATP. Future studies will now include determining the molecular identity of the underlying ion channel currents contributing to changes in membrane potential, and the relationship of calcium influx and membrane potential to pregnancy enhanced TRPC3 activation. Funded by NIH HL079020.

Arriaga, S., Wiltbank, MC.

Oxygen homeostasis involves a series of genes apt to respond and modulate in response to oxygen tension. Hypoxia inducible factor 1 (HIF-1) is a basic helix loop-helix (bHLH) transcription factor composed of α-β heterodimer. It binds to hypoxic response elements (HRE) on promoters that contain the sequence NCGTC to upregulate genes. Hypoxia is shown to stabilize HIF-1α by preventing its degradation, but under normoxic conditions, growth factors have been shown to increase HIF-1 activity by increasing HIF-1α translation. Oxygen tension in the developing follicle has brought about different mathematical models to explain oxygen diffusion across the relative avascular structure of a follicle. A trend in these models show that during two stages in a follicles' life, early and late antral, oxygen levels are hypoxic, reaching a minimum of 11 mmHg. We hypothesize that during follicle deviation when oxygen levels are relatively low, HIF -1 triggers gene expression of luteinizing hormone receptor (LHCGR). Isolated bovine granulosa cells from small follicles <5 mm were cultured in 3% and 20% O2. LHCGR mRNA is upregulated after 4 days in hypoxic culture, treated with 15% follicular fluid from dominant follicles.   Induction of LHCGR in a hypoxic environment must be a physiological occurrence during follicular deviation, a time in which LHCGR first appear on granulosa cells.

Soma Banerjee, Arvinder Kapur, Kai Ludwig, Hannah Van Galder, Mildred Felder, Kevin Eliceri, and Manish S. Patankar.

Tumor cells employ several redundant mechanisms to evade and suppress immune responses. In this study are investigate a novel mechanism by which tumor cells may incapacitate the immune cells by studying the metabolism of immune cells that are co-cultured with ovarian tumor cells. Tumor cells, by expressing high levels of glucose transporters and by developing specific mechanisms to sustain their proliferation by fermenting glucose to lactic acid, consume the available glucose and other carbohydrate nutrients at a very high rate. We therefore hypothesize, that immune cells in the tumor microenvironment, because they have not developed such adaptive mechanisms, are unable to gain and metabolize sufficient carbohydrate nutrients. Lack of nutrients inhibits immune cell proliferation and instead, forces them to survive via autophagy leading to severe loss of mitochondria, the organelle most required for a robust immune response. In essence, we are proposing that tumor-induced immune cell starvation may be yet another mechanism employed by tumors to suppress and evade the immune system. We will provide data on the expression of pyruvate kinase M2 (PKM2) isoform in ovarian cancer cell lines and immune cells. The PKM2 enzyme is overexpressed in most tumors and has recently been shown to be a major adaptation that allows cancer cells to produce high levels of NADH by metabolizing the pyruvate generated in glycolysis to lactic acid. Our data indicates high levels of PKM2 expression in a panel of ovarian cancer cell lines. We are also characterizing ovarian cancer cells and immune cells for the expression of glucose transporters (GLUT1-4) and the relative expression of these transporters in immune cells. We are also estimating the kinetics of glucose uptake by ovarian cancer cells versus immune cells. Flow cytometry, and fluorescent microplate assays have been conducted to look at the uptake of fluorescently-tagged deoxyglucose. Finally, we will also discuss future plans for dual-photon microscopy studies to determine glucose uptake and NADH formation in ovarian cancer cells and immune cells that are maintained under live co-culture conditions.

Heather A. Bankowski, Jennifer Krupp, Dinesh Shah, Ian Bird

Objective: Normal umbilical vein endothelium (UV Endo) develops sustained Ca2+ signaling to support NO mediated vasodilation and so fetal growth. Our previous observations of UV Endo of fresh umbilical cord vessels show the sustained NO production characteristic of normal pregnancy is lacking in DM and PE. While the initial peak and sustained Ca2+ burst response is similar in normal and DM UV Endo, Ca2+ burst signaling is notably impaired in PE UV Endo. Our hypothesis is that these changes in response are due to reprogramming of cell function or simply due to active suppression by local factors/inflammatory mediators.

Study Design: DM in pregnancy was categorized as pre-existing DM (DM) or gestational DM (GDM). HUVEC isolated from normal (N=7), PE (N= 6), DM (N=5) and GDM (N=5) subjects were imaged using Fura-2 to detect Ca2+ in real time in response to stimulation with ATP (100 uM). Like normal UV Endo in vivo, normal HUVEC in vitro showed repeated Ca2+ bursts for up to 30 minutes. While intact PE UV Endo lack sustained Ca2+ bursts in vivo, in PE HUVEC in vitro Ca2+ bursting is restored and frequency enhanced relative to control, but with smaller burst size and lower basal Ca2+. DM HUVEC have Ca2+ bursts that are smaller with lower basal Ca2+ than control and are similar to those from PE patients. Surprisingly, HUVEC from GDM pregnancies show decreased burst number and decreased basal Ca2+.

Conclusion: We herein report that when UV Endo of PE, DM and GDM pregnancies are removed from the in vivo environments and the isolated HUVEC maintained in culture in vitro to passage 3, they all manifest sustained Ca2+ burst responses which are more rapid relative to control HUVEC, suggesting programmed changes in cell signaling. In PE the apparent recovery of function in vitro relative to loss of sustained bursts in vivo implies PE is a suppression of function by local factors, rather than a failure of programming of cell signaling. In DM sustained Ca2+ burst signaling in HUVEC in vitro is consistent with previously reported impaired NO production occurring at the level of active nitric oxide synthase, presumably due to local damage by reactive oxygen species. GDM HUVEC have more impaired Ca2+ burst signaling in vitro relative to DM HUVEC, suggesting unique reprogramming of cell signaling and distinguishing this group from subjects with longstanding DM.

Poster - Pregnancy Enhancement of Endothelial Cell Function is Blocked by VEGF-165 Pretreatment in a Manner Reversible by the MEK/ERK Inhibitor U0126 in Uterine Artery (UA) Endothelial Cells

Derek S. Boeldt, Mary A. Grummer, Ronald R. Magness, Fu-Xian Yi, Ian M. Bird

Introduction: Pregnancy-related increases in endothelial nitric oxide (NO) production are necessary for enhanced vasodilation and so increased blood perfusion of the uterus. Failure of this adaptation can result in preeclampsia and associated fetal growth restriction. Increases in sustained phase Ca2+ signaling are critically linked to increased endothelial nitric oxide synthase (eNOS) activity in response to agonists such as ATP, which stimulates repeated Ca2+ bursts in UA endothelial cells (UAEC) in a manner dependent on Cx43 gap junction function. Vascular endothelial growth factor (VEGF), which is elevated in preeclamptic pregnancies, is a known inhibitor of Cx43 function and we have recently reported VEGF inhibits Ca2+ burst and NO production on subsequent ATP challenge in intact UA endothelium. Herein we examine if VEGF inhibition of subsequent ATP stimulated sustained phase Ca2+ signaling in UAEC is associated with Cx43 phosphorylation and whether it is through VEGFR1 or VEGFR2. **Methods:** Primary UAEC (passage 4) from nonpregnant (NP) and pregnant (P) ewes were grown to 100% confluence on 35 mm glass bottom dishes. Cells were then loaded with Fura-2 and imaged under stimulation with ATP for 30 minutes (control). After washing, the same cells were pretreated as below, followed by a second ATP stimulation. Cx43 data phosphorylation data was acquired by western blot. Results: Pretreatment of P-UAEC with VEGF-165, VEGF-E (VEGFR2-specific), or a combination of VEGF-E and PlGF significantly inhibited ATP stimulated sustained phase Ca2+ bursts (79.6±3.5, 80.7±3.3, 80.3±2.9 respectively, expressed as % control), while PlGF (VEGFR1-specific) alone did not differ from vehicle (91.7±3.8, 90.3±3.2). Incubation with the MEK/ERK inhibitor U0126 completely reversed VEGF-165 inhibition (89.8±3.3). Western blot analysis for Cx43 phosphorylation revealed phosphorylation at s279/282 in response to VEGF-165 and full reversal by U0126. **Conclusion:** We have previously shown that ATP stimulated sustained phase Ca2+ bursts are dependent upon Cx43 function. Herein we propose that VEGF-165 mediates inhibition of sustained phase Ca2+ bursts through VEGFR2, mediated via MEK/ERK signaling, likely via direct phosphorylation of Cx43, with s279/282 being the key phosphorylation site. Funded by NIH HL079020, T32HD41921, HD38843.

