IFPA Meeting 2015 Workshop Report I: placental mitochondrial function, transport systems and epigenetics.

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Abstract

Workshops are an important part of the IFPA annual meeting as they allow for discussion of specialized topics. At IFPA meeting 2015 there were twelve themed workshops, three of which are summarized in this report. These workshops covered areas of placental regulation and nutrient handling: 1) placental epigenetics; 2) placental mitochondrial function; 3) placental transport systems.

1 Placental Epigenetics

Chairs: Karen Moritz and Sam Buckberry

Speakers: Sam Buckberry, Tina Bianco-Miotto, Suyinn Chong, Courtney Hanna and Richard Saffery

1.1 Outline

Deciphering the role of the epigenome in regulating placental function will be critical to our understanding of how the environment can influence *in utero* development and pregnancy outcome. Numerous studies have already highlighted important links between altered epigenetic regulation in the placenta and diseases of gestation and early life. The aim of this workshop was to explore the intersection of nature and nurture in placental development and function, and examine the recent advances in placental epigenetics.

1.2 Summary

Sam Buckberry presented emerging methods for obtaining a comprehensive placental epigenomic profile. Our perspective concerning the features and modifications of DNA and chromatin has recently shifted to a more complex, but also more dynamic view of the epigenome, as a finely-tuned system composed of many layers and landmarks that together regulate a variety of cellular functions. In particular, the Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq) was highlighted as a powerful technique for profiling DNA accessibility, nucleosome positioning and DNA binding proteins in placental tissue and cell lines.

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72 **Suyinn Chong** discussed the interplay between environmental exposures *in utero*, epigenetics
73 and microRNAs and their impact on gene expression and, ultimately, phenotype of the
74 offspring. Data from an inbred mouse model of early gestational ethanol exposure was
75 presented. Gene expression analysis in the hippocampus revealed sex- and age-specific up-
76 regulation of solute carrier family 17 member 6 (*Slc17a6*), which encodes vesicular glutamate
77 transporter 2 (VGLUT2). Transcriptional up-regulation correlated with decreased DNA
78 methylation and enrichment of histone H3 lysine 4 trimethylation at the *Slc17a6* promoter. In
79 contrast to *Slc17a6* mRNA expression, hippocampal VGLUT2 protein levels were significantly
80 decreased in adult ethanol-exposed offspring, suggesting an additional level of post-
81 transcriptional control. MicroRNA expression profiling in the hippocampus identified several
82 ethanol-sensitive microRNAs, of which miR-467b-5p was predicted to target *Slc17a6*. *In vitro*
83 reporter assays showed that miR-467b-5p specifically interacted with the 3' UTR of *Slc17a6*,
84 suggesting that it contributes to the reduction of hippocampal VGLUT2 *in vivo*. In summary,
85 prenatal ethanol exposure has complex transcriptional and post-transcriptional effects on
86 *Slc17a6* (VGLUT2) expression in the mouse hippocampus.

87 **Courtney Hanna** presented data on the role of imprinting in placental development. The
88 maternal and paternal copies of the genome are both required for mammalian development
89 primarily due to imprinted genes, i.e. those that are mono-allelically expressed based on
90 parent-of-origin. Imprinted genes are essential for placental and fetal development, and may
91 have evolved with placentation in eutherian mammals. Recent data has shown that imprinting
92 in the human placenta is more extensive than previously reported and appears to be almost

entirely regulated by maternally-inherited DNA methylation. An example of this is at the *RASGRF1* gene, which is known to have paternally-inherited DNA methylation upstream of the gene in the mouse, but in human placenta appears to have a gene promoter with uniquely acquired maternally-inherited DNA methylation. The importance of this imprinting on placental function is not fully understood, but raises intriguing questions about the evolution and regulation of imprinting in the placenta.

Tina Bianco-Miotto discussed the challenges of studying DNA methylation in the placenta. The placenta is a transient, extra-embryonic organ primarily regulated by the fetal genome and shared between mother and fetus. For these reasons, the epigenetic mechanisms involved in regulation of gene expression within the placenta may not be under the same constraints as other organs. DNA methylation studies in the placenta are complicated by the fact that: i) different cell types that make up the placenta have different DNA methylation patterns and ii) the placenta methylome is hypomethylated in comparison to other organs within the body, and these hypomethylated regions occur in large domains. It was highlighted that further research investigating gene expression and DNA methylation in different placental cell types across gestation is required.

