IFPA meeting 2016 workshop report I: Genomic communication, bioinformatics, trophoblast biology and transport systems

Albrecht, Christiane; Baker, Julie C.; Blundell, Cassidy; Chavez, Shawn L.; Carbone, Lucia; Chamley, Larry; Hannibal, Roberta L.; Illsley, Nick; Kurre, Peter; Laurent, Louise C.; McKenzie, Charles; Morales-Prieto, Diana; Pantham, Priyadarshini; Paquette, Alison; Powell, Katie; Price, Nathan; Rao, Balaji M.; Sadovsky, Yoel; Salomon, Carlos; Tuteja, Geetu; Wilson, Samantha; O'Tierney-Ginn, P. F.

Published in:
Placenta

DOI:
10.1016/j.placenta.2017.01.103

IFPA Meeting 2016 Workshop Report I: Genomic communication, bioinformatics, trophoblast biology and transport systems.

Christiane Albrecht, University of Bern, Switzerland

Julie C. Baker, Stanford University School of Medicine, USA

Cassidy Blundell, University of Pennsylvania, USA

Shawn L. Chavez, Oregon Health and Science University, Oregon, USA

Lucia Carbone, Oregon Health and Science University, Oregon, USA

Larry Chamley, University of Auckland, New Zealand

Roberta L. Hannibal, Stanford University School of Medicine, USA

Nick Illsley, Hackensack University Medical Center, New Jersey, USA

Peter Kurre, Oregon Health and Science University, Oregon, USA

Louise C. Laurent, University of California, San Diego, USA

Charles McKenzie, University of Western Ontario, Ontario, Canada

Diana Morales-Prieto, University of Jena

Priyadarshini Pantham, University of Illinois at Urbana-Champaign, Illinois, USA

Alison Paquette, Institute for Systems Biology, USA

Katie Powell, University of Sydney, Australia

Nathan Price, Institute for Systems Biology, USA

Balaji M. Rao, North Carolina State University, USA

Yoel Sadovsky, Magee-Women's Research Institute, Pennsylvania, USA

Carlos Salomon, University of Queensland, QLD Australia

Geetu Tuteja, Iowa State University, Iowa, USA

Samantha Wilson, University of British Columbia, Canada

PF O'Tierney-Ginn, Center for Reproductive Health, MetroHealth Medical Center, Case Western Reserve University, Cleveland, Ohio, USA

Working title: IFPA 2016 workshops I

Key Words: transport; trophoblast; bioinformatics; exosomes; OMICS, DNA methylation

PFOG edited this manuscript based on contributions from the other authors.

Address for correspondence: Dr. Perrie F O'Tierney-Ginn

Center for Reproductive Health

MetroHealth Medical Center

2500 MetroHealth Drive, R358

Cleveland, Ohio, USA 44109

Email: poginn@metrohealth.org

Phone: 1-216-778-8983

Fax: 1-216-778-8282

Abstract

Workshops are an important part of the IFPA annual meeting as they allow for discussion of specialized topics. At IFPA meeting 2016 there were twelve themed workshops, four of which are summarized in this report. These workshops covered innovative technologies applied to new and traditional areas of placental research: 1) genomic communication; 2) bioinformatics; 3) trophoblast biology and pathology; 4) placental transport systems.

1 Genomic communication

Chair: Yoel Sadovsky

Speakers: Larry Chamley, Peter Kurre, Nathan Price, Alison Paquette, and Carlos Salomon

1.1 Outline

While circulating RNAs, either bound by plasma proteins or packaged within extracellular vesicles (EVs), may transmit information to researchers about tissue function, disease, and organismal wellness, recent data indicate that these messages play a key role in local and distant cell communication. Using a series of targeted and provocative exchanges, this workshop centered on the transfer of RNAs within EVs and their entry into target cells, the use of minimally invasive, circulating RNA biomarkers for disease monitoring, and the integration of these data into longitudinal assessment of pregnancy health, projecting a futuristic view of "scientific wellness."