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Cytochrome P4501b1 (Cyp1b1) is known as a key mediator in the activation of PAHs to reactive metabolites that initiate cancer and effect immunosuppression. Cyp1b1 is highly expressed in multipotential progenitor cells, in endothelia from various tissues and in macrophage. In endothelia, Cyp1b1 deletion produces increased oxidative stress, which then disrupts capillary morphogenesis, a model of angiogenesis. This disruption is reversed by lowering oxygen levels and by addition of N-acetyl cysteine. Cyp1b1 deletion in mice suppressed diet-induced adiposity, improved insulin sensitivity and blocked hepatic steatosis caused by high dietary fat. Although scarcely expressed in hepatocytes, expression of over 2,000 genes was affected in the liver ($p<0.025$). An appreciable number of responses to high dietary fat are mimicked by Cyp1b1 deletion, while others such as SCD1 suppression are appreciably enhanced by the combination. Among these responses, 31/33 Cyps that are detectable in liver responded to either dietary fat, Cyp1b1 deletion or the combination. This group includes two of the most responsive genes, Cyp4a10 and Cyp4a14, which catalyze fatty acid w-hydroxylation. One of the largest responses is a 6-fold suppression of PPARg by Cyp1b1 deletion, accompanied by near complete loss of constitutive expression of 30 genes that respond to activation of PPARa. Liver PPARg stimulates hepatic lipogenesis, while PPARa stimulates genes associated with fatty acid metabolism. Constitutive PPARg expression and PPARa activation may therefore depend on Cyp1b1 metabolism outside the hepatocyte. The loss of PPAR activity may account for the surprisingly low oxidative stress and steatosis. A model is developed in which Cyp1b1-deficiency in non-parenchymal cells of liver sinusoids modifies signaling to hepatocytes.

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Background: Exosomes are membranous vesicles released by a variety of cell types into the extracellular space. Exosomes typically measure between 40 and 100 nm in diameter and likely contain myriad soluble proteins involved in cellular signaling, cell division, protection and in certain cases, immune evasion. Current theories suggest that exosomes are endocytosed products repackaged as intraluminal vesicles and re-released into the extracellular environment. Hypothesis: We hypothesize that cancer cells release exosomes as a defense mechanism in order to dilute the body's immune response. Exosomes released from tumor cells may function as satellite decoys that prevent immune cells from disrupting the tumor. **Methods**: After a five stage differential centrifugation protocol on human ascites fluid, both the pellet and the supernatant are screened for the presence of Mucin 16 (MUC 16), a known ovarian cancer marker and cell surface protein by western blot analysis. The exosomes are characterized by Scanning Electron Microscopy and their proteome will be exhaustively analyzed by mass spectrometry. **Results**: Western blot analysis using two antibodies indicated the presence of MUC 16 on exosomes culled from human ascites. Exosomes have also been isolated from OVCAR-3 conditioned media and efforts are underway to identify the presence of cancer-specific biomarkers in these exosomes. Preliminary proteomic analysis has demonstrated that our exosomes conform to the empirical definition in terms of contained proteins. **Future Direction**: Further, more detailed, proteomic analysis in addition to scanning electron microscopy will be conducted to confirm that these microparticles are in fact, the first line of defense of the tumor against the body's immune response. Additionally, as a reaction to the body's immune response, exosomes are likely a product of stress. Experiments are ongoing to determine exactly what type of stress, chemical or biological, are able to elicit this phenomenon from tumor cells.

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An oncogenic mutation of the epidermal growth factor receptor (EGFR) discovered in glioblastoma results in a high fidelity duplication of the kinase domain. Whereas wild-type EGFR (WT-EGFR) is expressed at the plasma membrane and is dependent on ligand binding and dimerization for activation and internalization, the tandem kinase duplication mutant (TKD-EGFR) is primarily localized intracellularly in monomeric form and exhibits constitutive tyrosine kinase activity. Recent x-ray crystallographic evidence strongly suggests that activation of WT-EGFR is dependent on the formation of an asymmetric kinase domain dimer, with one monomer acting as an "activator" while the other serves as a "receiver." Disruption of the dimer interface of WT-EGFR has been shown to prevent receptor autophosphorylation. In the case of TKD-EGFR, the presence of two kinase domains occurring in the same monomer may offer a novel in-cis dimerization mechanism for its constitutive phosphorylation. Using site-directed mutagenesis, we are currently creating TKD-EGFR variants possessing point mutations at residues critical for asymmetric dimer formation. If the constitutive phosphorylation of TKD-EGFR is dependent on the formation of an in cis dimer, we hypothesize that destabilization of the dimer interface will result in the elimination of its basal kinase activity

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In the past 10-15 years the worldwide dairy industry has faced significant decline in reproductive performance among its high producing dairy cattle. Declining fertility is comprised of many influential factors, one of which being genetics. Recently, a growing number of large-scale transcriptomic studies have provided evidence of a complex genetic network in the developing pre-implantation embryo; however, there is limited information regarding a family of genes with established ties to early development. Imprinted genes play a critical role in early embryonic, placental, and neonatal growth. Evidence for the importance of proper imprinted gene expression can be seen in animal model studies where disruption or knockouts of particular genes have resulted in low postnatal survival rates in mutant progeny. We hypothesize that imprinted genes play a critical role in the development of the bovine pre-implantation embryo. Our lab has an established bovine embryo model for genetic and genomic interrogations of embryos that develop to the blastocyst stage vs. those that show abnormal and arrested development (deemed degenerate embryos) by day 8 in cattle. Using quantitative real-time PCR eight imprinted genes were detected in the blastocyst and degenerate populations with quantifiable differential expression. NDN, TSSC4, UBE3A, PEG3, and MKRN3 were found to be up-regulated in degenerative embryos showing average 1.5 ± 0.17-fold, 2.0 ± 0.22-fold, 2.0 ± 0.31-fold, 2.4 ± 0.30-fold, and 2.8 ± 0.26-fold differences between pools, respectively. The genes MAGEL2, NAP1L5, and IGF2R showed average 1.3 ± 0.04-fold, 1.5 ± 0.2-fold, and 2.5 ± 0.57-fold up-regulation in blastocysts, respectively. Of those differentially expressed, MKRN3 was the only gene to show statistically significant differences in expression between blastocyst and degenerate embryos ($P = 0.031$). An increasing number of studies have shown the importance and increased sensitivity of imprinted gene expression during the early developmental stages with a 2-3 fold change resulting in early embryonic lethality. Therefore, although the range of fold change in this study is relatively small, the biological significance at this level may result in a detrimental and degenerated phenotype. Thus, these genes serve as candidates for current functional investigations involving RNA interference (RNAi) to assess impacts on embryo's development at the single gene level.

KP Flores, SE Blohowiak, PJ Kling.