Richard Saffery presented data concerning telomere length regulation in the human placenta. Previous studies have identified longer telomeres in the placenta relative to somatic cells, though the mechanisms underlying this remain unclear. Telomere length is regulated by a complex interplay of genomic features, non-coding RNAs, various proteins and enzymes. Of note, the multi copy sub-telomeric long non-coding RNA TERRA, transcribed from a CpG rich gene promoter towards the terminal ends of chromosomes, inhibits the actions of telomerase

in association with shorter telomeres. Placental telomeres were longer relative to matched cord blood. Additionally, DNA methylation and gene expression status of several TERRA genes was examined. In contrast to what might be anticipated, placental TERRA genes were hypomethylated and more highly expressed in the placenta relative to cord blood, raising the possibility of a telomerase-independent mechanism of telomere elongation in the placenta.

1.3 Conclusions

Understanding epigenetic regulation of placental function is likely to yield important information on how the placenta influences *in utero* development and pregnancy outcome. This has particular relevance in complicated pregnancies where placental function may be compromised. Future studies in this area need to not only consider changes in DNA methylation but the role of the epigenome more broadly, including the roles of histone modifications, chromatin accessibility and interactions of microRNAs. Finally, given the complexity of the placenta, careful consideration of the different cell types that make up the placenta needs to be incorporated into experimental design and statistical analysis.

2 Placental mitochondrial function

Chairs: Leslie Myatt and Tony Perkins

Speakers: Larry Chamley, Olivia Holland, Leslie Myatt and Amanda Sferruzi-Perri

2.1 Outline

In addition to supplying cellular energy, mitochondria have many important roles in placental function including regulation of membrane potential, apoptosis, calcium signaling, steroid synthesis and production of reactive oxygen species (ROS). There is accumulating evidence for defects in mitochondrial respiration/function in pregnancies complicated by preeclampsia, growth restriction, obesity and gestational diabetes, although a mechanistic role for mitochondrial dysfunction in these conditions has yet to be shown. Attention is now focusing on strategies to improve placental mitochondrial function, with the objective of improving pregnancy outcomes. This workshop discussed mitochondrial structure, methods for studying mitochondrial respiration and function, evidence for mitochondrial dysfunction in pregnancy pathologies and therapeutic strategies (i.e. anti-oxidants, microRNAs).

2.2 Summary

Larry Chamley discussed the effects of antiphospholipid antibodies on the mitochondria in the syncytiotrophoblast. Antiphospholipid antibodies are a family of autoantibodies that are major risk factors for recurrent miscarriages and preeclampsia. The antibodies react with a complex antigen consisting of a negatively charged phospholipid and a phospholipid-binding protein. One of the most clinically-relevant groups of antiphospholipid antibodies is anticardiolipin

antibodies. Cardiolipin is a negatively charged lipid that is found mainly in the inner mitochondrial membrane in eukaryotic cells where it is an anchor for cytochrome C. Professor Chamley reported that these antibodies are internalized into the syncytiotrophoblast in a receptor-mediated, antigen-dependent fashion and that once internalized within syncytiotrophoblast, the antibodies bind to the mitochondria and appear to induce mitochondrial swelling. Oxygen analysis indicated that antiphospholipid antibodies caused an increase in mitochondrial leakage as well as release of cytochrome C. These effects on the mitochondria were accompanied by extrusion of an increased amount of extracellular vesicles, especially large multinucleated syncytial nuclear aggregates, from the syncytiotrophoblast. Since it is known that the number of extracellular vesicles extruded from the syncytiotrophoblast is increased in preeclampsia, these effects of antiphospholipid antibodies on mitochondria in the syncytiotrophoblast may explain, in part, why antiphospholipid antibodies are a strong risk factor for this pregnancy syndrome.