1.2 Summary

Larry Chamley discussed the role of RNAs in syncytial nuclear aggregates/trophoblast debris in fetal control of maternal physiology. The syncytiotrophoblast layer of the human placenta extrudes a wide variety of EVs into the maternal blood, ranging in size from multinucleated syncytial nuclear aggregates (SNAs) and other trophoblast debris, to nano-vesicles. Dr. Chamley's group found that SNAs/trophoblast debris contain multiple RNA species including fragments from mRNA, snRNA, piRNA and tRNA, as well as intact mature miRNA. When SNAs/trophoblast debris from normal first trimester placentae were incubated with endothelial cells, the endothelial cells substantially altered their transcriptome and expressed placenta-

specific genes, such as chorionic gonadotropin and placental lactogen, and their angiogenic capacity was increased. Such changes may be important for normal maternal physiological adaptations to pregnancy. Small RNA species in SNAs/trophoblast debris significantly differ between normal and preeclamptic placentae. The finding that functional miRNAs could be delivered to endothelial cells via SNAs/trophoblast debris, suggests that changes in the miRNA cargo of SNAs/trophoblast debris may, in part, be responsible for the inappropriate endothelial cell responses in preeclamptic pregnancies.

Peter Kurre discussed evidence that EVs contribute to intercellular genomic communication using the crosstalk between leukemia cells and hematopoietic stem cells as a paradigm. His work illustrated key concepts of vesicle-mediated transfer of RNA in phenotypically regulating diverse cell populations in the bone marrow compartment by EV-transferred miRNA. The studies revealed both paracrine and endocrine trafficking of EVs. The presentation highlighted approaches to the study of transfer and regulation, and identified opportunities for EVs as a platform for miRNA biomarkers.

Nathan Price outlined a strategy to leverage genomic and other data for optimizing personal wellness. Two fundamental challenges to pregnancy research are: 1) a general paucity of longitudinal molecular data; 2) difficulties in developing potential therapies due to the sensitive nature of launching clinical trials during pregnancy. Recently, Lee Hood and Dr. Price have completed a pilot study — the Pioneer 100 — for a 100K wellness project. The aim of this project is to build a discipline called "scientific wellness." Dense, dynamic, personal data clouds will be created for each individual, including their whole genome sequence as a baseline, adding repeated measurements of clinical chemistries, metabolites, proteomes, microbiomes,

and data from wearables, over time. These data will be interrogated to identify actionable possibilities for individuals to help optimize wellness and reduce disease risk. Dr. Price discussed how such an approach could result in valuable, dense longitudinal data for the field and provide a low-risk strategy for improving pregnancy outcomes, such as pre-term birth.

Alison Paquette discussed genome-scale analysis of miRNA regulation in preterm labor (PTL). Dr. Paquette's group performed global miRNA and mRNA profiling in both monocytes and whole blood of women who experienced preterm labor (N=15) matched to non-pathological controls (N=30), as a part of the Ontario Birth Cohort. They identified differentially expressed miRNAs, mRNAs and pathways associated with preterm labor using differential rank conservation (DIRAC). They identified 34 miRNAs associated with preterm labor in whole blood and monocytes. When comparing these data to an independent dataset of non-pathological pregnancies (N=25), they found that many miRNAs differentially expressed in PTL, were expressed in the placenta. miR-1299 expression, associated with PTL, was correlated between placenta and maternal plasma. This comprehensive profiling of miRNA and mRNA regulation identified specific biomarkers of preterm labor.

Carlos Salomon discussed optimizing methods to isolate and quantify placenta-derived exosomes from maternal circulation. Dr. Salomon's group has optimized methods to specifically isolate and quantify circulating placental exosomes in maternal circulation, using antibody-based enrichment of exosomes on magnetic beads and by Nanoparticle Tracking Analysis (NanoSight™) using quantum dots (Qdots) coupled with CD63 or placental alkaline phosphatase (PLAP) antibodies. They have validated the specific binding of PLAP-beads or PLAP-Qdots using exosomes isolated from syncytiotrophoblast (positive control) and plasma from non-pregnant

women (negative control). They determined that ~12% and ~20% of the total circulating exosomes are from placental origin in early gestation (i.e. ~10-12 weeks) and third trimester (i.e. >32 weeks), respectively. These methods may help profile and characterize exosomes from placental origin under normal and pathological conditions.

1.3 Conclusions

Observations discussed at this workshop suggest a central role for exosomes and syncytiotrophoblastic aggregates in influencing the biology of target tissues during pregnancy as well as during the process of carcinogenesis (such as leukemia). The use of vesicular circulating RNA biomarkers (mainly microRNAs) for disease monitoring, and the integration of these data into longitudinal assessment of pregnancy health, projects a futuristic view of "scientific wellness".