Background: Nutritional iron deficiency (ID) during fetal or early postnatal development can alter brain growth and function, especially in the hippocampus. With both rapid growth and erythropoietic need, iron stores in premature infants may be depleted. Erythropoietin (Epo) stimulates erythropoiesis and is a drug to clinically treat the anemia of prematurity, but Epo shifts body iron utilization to red cells. Epo receptors are present on immature brain oligodendrocytes, but Epo may preferentially withhold iron from developing brain in ID. Conversely, Epo also benefits brain development by promoting oligodendrocyte differentiation to myelin-producing cells. **Objective:** The goal of this study was to determine whether erythropoietic doses of Epo alter brain iron or structure under differing iron status. Methods: Sprague-Dawley newborn rats were used to model premature newborns. 4 groups were treated P4-P12: dam fed (iron-sufficient, IS), IS+Epo (425U/kg/d SQ), ID (artificial milk via gastronomy) and ID+Epo. All groups were given ferrous sulfate 6 mg/kg/d. We measured tissue iron and blood indices of iron/erythropoiesis. Fixed brain tissues were stained with Prussian Blue, Luxol Fast Blue and myelin basic protein (MBP). On photomicrographs, we measured pyramidal cell density in the hippocampal CA1 area and estimated cerebellar, fimbria and hippocampal myelination. Results Brain wts (μg/g rat wt) and iron content (μg/g rat wt) were lower in ID vs. IS, $p<0.05$, with IS+Epo slightly higher than IS, $p<0.05$, but ID+Epo similar to ID. Brain iron content was correlated to brain wt, but was also correlated to plasma Epo, $p<0.05$. In the CA1 region, pyramidal densities in ID trended lower, but with no Epo effect. Oligodendrocytes had no storage iron. Qualitative Luxol myelin stain and MBP expression patterns support better myelination in IS, with improvement in +Epo groups. **Conclusions:** ID decreased brain wt and iron content, but erythropoietic-stimulatory doses of Epo either improved or left brain iron unchanged. Brain iron was directly related to steady-state plasma Epo levels, consistent with a study showing better neurological function in Epo-treated premature human infants exhibiting higher plasma Epo levels. Epo did not alter CA1 pyramidal cell proliferation, but improved oligodendrocyte differentiation to myelin producing cells. Additional studies should further explore cell differentiation and brain function.

Poster - Tcf19 is a Novel Transcription Factor Necessary for Beta Cell Proliferation

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Tcf19 is a largely uncharacterized transcription factor that is expressed during cell division, beginning at G1/S phase. In a mouse model of obesity-associated diabetes, we identified tcf19 within a group of cell cycle genes whose expression correlates with adaptive islet proliferation. In confirmation of these microarray data, we observed 3.3-fold upregulation of tcf19 mRNA in islets from obese non-diabetic C57Bl/6J (B6) mice vs. lean (n=5, p=0.01). This obesity-driven upregulation was not observed in islets from the diabetic BTBR strain. We next examined tcf19 expression across a panel of 13 lean B6 tissues and in obese B6 islet. Tcf19 is most highly expressed in islet, which is an unusual pattern of expression for a cell cycle reglator since numerous tissues have higher basal proliferation rates. In addition, in BioGPS human expression profiling, TCF19 is most highly expressed in pancreas (1). We have also identified a non-coding SNP between the B6 and BTBR strains within exon 1 of tcf19. In addition, in BioGPS human expression profiling, TCF19 is most highly expressed in pancreas. This is an unusual pattern of expression for a cell cycle regulator since numerous tissues have higher basal proliferation rates and suggests that tcf19 may play a more specific role in cell cycle regulation in the islet. The role of tcf19 in beta cell growth was then examined. After siRNA-mediated knockdown of tcf19 in INS-1 cells, a significant reduction in the number of viable cells was found. Proliferation was directly measured by 3H-thymidine incorporation and a 40% reduction was found with tcf19 knockdown (n=3, p=0.012). Tcf19 knockdown also led to a decrease in expression of many key cell cycle regulatory genes, including A-, B-, and E-type cyclins, mki67, pbk, cdca3, and bub1 (17-53%, n=3, p<0.05). In summary, tcf19 is necessary for beta cell proliferation and may play a role in obesity-driven proliferation in mouse islets.

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A gonadal steroid-independent increase in GnRH release triggers the onset of puberty in primates. In this study, we first examined the role of kisspeptin/kisspeptin-1 receptor signaling in the pubertal increase in GnRH release by measuring kisspeptin-54 release and the GnRH response to the kisspeptin agonist, human kisspeptin-10 (hKP-10), in microdialysates collected from the stalk-median eminence of prepubertal and pubertal monkeys using an in vivo microdialysis method. Subsequently, we examined whether ovarian steroid feedback can modulate kisspeptin/kisspeptin-1 receptor signaling by assessing the effects of ovariectomy (OVX) with or without estrogen replacement on kisspeptin-54 release and the GnRH response to hKP-10 using the microdialysis method in prepubertal and pubertal monkeys. Results are summarized: 1) A developmental change in kisspeptin-54 release occurred at puberty, as characterized by an increase in mean release, pulse frequency, and pulse amplitude, 2) OVX increased mean kisspeptin-54 release and pulse amplitude in pubertal, but not prepubertal, monkeys, 3) 10 nM hKP10 stimulated GnRH release similarly in both prepubertal and pubertal ovarian intact monkeys, while 100 nM hKP-10 stimulated GnRH release to a greater degree in prepubertal monkeys, 4) the GnRH response to 10 nM hKP10 remained the same after OVX in prepubertal monkeys, whereas OVX eliminated the hKP10-induced GnRH stimulation in pubertal monkeys, and 5) implantation of a silastic capsule containing estradiol partially reversed the effects of OVX on the hKP-10-induced GnRH stimulation in pubertal monkeys. These results suggest that the pubertal increase in kisspeptin-54 release is independent from ovarian steroid feedback, and that kisspeptin/kisspeptin-1 receptor signaling to GnRH neurons undergoes a striking developmental change from an ovarian steroid feedback-independent to - dependent state. Supported by NIH grants HD11355 and T32 HD041921.

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Previous studies have suggested that increases in circulating insulin may increase circulating progesterone (P4) either by reducing metabolism of P4 by the liver and/or increasing luteal P4 production. We tested the effect of insulin on circulating P4 by drenching cows with differing doses of propylene glycol (PG) to increase circulating insulin. Eight pregnant, second lactation Holstein cows were randomly assigned to 4 treatments in an incomplete Latin square design with two periods. Treatments of water or PG were administered every 6 h for 4 d as an oral drench to produce 4 treatment groups: I. Water every 6 h (WATER); II. 300 mL PG every 12 h and water at the intervening 6 h intervals (300X2); III. 150 mL PG every 6 h (150X4); IV. 300 mL PG every 6 h (300X4). Cows were fed ad-libitum twice daily at 0300 and 1500 h to coincide with one of the PG doses; residual feed was removed 6 h after each feeding. Blood samples were collected every 6 h immediately prior to treatments and intensively on Days 2 and 4 (0, 10, 20, 30, 60, 90, 120, 180, 240, and 300 min after drenching) to analyze circulating glucose, insulin, and P4. The intensive sampling was done after two consecutive treatments to allow analysis of PG effects both at the same time as feeding and in the absence of feeding. Daily milk production and dry matter intake were recorded and found to not differ among treatments. Insulin peaked dramatically after drenching with either 150 (2.7-fold) or 300 (4.4-fold) mL of PG compared to drenching with water, which did not result in a rise in insulin. This PG-induced increase in insulin was significant by 10 min after drenching and was maximal between 20 and 30 min after drenching. During the PG treatment in the absence of feeding, insulin decreased toward baseline between 1 h and 4 h after drenching. However, feeding at the time of drenching also caused an increase in insulin that reached maximum 2 h after feeding in all treatment groups. This resulted in a second increase in insulin in 300X4 and 150X4 cows and a single increase in 300X2 and WATER cows that did not reach basal levels before the completion of the intensive sampling period. Drenching with either 150 or 300 mL of PG induced a biologically small, but statistically significant increase in glucose concentrations. When insulin concentrations were analyzed in samples collected every six hours, it was found that 300X4 cows were significantly higher than 150X4 and WATER cows, but not 300X2 cows. Surprisingly, circulating P4 was not affected by treatment when analyzed either on a 6-h basis or during the intensive sampling period. Thus, although dramatic, acute increases of >2-fold in circulating insulin concentrations were induced by drenching with PG, there was no detectable change in circulating P4. This was unexpected and is not consistent with our original hypothesis that PG-induced increases in circulating insulin will increase circulating P4. Thus, acute insulin increases appear to not decrease hepatic P4 metabolism and/or increase luteal P4 production in pregnant, lactating dairy cows. However, the results of this work indicate that using propylene glycol at a rate of 300ml every 6 h is an effective way to increase circulating insulin concentrations and could be used in future research.