Leslie Myatt presented data on the effect of maternal adiposity and gestational diabetes on placental mitochondrial respiration. Maternal obesity is associated with an increase in pregnancy complications and programming of the fetus for metabolic syndrome and cardiovascular disease in later life. In adults, obesity is associated with increased production of ROS and mitochondrial dysfunction in liver and skeletal muscle. Increasing maternal adiposity was associated with a reduction in syncytiotrophoblast respiration *in vitro* and with increased ROS. However, mitochondrial ATP production, mitochondrial mass and DNA were decreased, together with significant attenuations in expression of mitochondrial complexes I-V. In type A2 gestational diabetics (GDM) (treated with either glyburide or insulin), but not in A1 GDMs

(treated with diet), mitochondrial respiration was further reduced compared to BMI-matched controls, whilst glycolysis was upregulated. Antioxidant therapy is currently being investigated to potentially improve respiration. Together, these data imply maternal adiposity and gestational diabetes are associated with significant mitochondrial dysfunction in the human placenta.

Amanda Sferruzzi-Perri discussed the effect of hypoxia on placental mitochondrial function. In many tissues mitochondria modify their function according to oxygen supply. However, little is known about the role of mitochondria in the placental response to hypoxia, a major cause of fetal growth restriction particularly at high altitude. Maternal hypoxia reduced placental mitochondrial oxygen consumption in mice, potentially sparing oxygen for fetal transfer at the expense of ATP-dependent placental processes. Whether changes in placental mitochondria are related to alterations in active transport is unclear, but theoretically this would depend on timing and severity of hypoxia. It was suggested that further work is required to identify the developmental and environmental regulation of placental mitochondria, reliance on glycolysis for ATP, and the signaling pathways involved.

Olivia Holland discussed therapeutic strategies for improving mitochondrial function. Increased placental oxidative stress is a feature of preeclampsia. Oxidative stress can harm mitochondria and impair their ability to produce ATP, affecting cellular functionality. At high levels, oxidative stress leads to the release of mitochondrial intermembrane space proteins that activate apoptosis, and can also initiate necrosis. Mechanisms such as endogenous antioxidant systems, stress activated-transcriptional regulators and the mitochondrial fission/fusion cycle help to maintain mitochondrial function. Therapeutic strategies targeting these processes have been

shown to counter the damaging effects of oxidative stress in several tissue systems and may have implications for the treatment of placental dysfunction.

2.3 Conclusions

Evidence is rapidly accumulating showing mitochondrial dysfunction in the placenta in situations associated with an adverse intrauterine environment, including hypoxia, obesity and GDM. As yet, the functional consequences of this altered mitochondrial function, for the placenta and fetus, remain to be elucidated. Indeed, can an increase in glycolysis compensate for the reduced activity of oxidative phosphorylation in ATP generation, and sustain fetal growth? The many roles of the mitochondria, particularly in steroid synthesis, suggest wider implications for the placenta beyond energy generation. There is also a need to understand the functions of placental mitochondria in mononuclear cytotrophoblasts as opposed to mitochondrial function in the syncytium. Syncytial mitochondria appear to be morphologically distinct and may have profoundly altered metabolic capacities. While the effects on placental function remain to be demonstrated, several approaches show promise in restoring mitochondrial respiration and may prove fruitful in a therapeutic manner.

3 Placental Transport and its Regulation

Chairs: Paul Dawson and Colin Sibley

Speakers: Cassidy Blundell, Elizabeth Cottrell, Rohan Lewis, Theresa Powell and David Simmons

3.1 Outline

The aims of this workshop were to: 1) address the biological roles and regulation of placental transport, and the impact of perturbed transport on placental, fetal and maternal physiology, 2) increase awareness of the known/potential clinical consequences of impaired placental transport and, 3) bring together research teams to foster collaboration and direct future biomedical and clinical research on placental transport systems.

3.2 Summary

Elizabeth Cottrell presented data concerning whether dietary nitrate supplementation in pregnancy may benefit uteroplacental function and fetal growth in a well-characterized model of fetal growth restriction (FGR), the endothelial nitric oxide synthase knockout (*eNOS*^{-/-}) mouse. Disturbances in NO bioavailability associate with FGR in humans. In animal models, low NO production can lead to impairments in uteroplacental blood flow and reduced placental nutrient transport. Supplementation with dietary nitrate, providing nitrite, a source of nitric oxide-generating potential, improves blood flow and cardiovascular function in non-pregnant individuals. These data demonstrate that maternal dietary nitrate supplementation, via beetroot juice, alters both maternal uterine artery and fetal umbilical artery vascular function in

the *eNOS*^{-/-} mouse, in a manner consistent with improved blood flow. Ongoing experiments aim to determine the mechanisms underlying these beneficial effects of dietary nitrate on vascular function, and to investigate whether and how this nutritional intervention strategy may also impact on placental nutrient transfer.