2 Bioinformatics and omics applied to the placenta

Chair: Lucia Carbone

Speakers: Diana Morales-Prieto, Priyadarshini Pantham, Katie Powell, Geetu Tuteja, Samantha Wilson

2.1 Outline

Rapid advances in omics technologies and associated bioinformatics tools have significantly influenced the placenta field. One of the ultimate goals of performing omics analyses is the identification of biomarkers that reflect the status of the placenta, the mother and the baby. Bioinformatics analysis and integration of such datasets, however, present many challenges. Firstly, references for placental transcriptomes, metabolomes, and epigenomes are missing, hindering the interpretation and integration of omics data. Secondly, the range of variability within the population is still unknown, thus, a baseline to evaluate adverse profiles is missing. During this workshop, scientists involved in the analyses of different types of omics data (e.g. epigenomes, microRNAomes and metabolomes), elaborated on the current methods used to obtain and analyze omics data and strategies used to deal with the issues raised above. Furthermore, some of the provocative questions raised in the placenta field when omics data are generated and analyzed, are beginning to be addressed.

2.2 Summary

Diana Maria Morales-Prieto presented data on the expression of Chromosome 14 miRNA cluster (C19MC) and C14MC miRNAs and their potential involvement in pregnancy disorders.

Inappropriate vessel transformation by trophoblast cells is associated with preeclampsia (PE) and intrauterine growth restriction (IUGR), while exacerbated trophoblast invasion occurs in placenta accreta. Human trophoblast cells express two large miRNA clusters: C14MC and C19MC. These miRNAs regulate human pregnancy by controlling trophoblast cell functions, including cell proliferation and invasion. miRNAs in these clusters were differentially expressed between normal and pathological placentas. For instance, miR-370 was upregulated in placenta accreta, and down-regulated in early-onset PE compared to controls. miRNAs from the same cluster had similar expression profiles suggesting dysregulation of entire families. C19MC and C14MC miRNAs could potentially be useful for molecular classification of pregnancy pathologies, but this will require further study.

Priyadarshini Pantham reported on the identification of a core placental transcriptome across 14 different species of placental mammals spanning the phylogenetic tree. The mRNA environment of the placenta was quantified using RNA-seq technology, and 1:1 *Homo sapiens* orthologs were identified. The core placental transcriptome was significantly enriched for pathways involved in epidermal growth factor receptor (EGFR) signaling. Study limitations included the inability to collect placental samples from all species throughout gestation, cellular heterogeneity, and animals without reference transcriptomes. The core placental transcriptome described may be critical for the organization and function of the placenta across these species.

Katie Powell discussed the use of metabolomics to identify novel predictive biomarkers of pregnancy complications. She described aspects of study design, including analysis of samples using nuclear magnetic resonance (NMR) spectroscopy, and methods for data analysis.

Advantages of this technology include the high degree of accuracy and precision in the measurement of multiple metabolites from a sample, high sample throughput, and low processing cost. Field limitations include the reduced sensitivity of low abundance metabolites via NMR spectroscopy and the low number of published validation studies confirming the role of individual metabolites identified in discovery studies. Metabolomics has the capacity to identify new biomarkers that will increase our understanding of disease processes and these biomarkers have the potential to be developed into clinical screening tests.

Geetu Tuteja presented challenges in ChIP-Seq data analysis, and strategies to overcome them. Although ChIP-Seq is becoming a routine method, best practices in experimental design and data analysis are often overlooked. ChIP-Seq requires multiple biological replicates and control data to obtain meaningful results. Read quality should be assessed and, if necessary, reads should be trimmed prior to sequence alignment. Software, used to identify protein-DNA interactions from ChIP-Seq data (peak-callers), were shown to give widely different results from the same input data, significantly affecting downstream analysis. Therefore, peak-callers should be chosen carefully, and parameters should be understood and set prior to running analyses.

Samantha Wilson discussed the potential of using placental epigenetic changes as biomarkers. The placenta shows a pattern of DNA methylation (DNAm) that is unique compared to other tissues. Fetal sex, gestational age, ethnicity, and cell type are important factors that influence placental DNAm. Thus, a change in placental DNAm may represent: 1) an active modification in DNAm within a cell type; or 2) a change in proportion of different cell types between placentas. For a placental-specific epigenetic biomarker to be usable, it cannot be masked by DNAm

signatures from maternal tissues, and should display sufficiently large changes in DNAm for detectability. To reflect protein expression, the methylated site must regulate gene expression, encode a protein, and the protein must be shed into the maternal circulation in large enough amounts to be detected. In the future, omics data must be integrated to achieve a better understanding of how all of these pieces interact with each other.