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Background: Iron is required for in utero development of the central nervous system. The fetus receives iron through a complex placental transport mechanism facilitated by membrane proteins, including divalent metal transporter 1 (DMT1). Interrupting this mechanism may compromise fetal neurodevelopment. Lead, in addition to possessing gender-specific, neurotoxic properties, interferes with iron-DMT1 binding. **Objectives**: The present study investigates the gender-specific, gestational interaction between iron and lead using the umbilical cord blood of newborns at-risk for iron deficiency. We hypothesized that cord blood lead levels (BLLs) would correlate to cord blood iron status in a gender-specific manner. Methods: In a prospective study of 259 term newborns at-risk for infantile iron deficiency, cord BLLs and iron measures were studied. Risk factors, which were evaluated using medical records and a postnatal retrospective stress questionnaire, included abnormal fetal growth, maternal diabetes mellitus (DM), psychosocial stress, and iron deficiency anemia (IDA). Cord blood iron status was established by measures of RBC iron (zinc protoporphyrin/heme), storage iron (plasma ferritin), and transport iron (plasma transferrin). Cord BLL was measured by Inductively Coupled Plasma Mass Spectrometry. **Results**: The cord BLLs were neither elevated nor gender-specific. Maternal DM and high maternal stress did not affect cord BLLs, while maternal IDA lowered cord BLLs, $p<0.05$. Collectively, cord BLLs correlated to worsening RBC iron, $p<0.01$, but a gender-specific analysis revealed males dominated this correlation. Maternal DM, stress, and IDA caused a direct correlation between cord BLLs and worsening RBC iron in males, while maternal IDA caused an inverse correlation in females. Cord BLLs were not correlated with measures of transport or storage iron. **Conclusion**: Our findings show males have a stronger correlation between impaired RBC iron and cord BLLs. Although BLLs were low, our work supports the previously suggested phenomenon that lead neurotoxicity is gender specific, with greater deficit in male neurodevelopment than female, despite comparable BLLs. Moreover, our work suggests an interaction between lead neurotoxicity and RBC iron in males. Maternal IDA, with prenatal iron prescribed, may be protective, causing lower BLLs. Our work supports that the interplay between iron and lead status early in infancy is complex, gender-specific, and requires further physiological study.

Kentaro Hayashi & Craig S. Atwood

Apolipoprotein E (APOE) ε4 allele is reported as the major risk factor for late-onset Alzheimer's disease (AD), though ~50% of AD patients do not carry the allele. The function of APOE is to transport cholesterol for luteinizing hormone (LH)-regulated steroidogenesis, and both LH and neurosteroids have been implicated in the etiology of AD. In our previous study, we scored AD DNA samples and age-matched control samples for APOE genotype and 14 single nucleotide polymorphisms (SNP) of LHB and LHCGR. Thirteen gene-gene interactions between the loci of LHB, LHCGR, and APOE were associated with AD which was detected using all three interaction analyses: linkage disequilibrium (LD), multifactor-dimensionality reduction (MDR), and logistic regression (LR). Newly, SORL1, a cell membrane protein of the low-density lipoprotein receptor family that adjusts the flow of amyloid-β precursor protein (AβPP) in neurons, has been identified as an AD risk factor. Since SORL1 may influence cholesterol metabolism and steroid synthesis, we examined two AD-associated SORL1 polymorphisms (rs2298813 and rs2282649) in DNA samples from our case-control cohort. We found an interaction between SORL1 (rs2282649) and gender, such that females with this SORL1 SNP were 1.4 fold more likely to have AD [OR = 2.2667 (0.9882, 5.1993); p=0.017]. Our future studies will examine SNPs in other steroidogenic pathway family member (SPFM) genes that might alter steroidogenesis and assess their association with AD. In addition we will increase our sample size by obtaining samples from the National Cell Repository for Alzheimer's Disease, Wisconsin Brain Donor Program and the Framingham Heart Study, from which GWAS data can also be obtained and analyzed. We will test for single main factor effects as well as gene-gene interactions using recursive portioning, logistic regression and multi-dimensionality reduction analyses. These studies will determine which gene-gene interactions predict AD.

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The current understanding of the cellular and molecular effect of hypoxia on endothelial cells is primarily obtained using cell models established under 20% O2. However, endothelial cells reside in a low oxygen microenvironment. Here, we established human umbilical vein endothelial (HUVE) cell cultured in normoxia (N; 20% O2) and hypoxia (H; 3% O2, which is within the range of physiological level of oxygen in human placentas). We tested if chronic hypoxia (~25 days) promotes FGF2-and VEGF-induced cell migration and proliferation via enhancing activation of the MEK/ERK1/2 and PI3K/AKT pathways and/or via elevation of HIF1α protein levels. Method: Cell migration and proliferation was evaluated by a Transwell system and a BrdU kit, respectively. Phospho-ERK1/2 & AKT were examined by Western blotting. PD98059 and LY294002 were used to determine the role of the MEK/ERK1/2 and PI3K/AKT pathways in HUVE cells cultured in N (N-HUVE) and H (H-HUVE). N-HUVE cells were infected with adenoviruses carrying HIF1α (AdHIF1a) or GFP (AdGFP). H-HUVE cells were transfect with a scrambled or HIF1α siRNA. Results: 1) Chronic hypoxia promoted HUVE cell proliferation and migration. 2) Both FGF2 and VEGF rapidly stimulated ERK1/2 and AKT phosphorylation in N and H. H greatly enhanced FGF2- and VEGF-induced ERK1/2 phosphorylation vs. N, whereas enhanced only VEGF- but not FGF-induced AKT phosphorylation. PD98059 and LY294002 inhibited FGF2- and VEGF-induced phosphorylation of ERK1/2 and AKT, respectively. LY294002 significantly decreased FGF2- and VEGF-induced H-HUVE and N-HUVE cell proliferation; however, PD98059 decreased FGF2- and VEGF-induced H-HUVE, but not N-HUVE cell proliferation. PD98059 and LY294002 significantly decreased FGF2- and VEGF-induced H-HUVE and N-HUVE cell migration. 3) As compared to the AdGFP or scrambled siRNA control, AdHIF1α and HIF1α siRNA, respectively, increased and decreased HIF1α protein levels. AdHIF1α enhanced FGF2- and VEGF-induced N-HUVE cell proliferation; however, HIF1α siRNA did not affect H-HUVE cell proliferation. Conclusions: chronic hypoxia promotes HUVE cell proliferation and migration via enhancing activation of the MEK/ERK1/2 and PI3K/AKT pathways, but not via elevation of HIF1α protein levels. MEK/ERK1/2 and PI3K/AKT pathways differentially regulate H-HUVE and N-HUVE cell migration and proliferation.

