

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

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

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39 **Abstract**

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

45

46 **1 Genomic communication**

47 **Chair:** Yoel Sadovsky

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

49 *1.1 Outline*

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

58 *1.2 Summary*

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

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

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

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

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

93 **Alison Paquette** discussed genome-scale analysis of miRNA regulation in preterm labor (PTL).

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

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

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

115 *1.3 Conclusions*

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

122

123 **2 Bioinformatics and omics applied to the placenta**

124 **Chair:** Lucia Carbone

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

127 *2.1 Outline*

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

140 *2.2 Summary*

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

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

153 **Priyadarshini Pantham** reported on the identification of a core placental transcriptome across
154 14 different species of placental mammals spanning the phylogenetic tree. The mRNA
155 environment of the placenta was quantified using RNA-seq technology, and 1:1 *Homo sapiens*
156 orthologs were identified. The core placental transcriptome was significantly enriched for
157 pathways involved in epidermal growth factor receptor (EGFR) signaling. Study limitations
158 included the inability to collect placental samples from all species throughout gestation, cellular
159 heterogeneity, and animals without reference transcriptomes. The core placental
160 transcriptome described may be critical for the organization and function of the placenta across
161 these species.

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

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

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

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

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

191 *2.3 Conclusions*

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

199

200 **3 Trophoblast Biology & Pathology**

201 **Chair:** Shawn L. Chavez and Julie C. Baker

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

203 *3.1 Outline*

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

218 *3.2 Summary*

219 **Shawn L. Chavez** discussed the role of endogenous retroviruses (ERVs) in primate placentation.

220 Although initially classified as “junk” DNA, several ERVs were found to maintain coding

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

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

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

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

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

259 *3.3 Conclusions*

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

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

266

267 **4. Transport NextGen: cool new stuff**

268 **Chair:** Nick Illsley

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

270 *4.1 Outline*

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

273 *4.2 Summary*

274 **Christiane Albrecht** introduced the successful establishment of a confluent human primary
275 trophoblast monolayer using Matrigel-coated Transwell® inserts. During 5-day culture,
276 trophoblasts showed polarization exhibiting a modest transepithelial electrical resistance (>1.2
277 $k\Omega \cdot \text{cm}^2$) and a size-dependent apparent permeability coefficient. The syncytialization progress
278 was characterized by gradually increasing levels of fusogen genes and hCG secretion. Electron
279 microscopy confirmed a confluent trophoblast monolayer with numerous microvilli and tight
280 junctions. Immunocytochemistry showed positivity for the cell-cell adhesion molecule E-
281 cadherin, and the tight junction protein ZO-1 between mononucleated cytotrophoblasts.
282 Studying the bidirectional transport of a non-metabolizable glucose derivative in presence of
283 the inhibitor phloretin indicated a carrier-mediated placental glucose transport mechanism
284 with asymmetric kinetics. Development of this model opens the way for integrated studies of
285 trans-syncytial transport and the analysis of its complex mechanisms and kinetics.

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

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

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

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

312 *4.3 Conclusions*

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

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