2.3 Conclusions

The discussions during and after this workshop centered on the use of OMICS for studying the placenta and the difficulties that scientists are currently experiencing. Although generating data is becoming common practice in many laboratories, the analysis still weighs on investigators. Analysts that have expertise to mine the data often lack an understanding of the biology and the complex questions that need to be addressed. Establishing a solid and lasting partnership between these two sides will be key to make sure that all useful information are obtained from the omics data.

3 Trophoblast Biology & Pathology

Chair: Shawn L. Chavez and Julie C. Baker

Speakers: Shawn L. Chavez, Roberta L. Hannibal, Louise C. Laurent, Balaji M. Rao

3.1 Outline

Normal placental development is largely dependent upon the differentiation and invasion of the trophoblast, which originates from the trophectoderm of the blastocyst prior to embryo implantation. Given that aberrant trophoblast development is a common phenomenon observed in pregnancy complications such as preterm labor, preeclampsia, and IUGR, much research emphasis is placed on the genetic, epigenetic, and chromosomal aspects regulating trophoblast function. Recent technological advances in genome-wide DNA methylation analysis and next generation sequencing (NGS), as well as the use of human pluripotent stem cells to assess trophoblast regulation, has provided considerable insight into normal placental development and the pathophysiology of these pregnancy-related diseases. The objectives of this workshop were to discuss NGS and other emerging approaches for assessing trophoblast competency at the single-cell and/or whole-genome level. We also discussed how this work has provided novel diagnostics to understand and predict placental misregulation. Lastly, we reviewed key trophoblast regulators, including endogenous retroviruses, and intracellular signaling pathways mediating trophoblast fate that are important for normal placental function.

3.2 Summary

Shawn L. Chavez discussed the role of endogenous retroviruses (ERVs) in primate placentation. Although initially classified as “junk” DNA, several ERVs were found to maintain coding

potential and play important biological roles in mammalian development. While ERV-W (Syncytin-1) and ERV-FRD (Syncytin-2) are important for normal trophoblast syncytialization, the precise function of ERV-K, the most recently acquired ERV in the human genome, remains unknown. Dr. Chavez highlighted previous reports of ERV-K mRNA and certain retroviral protein components in normal human placental tissues and discussed similar observations of single-cell ERV-K expression in rhesus monkey embryos and placentas. She also reported on the expression and potential function of ERV-K in a primate maternal infection model with or without antibiotic therapy. Her data suggests that ERV-K is active at the maternal-fetal interface and has a distinct role in normal human and non-human primate placental development.

Louise C. Laurent presented genomic approaches her lab has used to identify novel regulatory factors involved in trophoblast differentiation. In the first approach, microarray-based gene expression data from a broad range of tissue and cell types were analyzed to identify placenta and cytotrophoblast-specific transcripts. One such transcript was Grainyhead-like protein 1 homolog (GRHL1) and is the focus of ongoing functional analyses, using *in vitro* differentiation of human embryonic stem cells as a model system. Dr. Laurent also presented proof-of-concept for a single-cell transcriptomics approach to build regulatory networks associated with a stepwise differentiation system, using human embryonic stem cell differentiation to the pancreatic lineage as the test model.

Roberta L. Hannibal reported on trophoblast misregulation in placenta accreta. In accreta, the placenta abnormally invades uterine tissues. While prior uterine surgery is a risk factor, suggesting a uterine component, previous histopathology has also found defects in trophoblast cells. Dr. Hannibal sequenced multiple regions of placentas with and without accreta. She

found genes upregulated in the entire placenta of accreta cases. These genes are enriched for previously unidentified secreted and membrane molecules she has termed Accreta (ACC) #1-4. Their overexpression was confirmed using semi-quantitative immunofluorescence. To examine whether these upregulated proteins could be used as potential biomarkers for accreta, they will be measured in maternal plasma. Overall, this data suggests that uterine damage does indeed lead to trophoblast misregulation.