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Introduction: Endothelial proliferation, a sensitive index of angiogenesis, is a cardinal feature of gestational vasuclar adaptations such as angiogenesis. We recently reported that the catecholestradiols 2- & 4-hydroxyestradiol (2-OHE2 & 4-OHE2) increase pregnant uterine artery endothelial cell (P)-UAEC proliferation/angiogenesis via estrogen receptor-independent mechanisms; thus the underlying receptors & signaling mechanisms are unknown. Since catecholestradiols exhibit structural similarities and functional interactions with catecholamines norepinephrine (NE) & epinephrine and have affinities for α- and β-adrenergic receptors (ARs), we investigated if α- or β-ARs mediate 2-OHE2 or 4-OHE2-induced proliferation of P-UAECs and the involvement of adrenergic catecholamines to these effects. Therefore, we hypothesized that 2-OHE2 & 4-OHE2 mediate in vitro P-UAEC proliferation via either α-AR or β-AR G-protein-coupled receptor (GPCR) signaling pathways (cAMP or MAPKs) & thus NE & E will partly modulate these effects. **Methods**: Identification of AR subtypes (α1, α2, β1, β2 and β3) in P-UAECs was performed by Western Blotting. Dose-response was evaluated with NE or E (0, 0.1, 1, 10 0r 100nM) or with treatments (0.1nM, 24hr) of 2-OHE2 or 4-OHE2 in combination with NE or E. The interactive effects of 2-OHE2 and 4-OHE2 with NE and E on P-UAECs mitogenesis with or without AR blockers (10μM, 1hr; α-ARs, phentolamine; β-ARs, propanolol; α1-ARs, Prazosin; α2-ARs,Yohimbine; β1-ARs, Betaxolol; β2-ARs, ICI 118,551; β3-ARs, SR 59230A) or MAPK blockers (p38, SB203580; p42/44, PD98059). Following AR subtype specificity determination, activation of β-ARs were evaluated (1μM, 1hr) with Isoproterenol (both β2/β3ARs), Formoterol (β2-ARs), or BRL37344 (β3-ARs). Proliferation was evaluated utilizing BrdU. cAMP production was assessed by ELISA (validated w/Forskolin). Results: P-UAECs express α2, β2 & β3, but not α1 & β1-ARs. 2-OHE2 & 4-OHE2 increased (group X dose, P<0.01) P-UAEC mitogenesis & this was unaltered by phentolamine (P> 0.05). Propanolol, ICI 118,551, SB203580, & PD98059 abrogated (Group X antagonist P<0.01) proliferative effects of 2-OHE2 & 4-OHE2; lesser inhibition seen with SR 59230A. NE dose-dependently increased P-UAEC proliferation, whereas E induced proliferation decreased with increased doses. NE & E-induced proliferations were inhibited by propanolol, but not by phentolamine. Combined treatments of 2-OHE2 or 4-OHE2 with NE or E increased (P<0.01) proliferation compared to 2-OHE2, 4-OHE2, NE or E alone. Nonselective activation of β-ARs by Isoproterenol as well as selective activation of β2- and β3-ARs by Formoterol and BRL37344 respectively all dose-dependently induced P-UAEC proliferation. 2-OHE2, 4-OHE2, NE, E & Forskolin all induced significant production of cAMP in P-UAECs (dose X time, P< 0.01). 2-OHE2 & 4-OHE2 treatments induced time course-specific phosphorylation of p38 & p42/44 MAPKs. **Conclusions**: We provide novel evidence that the endothelial β2- and β3-ARs are critically involved in the regulation of catecholestradiol- and catecholamine-induced endothelial proliferation and this interaction points to the convergence of catecholestradiols and catecholamines in promoting vascular angiogenesis in pregnancy vasuclar physiology and in impaired angiogenesis such as in preeclampsia. NIH HL49210, HD38843, HL87144, R25-GM083252, T32-HD041921-07

Brian P. Kenealy, Kim L. Keen, and Ei Terasawa

Previously, we have reported that 17β-estradiol (E2) causes a rapid stimulatory action in primate GnRH neurons. E2 increases the frequency of intracellular calcium ($[Ca2+]i$) oscillations, their synchronization, as well as GnRH release within 10 minutes. This rapid E2 action is, in part, mediated by a G-protein coupled receptor, GPR30, as a GPR30 agonist, G1, mimicked E2 action while GPR30 specific siRNA blocked E2-induced $[Ca2+]i$ oscillations (Noel et al., Mol Endocrinol 2009). However, the source of an E2-induced increase in $[Ca2+]i$ concentration is unknown. Therefore, the aim of this study is to determine 1) whether entry of extracellular calcium plays a role and/or 2) whether E2 signaling results in calcium release from intracellular calcium stores.   In order to investigate the sources of calcium involved in E2 rapid action, four experiments were performed. 1) To determine the role of extracellular calcium on E2-induced GnRH release, a low calcium buffer was perifused starting 5 min prior to a 20 min E2 application and continued for 60 min after E2 administration, while perifusates were collected and GnRH in perifusates were measured by RIA. 2) The involvement of inositol 1,4,5-triphosphate-receptor (IP3-R) sensitive $[Ca2+]i$ stores on GnRH release was determined by perifusing the PLC inhibitor, U73122 (10 µM) starting 10 min prior and during a 20 min E2 application, followed by a 60 min wash out period and GnRH in perifusates were measured by RIA. 3) The role of IP3-R sensitive stores in rapid E2-induced increase in $[Ca2+]i$ was assessed by application of U73122 (10 µM) 5 min prior and during a 10 min application of E2, $[Ca2+]i$ levels were measured by calcium imaging using fura-2 AM and a mixture of pluronic F-127. 4) Using calcium imaging, the role of RyR sensitive $[Ca2+]i$ stores in E2-induced increase in $[Ca2+]i$ was determined by application of the RyR inhibitor, ryanodine (50 µM), 10 min prior and during a 10 min application of E2.   Results are as follows: 1) E2-induced GnRH release failed to occur in a low calcium buffer; 2) Treatment with U73122 (10 µM) did not block E2-induced GnRH release or $[Ca2+]i$ oscillations; and 3) Ryanodine (50 µM) significantly blocked E2-induced changes in $[Ca2+]i$ oscillations while ryanodine alone had no effect. The results are interpreted to mean that entry of extracellular calcium and release of RyR sensitive stores, but not IP3-R sensitive stores, are important for the rapid stimulatory action of E2 in primate GnRH neurons.