Rohan Lewis discussed the regulation of placental amino acid transfer by flow, transporters and metabolism. Membrane transporters are essential for placental amino acid transfer, but the contribution of other factors such as blood flow or metabolism is less clearly defined. An experimental and computational modelling approach was used to explore the determinants of amino acid transfer across the isolated perfused human placenta. This analysis suggested that blood flow is not a major determinant of placental amino acid transfer but that other factors, most likely amino acid metabolism, may be rate limiting for transfer. This has implications for the regulation of placental amino acid transfer.

Cassidy Blundell presented a microengineered system of the human placental barrier as a potential model to study placental transport and regulation. This human organ-on-a-chip model developed by Dan Huh's group at Penn Engineering, University of Pennsylvania, comprises a compartmentalized microdevice containing upper and lower microchannels separated by a polymeric membrane that enables precise microfluidic control over co-cultured bilayers of primary placental cells or decidualized trophoblast cell lines. Flow of culture media into the upper and lower micro-channels is a surrogate of maternal and fetal blood flow, respectively, and has the potential to recapitulate transport systems at the maternal-fetal interface.

David Simmons presented recent lessons on placental sulfate transport from mouse studies. The fetus has a limited capacity to generate sulfate from sulfur-containing amino acids, and is thought to rely on access to maternal serum sulfate for normal development. It was found that the sulfate transporter *Slc13a4* is essential for normal fetal development in the mouse. Homozygous deletion of *Slc13a4* results in a variety of developmental abnormalities in tissues with known requirements for sulfate metabolism, such as the skeleton, palate, eye, and vasculature. Retaining *Slc13a4* expression in the placenta, but not the embryo, rescued all of the phenotypes, indicating placental *Slc13a4* expression in particular is required for embryonic development.

Theresa Powell addressed the role of fatty acids as both nutrients and signaling molecules in the placenta. Lipids are essential nutrients for fetal growth and are especially critical for brain and eye development. Mechanisms for placental transfer of lipids and their regulation remain unclear. Greater expression in the basal membrane compared to the microvillus membrane for both fatty acid transport proteins 2 and 4, and inhibition of lipoprotein lipase by Angiopoietin-like 4 were presented. A role for fatty acid species as regulators of placental function was demonstrated using both cultured primary human trophoblast cells and maternal omega-3 supplementation by demonstrating differential effects of oleic acid and docosahexaenoic acid on syncytiotrophoblast nutrient transport capacity.

3.3 Conclusions

This workshop raised questions on what are the next big issues for placental transport research, and what transport systems should we try to understand better? To address these questions, this workshop identified the need for our research to overlap and integrate the function and regulation of placental transporters. For example, the interaction of fatty acid transport on amino acid transfer capacity highlights the need to test a mix of nutrients in our *in cellulo* and animal model systems, which would more accurately reflect the *in vivo* situation. In addition, the contribution of intracellular metabolism to net nutrient supply is rather underappreciated, as highlighted in Rohan Lewis' presentation on amino acid transfer. Furthermore, dietary contributions such as nitrate deficiency to impairments in uteroplacental blood flow and reduced nutrient transfer, is an example of systems biology impacting on placental transport. Importantly, animal models have provided a wealth of knowledge for the consequences of perturbed nutrient transfer, as shown for the non-redundant role of the Slc13a4 sulphate transporter in fetal development. As clinical interest in placental transport systems expand, our research will benefit from new and more cost effective methodologies, such as an omics approach as well as the microengineered placental barrier system described in this workshop. In summary, this workshop highlighted the need for bringing together our placental transport research field to work collaboratively with an integrative and systems biology approach to more accurately recapitulate transport function and its regulation at the maternal-fetal barrier.

There is no conflict of interest.

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