Balaji M. Rao discussed pluripotent stem cell models of human placental development. Trophoblasts derived from human pluripotent stem cells (hPSCs) are a promising *in vitro* model system for studying early trophoblast development. However, a consensus must be reached as to which markers should be used to confirm that hPSC-derived trophoblast subtypes are similar to trophoblasts *in vivo*. Dr. Rao's group determined that the expression of certain DNA methyltransferases and chromatin remodeling genes is largely consistent between trophoblasts produced *in vitro* and *in vivo*. They also demonstrated that hypomethylation of the E74-Like ETS Transcription Factor-2b promoter and down-regulation of human leukocyte antigen class I antigens, is observed in hPSC-induced trophoblasts. This suggests that *in vitro*-derived trophoblasts possess similar properties as their *in vivo* counterparts.

3.3 Conclusions

Despite the ethical, legal, and technical challenges of studying early human placental development, recent advances in single-cell and/or whole-genome analyses as well as the use of hPSC-derived trophoblasts has provided considerable insight into the regulation of trophoblast differentiation. With ongoing and future efforts, the precise molecular mechanisms

mediating normal trophoblast function, and how it is disrupted across pregnancy-associated diseases, may be elucidated.

4. Transport NextGen: cool new stuff

Chair: Nick Illsley

Speakers: Christiane Albrecht; Cassidy Blundell; Che-Ying Kao; Charles McKenzie

4.1 Outline

This workshop looked at several new technologies that are becoming available for research into placental transport and provide new opportunities for investigation.

4.2 Summary

Christiane Albrecht introduced the successful establishment of a confluent human primary trophoblast monolayer using Matrigel-coated Transwell® inserts. During 5-day culture, trophoblasts showed polarization exhibiting a modest transepithelial electrical resistance (>1.2 $k\Omega \bullet cm^2$) and a size-dependent apparent permeability coefficient. The syncytialization progress was characterized by gradually increasing levels of fusogen genes and hCG secretion. Electron microscopy confirmed a confluent trophoblast monolayer with numerous microvilli and tight junctions. Immunocytochemistry showed positivity for the cell-cell adhesion molecule E-cadherin, and the tight junction protein ZO-1 between mononucleated cytotrophoblasts. Studying the bidirectional transport of a non-metabolizable glucose derivative in presence of the inhibitor phloretin indicated a carrier-mediated placental glucose transport mechanism with asymmetric kinetics. Development of this model opens the way for integrated studies of trans-syncytial transport and the analysis of its complex mechanisms and kinetics.

Cassidy Blundell presented work on the development and characterization of the placenta-on-a-chip, a microengineered model that reconstitutes the bilayer structure of the human placental barrier. This system enables compartmentalized co-culture of trophoblast and endothelial cells in a dynamic flow environment. Preliminary studies of glucose transport were performed and the rate of maternal-to-fetal glucose transfer in the placenta-on-a-chip matched rates measured in an *ex vivo* placental perfusion model. This work illustrates the potential for leveraging this microphysiological platform for studying placental transport.

Che-Ying Kuo discussed engineering diffusion of chemoattractants in bioprinted tissues. The development of a chemotactic gradient plays a critical role in regulating trophoblast invasion that, if not properly regulated, can lead to preeclampsia. Dr. Kuo's group has recently leveraged the advantages of bioprinting (e.g. spatial control of biomaterials) and created a novel Bioprinted Placenta Model with a chemotactic gradient to study trophoblast migration. In this workshop, Dr. Kuo presented methods used to establish the chemotactic gradient, including 3D Bioprinting, time-lapse fluorescent imaging and mathematical modeling. This method may be extended to study other transport phenomena during trophoblast invasion, such as cell migration and invasion.

Charles McKenzie presented prospects for non- invasive measurement of placental metabolic and transport processes with Hyperpolarized Magnetic Resonance Imaging (MRI)**.** Hyperpolarised MRI of ^{13}C labeled substrates is an emerging new technology for imaging placental metabolism and transport *in vivo*. This technology images the distribution of molecules, such as [1-^{13}C]pyruvate, in real time, allowing the dynamics of metabolism and transport to be investigated without the use of invasive techniques that could disturb placental

physiology. Importantly, this technique uses stable, non-radioactive isotopes so it is safe to use repeatedly, allowing investigation of changes in metabolism and transport across gestation. It also has the potential for use in humans, making *in vivo* metabolism and transport processes in the human placenta observable for the first time.

4.3 Conclusions

The new techniques described in this workshop have promise for revolutionizing studies of transport. These models present the possibility, for the first time, of analyzing transport in complex structures, beyond the previous "black-box" approach, which characterizes methods such as lobule perfusion. The ability to track objects, from metabolites up to cellular size, in multicellular systems will significantly advance the fields of transport and metabolism and provide even greater translational relevance.