Carly Kibbe, Emery Bresnick and Anath Shalev

Chronic hyperglycemia, a hallmark of both type 1 and type 2 diabetes mellitus, leads to a number of pathological problems including beta cell apoptosis. We have previously shown that human pancreatic islet cells exposed to high levels of glucose have increased expression of thioredoxin-interacting protein (TXNIP). TXNIP binds and inhibits thioredoxin, a redox regulator, resulting in increased cell apoptosis. Using mouse models, we recently found that TXNIP deficiency inhibits beta cell apoptosis and protects against type 1 and type 2 diabetes. These results suggest that TXNIP is an attractive target for therapies aimed to stop beta cell apoptosis in diabetic patients, however more must be known about the mechanisms regulating TXNIP. The forkhead box O1 transcription factor (FOXO1) has been reported to regulate the expression of TXNIP, but the effects seem to be tissue-dependent and the effect of FOXO1 on TXNIP expression in the beta cell remains to be elucidated. Therefore, we first demonstrated that FOXO1 binds the TXNIP promoter in both human islet cells and rat insulinoma (INS-1) cells, as assessed by Chromatin Immunoprecipitation (ChIP) assays. Using transient transfections of a FOXO1 expression plasmid we further showed that overexpression of FOXO1 in INS-1 cells significantly decreases endogenous TXNIP transcript levels. Using luciferase reporter assays we also demonstrated that FOXO1 overexpression reduces TXNIP promoter activity in INS-1 cells. However, a promoter deletion analysis revealed that rather than the known consensus FOXO1 binding site a downstream E-box-like motif was responsible for FOXO1 regulation of TXNIP. In fact, we were able to show that this E-box-like motif is not only necessary, but also sufficient for FOXO1 mediated repression of TXNIP. We have previously shown that glucose-induced TXNIP expression is mediated through carbohydrate response element-binding protein (ChREBP) binding to this E-box-like motif in the TXNIP promoter. Using another set of luciferase reporter assays we demonstrated that FOXO1 overexpression blocks glucose-induced TXNIP expression. Furthermore, using ChIP assays we showed that FOXO1 overexpression is able to block the glucose-induced increase in ChREBP binding to the TXNIP promoter. Using a luciferase reporter assay employing a FOXO1 DNA-binding mutant (FOXO1 H215R) we further showed that DNA-binding is required for FOXO1 mediated repression of TXNIP. Together, these results demonstrate for the first time that FOXO1 down regulates TXNIP expression in pancreatic beta cells, and suggest that FOXO1 acts by competing with ChREBP for DNA binding.

Yan Li, Kai Wang, Yi-zhou Jiang, Cai-feng Dai, Manish S. Patankar, Jiasheng Song, Jing Zheng

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor, mediating many biological processes and toxic effects upon binding to its ligands. One of classic AhR ligands is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), an environmental carcinogen. Little is known about roles of the AhR in human ovarian cancer. Herein, we examined expression of the AhR in human normal and malignant ovarian tissues using the tissue microarray and determined if the AhR participated in mediating ovarian cancer growth in vivo and in vitro using a mouse xenograft model and OVCAR-3 and SKOV-3 cells, two human ovarian cancer cell lines. We found that the AhR was immunolocalized in many histotypes of ovarian cancer and was expressed in OVCAR-3 and SKOV-3 cells, but not in normal ovarian tissues. As compared with the control (n = 8 animals), 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE, an endogenous AhR ligand, at 80 mg/kg body weight) time-dependently suppressed ($p < 0.05$) growth of OVCAR-3 cells (by > 50% in size at Day 28 of treatment; n = 8) in mice without any significant side effects. Both ITE and TCDD in vitro also dose- and/or time-dependently inhibited ($p < 0.05$) OVCAR-3, but not SKOV-3 cell proliferation, while only ITE, but not TCDD inhibited ($p < 0.05$) SKOV-3 cell migration. The estimated IC50 for OVCAR-3 cell proliferation were 0.2 and 4.6 nM, respectively for ITE and TCDD. A single dose of ITE and TCDD decreased ($p < 0.05$) AhR protein levels and increased ($p < 0.05$) CYP1A1 protein levels in SKOV-3 and OVCAR-3 cells, indicating activation of the AhR. Knockdown of the AhR using the special AhR siRNA blocked ($p < 0.05$) both ITE-inhibited and TCDD-inhibited OVCAR-3 proliferation and ITE-suppressed SKOV-3 migration. These data indicate that ITE and TCDD can differentially suppress human ovarian cancer cell proliferation and/or migration via the AhR, in which ITE is ~ 23 fold more potent than TCDD in its effect on anti-cancer cell proliferation. Thus, given its naturally producing feature and specific binding to the AhR, ITE can potentially be used for therapeutic intervention of human ovarian cancer, the most lethal gynecological cancer. Supported by the NIH HL64703 and HD38843 (JZ).

Maguire M, Bushkofsky J, Jefcoate CR

Cytochrome P450 1b1 (Cyp1b1) is a mono-oxidase metabolizing enzyme with a wide variety of substrate affinity including xenobiotics, estrogen, and retinol. It is expressed in endothelial cells, macrophage and numerous tissues including, the eye, liver, kidney, brain and sex-steroid responsive tissues, such as the breast and prostate. Cyp1b1 has the ability to catalyze the conversion of retinol (Vitamin A) to retinaldehyde and further to the bioactive metabolite, retinoic acid; however, it cannot metabolize RA into inactive compounds as performed by the Cyp26 family. In addition the embryonic expression pattern overlaps with certain retinoid-producing tissues which do not express the main RA generating enzymes of the Raldh family. In particular the hindbrain, expresses Cyp1b1 predominantly in rhombomere 4. When Cyp1b1-/- mice are challenged in utero with a maternal Vitamin A deficient (VAD) diet beginning in early gestation and maintained through the postnatal period, pups exhibit a decreased weight at weaning. It is unclear yet as to whether this is attributable to an overall decrease in size or distinct organ development. The combined insult of the Cyp1b1 deficiency and the VAD diet induces changes in the growth and development of the embryo that are present at weaning. In the future it will be advantageous to elucidate some of the gene expression patterns of major players in the retinoid pathway and their changes in a Cyp1b1 deficient mouse with and without exposure to a VAD diet.

Poster - Stability of Zinc Protoporphryin/Heme Ratios (ZNPP/H) on Filter Paper as a Newborn Screen for Tissue Iron Deficiency (ID)

S. L. Marmer, M.E. Chen, B.A. Fischer, S. E. Blohowiak, P.J. Kling

Background: ID, a common nutrient deficiency, impairs brain development. With earlier identification of at-risk children, long-term neurocognitive deficits could be prevented. ZnPP/H is an available, cost effective, and sensitive biomarker of iron status. ZnPP/H, a candidate for newborn screening of iron deficiency, is measurable on washed umbilical cord blood and potentially on filter paper blood spots. Bilirubin interferes with ZnPP/H readings, but interference is reduced with Bilirubin Oxidase (BO). Objective: To examine whether ZnPP/H from filter paper specimens is comparable to washed blood specimens. **Methods:** De-identified cord blood samples were collected at birth in EDTA and whole blood and washed ZnPP/H ratios were measured by hematofluorometry. Blood was spotted onto Whatman 903 filter paper. Specimens were dried and eluted from the paper using different reagents; PBS elution (control), superoxide dismutase (SOD) and catalase to prev ent oxidation of heme from lysed RBCs, and BO to prevent heme breakdown to bilirubin and other autofluorescent molecules. All treatments were repeated at 1, 2, and 7 days to measure the ratio's stability over time. **Results:** Filter paper ZnPP/H ratios were higher when eluted, compared to rinsed blood. With $\leq$ 24 hrs on filter paper, elution with PBS was highly correlated with rinsed blood R2=.74, p<0.01, with slight improvement from SOD/Catalase, R2=.81, p<0.01, as well as BO, R2=.85, p<0.01. With increased time on filter paper, variability of the readings increased and the correlations were weakened. Compared to control PBS, SOD/Catalase and BO yeildeded stronger correlations which persisted over time. **Conclusion:** With increasing time on filter paper ZnPP/H readings deteriorated. The relationship between filter and washed ZnPP/H deteriorated less rapidly when eluted with either SOD/Catalase or BO. Although it is unclear what biochemical mechanisms are responsible for this deterioration, our treatments prevented both heme oxidation and heme breakdown. Further studies are needed to determine the biochemical changes in ZnPP and heme on filter paper, but using filter ZnPP/H on dried blood spots may still be feasible.

Poster - Effects of Pregnancy on Uterine Artery Connexins: A Study of Domain Specific Partitioning

Timothy J Morschauser, Jayanth Ramadoss, Wu-xiang Liao, Dong-bao Chen, Ian M Bird, Ronald R Magness

INTRODUCTION: Uterine vascular adaptations facilitate rises in uterine blood flow (UBF) during pregnancy which is partly mediated by gap junction (GJ) connexin (Cx) proteins and eNOS. In systemic vasculature, the caveolar (cav) subcellular domain, plays a role in Cx GJ communication. However, no study has investigated the partitioning of Cx proteins to the cav domain, the home for eNOS, & their dynamics upon ATP treatment. It is currently thought that ATP regulates eNOS in a manner dependent on Cx function. **HYPOTHESIS**: We hypothesized that compared to the nonpregnant (NP) uterine artery endothelium (UAE), GJ proteins Cx 43 & 37 expression is elevated during pregnancy (P) and will exhibit domain-specific partitioning between cav & noncav subcellular pools. **METHODS**: UAE from uterine vessels were isolated from NP (luteal,n=7) & P (120-130d,term=147d;n=31) ewes. Domain-specific studies were performed using cultured uterine artery endothelial cells from P ewes. Domain partitioning was studied in response to ATP (100mM,5 min). Cav enrichment was done using sucrose gradient ultracentrifugation. Data were analyzed using LC/MS/MS & validated by Westerns. **RESULTS**: Compared to luteal, Cx43 & Cx37 protein levels in UAE from P ewes increased by 25.8 & 10.3 fold, respectively ($P<0.001$). Subcellular domain analyses in P UAECs via LC/MS/MS revealed the presence of Cx43, but not Cx37 in the cav. Western validation of Cx43 showed a significant main effect of subcellular domain; Cx43 expression was 2.9 fold more abundant in the cav vs. noncav pool ($P=0.006$). An effect of ATP or interaction was not noted. However, pairwise analysis revealed a significant domain-specific ATP effect; ATP led to a 1.7 fold increase in the cav Cx43 distribution ($P=0.013$). Cx37 was not detected in the cav domain but was located only in the noncav pool and was unaltered by ATP. **CONCLUSIONS**: There is a P-associated increase in UAE expression of Cx43 & Cx37. A subcellular domain distribution was noted with both Cx43 & Cx37 expression; Cx43 is predominately located in the cav & Cx37 in the noncav pool. ATP resulted in an increase in cav Cx43 levels suggesting rapid trafficking of Cxs to the caveolae and an interaction with caveolar eNOS. These data suggest an important role for Cxs in delineating mechanisms for increases in UBF during pregnancy.NIH HL49210,HD38843,HL87144

Phillips AK, Roy SC, Fischer BA, Blohowiak SE, Coe CL, Kling PJ

Introduction: Up to 30% of women of childbearing-age in the U.S. are obese. Obesity increases risk for poor iron status in non-pregnant adults, because obesity-related inflammation impairs intestinal iron absorption. In the last trimester of pregnancy, iron needs increase 4-fold. The placenta uses the same iron transporters as the intestine, but the effects of maternal obesity on newborn iron status are unknown. **Hypothesis**: Because obesity impedes intestinal iron absorption, we hypothesize that maternal obesity impedes placental iron transport to the fetus. **Methods**: In a study of newborn iron deficiency anemia (IDA), sensitive measures of cord blood iron included zinc protoporphyrin/heme (ZnPP/H) and reticulocyte-enriched ZnPP/H (RE ZnPP/H). RE ZnPP/H measures iron in reticulocytes, the youngest red cells, thereby improving ZnPP/H sensitivity. Cord C-reactive protein (CRP) assessed neonatal inflammation. Maternal body mass index (BMI) was calculated and BMI $\geq 30 kg/m2$ defined obesity. Results: 281 mother/newborn pairs underwent informed consent and were studied. Higher cord RE ZnPP/H was seen with maternal obesity at delivery ($p<0.05$). BMI at delivery and RE ZnPP/H exhibited a weak direct linear relationship ($R=0.14$, $p<0.05$). The prevalence of maternal IDA was similar in obese and non-obese mothers. No relationship was observed between BMI and cord CRP. Diabetes was more prevalent with obesity ($p<0.05$). **Conclusions**: This study is the first to observe a link between maternal obesity and impaired newborn iron status, a vital finding due to the prevalence of obesity. Our results support iron transport reduction in late pregnancy when iron needs are highest. It is unlikely that longstanding maternal IDA caused the fetal iron impairment, as cord iron status was similar in mothers with and without IDA. Fetal CRP was not elevated, but maternal inflammation might independently prevent the massive iron absorption necessary at the end of pregnancy. Obtaining maternal blood may be useful to examine this potential mechanism. The co-morbid conditions of diabetes and obesity may contribute as well, given the known risk for impaired iron status in infants born to women with diabetes. This relationship was previously unrecognized and further research is required to determine the full impact of maternal obesity on infant wellbeing.

Quilling AT, Pittner C, Blohowiak SE, Kling PJ

BACKGROUND: Transferrin, a protein that transports iron, is found in the small intestine and is critical to iron metabolism in mammals. Concentrations of transferrin and its receptor may be influenced by erythropoietin, a hormone found in milk that stimulates iron usage in red blood cells. This experiment is designed to examine how transferring (Tf) and transferrin receptor (TfR) concentrations in newborn rats are affected by ingested doses of erythropoietin (Epo). Our hypothesis was that both Tf and TfR concentrations would be greater in both iron-deficient and normal rats that were fed Epo in milk. **METHODS**: Both iron-deficient (ID) and normal (Dam) rats were treated with either Epo or a control treatment for twelve days following birth. Concentrations of blood Tf and intestinal TfR were then measured using immunoassays, Western blots and immunohistochemistry staining. Total body weights and duodenal weights were also recorded. **RESULTS**: Total body weights were not significantly different between treatment groups. Duodenal weights were greater in ID rats given both treatments than in Dam rats. TfR expression was significantly higher in ID rats given Epo than in ID rats given control treatments. More results are currently being obtained. **CONCLUSIONS**: Epo appears to increase TfR concentration in the duodenum. This implies that Epo may have potential as an adjunct treatment for iron deficiency in newborns.

Mary Y. Sun, Jason M. Habeck, Jill M. Koch, Katie Meyer, Ronald R. Magness, Pamela J. Kling

Background: In humans and rodents, transferrin receptor (TfR) is regulated by nitric oxide (NO)-associated iron regulatory proteins (IRP) 1 & 2. TfR-mediated placental iron transport is further controlled by fetal liver iron status. However, little is known if NO/endothelial NO synthase (eNOS) regulates placental TfR expression during normal or compromised (IUGR) pregnancies. We hypothesized that, in compromised pregnancies, placental TfR expression is altered and associated with eNOS expression. **Methods**: Compromised IUGR sheep fetuses (USR) were produced with triplet or quadruplet gestations or twins in a space restricted uterus (single unilateral uterine horn ligation; Meyer et. al, BOR 2010). USR fetuses were compared to non-space restricted (NSR) controls at gestation day (GD) 120 and 130 (term=147). Fixed placentomes were stained in hematoxylin, DAB, and a CD-71 TfR antibody for immunohistochemistry (IHC). Immunoblotting was performed for TfR and eNOS expression. Liver tissue iron was quantified with a non-heme iron assay. **Results**: IHC and immunoblot showed TfR in both NSR and USR placentomes, but the most staining was at GD130 in the NSR group. eNOS immunoblot expression increased with gestation and was directly correlated with TfR expression (r=0.726 P=0.0003). Additionally, the pattern of fetal liver iron content paralleled that of placentome TfR expression. **Conclusions**: Our data support that, in addition to fetal liver iron, placental eNOS appears to be involved in the regulation of TfR expression. Investigation of placental iron transport should interrogate the interaction of eNOS and TfR in normal and compromised sheep pregnancies. NIH HL49210, HD38843, HL87144+Supplement

Chanel Tyler, Jennifer A. Belisle, Christine Trautman, Jennifer A.A. Gubbels, Joseph P. Connor, and Manish S. Patankar

During immune surveillance immune cells sample different antigens and respond to these molecules by directly binding to them via cell surface receptors, internalizing and processing the antigens, displaying specific epitopes of the antigens to harness immune responses from other mediators. Interactions with the antigens result in specific alterations of the immune cell transcriptome and proteome. We hypothesized that the changes in the transcriptome and proteomes of the immune cells in response to the pathogenic or cancer antigens may serve as disease-specific biomarkers. Here, we provide evidence in support of this hypothesis by comparing the binding of the mucin MUC16, a carrier of the tumor marker CA125, and the immune cell receptor Siglec-9 in ovarian cancer patients, healthy pregnant women and patients with preeclampsia. MUC16 binds to Siglec-9 via its •2-3-linked sialic acid residues and is retained on the surface of select subsets of immune cells. Immune cells from the peripheral blood of three cohorts indicate a distinct pattern of binding of MUC16 to the CD16pos/CD56dim and CD16neg/CD56pos NK cells. In ovarian cancer patients the binding occurs predominantly to the CD16pos/CD56dim NK cells, equal binding is observed among the two subsets in healthy pregnant women, whereas in preeclampsia, significantly higher levels of MUC16 are found on CD16neg/CD56pos NK cells. These differences in the binding pattern of MUC16 indicate that the detection of specific molecules in the total immune cell population followed by their presence in specific subsets of immune cells may provide a novel two-tiered approach for detecting biomarkers for early detection and monitoring of benign and malignant diseases.

Name	Research Interests
Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome
Alarid, Elaine	Estrogen response
Atwood, Craig	Hormone regulation of aging and Alzheimer's Disease
Barry, Terence	Aquaculture, fish reproduction
Bertics, Paul	Cell regulation and proliferation by growth factors
Bird, Ian	Uterine blood flow, eNOS, Adrenal Steroidogenesis.
Blank, Robert	Identifying genes that contribute to differences in bone's biomechanical performance
Bosu, William	Folliculogenesis, Corpus luteum function
Cezar, Gabriela	Stem cell safety
Davis, Dawn	Basic and translational research on diabetes and obesity
Downs, Karen	Developmental and genetic control of fetal and extraembryonic lineage formation during mouse gastrulation, use of mammalian stem cells in gene therapy
Drezner, Marc	Phosphatonins, Hormones
Duello, Theresa	Health Disparities in Underrepresented Populations
Ginther, Oliver	Equine reproduction
Golos, Thaddeus	Placenta biology, stem cells
Jefcoate, Colin	StAR protein
Jorgensen, Joan	Gonad formation
Kessel, Julie	Neonatology
Khatib, Hasan	Genomic imprinting, genetic development of embryos in cattle, genetic traits that impact health and milk quality in cattle.
Kling, Pamela	Neonatal development, Growth factors
Kreeger, Pamela	The use of mathematical, and computational techniques to address cellular signaling questions relevant to women's health

Name	Research Interests
Levine, Jon	Polycystic Ovary Syndrome
Liu, Bo	Molecular mechanism underlying vascular inflammation, molecular mechanism underlying occlusive vascular diseases, and development of new materials for biomedical applications (gene delivery and vascular grafts)
Magness, Ronald	Shear stress
Martin, Thomas	Cell Signaling, neuropeptides
Ntambi, James	Genetic regulation of metabolism
Odorico, Jon	Stem cells, Pancreatic islet development
Parrish, John	Sperm regulation and function, Equine Reproduction
Patankar, Manish	Epithelial Ovarian Cancer (EOS)
Pelegri, Francisco	Cellular and molecular level processes involved in early vertebrate development
Peterson, Richard	Prostate disease
Salih, Sana	Molecular Determinants of Oocyte Development, Fertilization, and Early Embryogenesis in Humans
Schuler, Linda	Prolactin, Growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension
Terasawa, Ei	Neuroendocrinology
Thomson, James	Stem Cells
Vezina, Chad	Prostate Disease
Watters, Jyoti	Molecular mechanisms employed by microglia, Central Nervous System
Wiltbank, Milo	Hormonal interaction; intracellular regulation of cell death and steroidogenesis in the corpus luteum; regulation of ovarian function in dairy cattle.
Xu, Wei	Dissecting the epigenetic mechanisms controlling estrogen responsiveness
Zheng, Jing	Endothelial cell function

Student	Degree Goal	Research Advisor
Roxanne Alvarez	Ph.D.	Bird
Soma Banerjee	M.S.	Patankar
Heather Bankowski, DO	M.S.	Bird
Derek Boeldt	Ph.D.	Bird
Justin Bushkofsky	Ph.D.	Jefcoate
Nick Claussen	Ph.D.	Patankar
Luca Clemente	Ph.D.	Bertics
Jeff Denney, MD	Ph.D.	Shah
Ashley Driver	Ph.D.	Khatib
Danielle Fontaine	Ph.D.	Davis
Kate Guerriero	Ph.D.	Terasawa
Katherine Hackbart	Ph.D.	Wiltbank
Kentaro Hayashi	Ph.D.	Atwood
Beverly Hutcherson	M.S.	Abbott
Igor Iruretagoyena, MD	Ph.D.	Shah
Yizhou Jiang	Ph.D.	Zheng
Omar Jobe	Ph.D.	Magness
Brian Kenealy	Ph.D.	Terasawa
Carly Kibbe	Ph.D.	Shalev / Bresnick
Jasmin Kristianto	Ph.D.	Blank
Jennifer Krupp, MD	M.S.	Bird
Jinwoo Lee	Ph.D.	Jefcoate
Samantha Lewis	Ph.D.	Jorgensen
Yan Li	Ph.D.	Zheng
Ka Yi Ling	Ph.D.	Downs
Meghan Maguire	Ph.D.	Jefcoate
Matt Millette	Ph.D.	Dent
Tim Morschauser	M.S.	Magness
Gopika Nair	Ph.D.	Odorico
Mayra Pastore	M.S. / Ph.D.	Magness
Ann Rozner	Ph.D.	Golos
Soraya Silva	M.S. / Ph.D.	Wiltbank
Mian Shazhad, MD	Ph.D.	Patankar
Chanel Tyler, MD	Ph.D.	Patankar
Lei Wang	Ph.D.	Bertics

[5/1/2004 – 4/30/2014]

List of Trainees (2004 - 2009)

- Dr. Jacqueline Cale (I. Bird)
- Dr. Behzad Gerami-Naini (T. Golos)
- Dr. Nicole Korpi Steiner (P. Bertics)
- Dr. J. Christina Pattison (I. Bird)
- Dr. Amy Reeder (J. Parrish)
- Dr. Jessica Drenzek (T. Golos)
- Dr. Sekoni Noel (E. Terasawa)
- Dr. Jennifer Arens Gubbels (M. Patankar)
- Dr. Maria Giakoumopoulos (T. Golos)
- Justin Bushkofsky, PhD in progress (C. Jefcoate)
- Derek Boeldt, PhD in progress (I. Bird)
- Kate Guerriero, PhD in progress (E. Terasawa)

List of Trainees (2009-2014)

- Derek Boeldt, PhD in progress (I. Bird)
- Kate Guerriero, PhD in progress (E. Terasawa)
- Ann Rozner, PhD in progress (T. Golos)
- Dr. Chanel Tyler, PhD in progress (M. Patankar)
- Samantha Lewis, PhD in progress (J. Jorgensen)
- S. Omar Jobe, PhD in progress (R. Magness)
- Katie Hackbart, PhD in progress (M. Wiltbank)
- Brian Kenealy, PhD in progress (E. Terasawa)

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