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IFPA Meeting 2013 Workshop Report II: Use of 'omics' in understanding placental development, bioinformatics tools for gene expression analysis, planning and coordination of a placenta research network, placental imaging, evolutionary approaches to understanding pre-eclampsia



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ABSTRACT

Workshops are an important part of the IFPA annual meeting as they allow for discussion of specialized topics. At the IFPA meeting 2013 twelve themed workshops were presented, five of which are summarized in this report. These workshops related to various aspects of placental biology but collectively

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covered areas of new technologies for placenta research: 1) use of 'omics' in understanding placental development and pathologies; 2) bioinformatics and use of omics technologies; 3) planning and coordination of a placenta research network; 4) clinical imaging and pathological outcomes; 5) placental evolution.

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1. Use of 'omics' in understanding placental development and pathologies

Chairs: Martin Post and Greg Rice

Speakers: Leonardo Ermini, Pamela Hoodless, Carlos Salomon, Wendy Robinson

1.1. Outline

Our understanding of the molecular events that mediate both normal placental function and response to challenges to maternal homeostasis; our ability to predict and diagnose placental dysfunction; and the appropriateness of clinical management decisions are all informed by the quality and breadth of the data provided. Systems biology approaches afford the opportunity to synthesize a more integrated and dynamic insight into placental development and function. Risk assessment and diagnostic tests that are based upon mathematical algorithms that combine information from multiple variables into a single probability index are fundamental to the delivery of precision medicine. Platform technologies that deliver multivariate and multiplex data (*i.e.* "omics") are pivotal in delivering such outcomes. This workshop, thus, focused on selected 'omics' approaches and platforms of relevance to placentology, including RNA-Sequencing (RNA-Seq), DNA methylation profiling, lipidomics and nanovesicles as a new 'omics' target.

1.2. Summary

Wendy Robinson discussed DNA methylation and the placental methylome. DNA methylation profiling of the human placenta can have application to basic biology, clinical and epidemiological questions. Placental methylation is quite distinct from somatic tissues; while globally decreased, methylation is higher at some regions, such as CpG island promoters. Placenta is also unique amongst normal tissues in the presence of "partially methylated domains", large blocks of sequence along the chromosomes that show distinctly lower methylation. Application of array and sequencing based technologies can identify differences between pathological and normal placentas, for example early onset pre-eclampsia is associated with a unique methylation profile; the detection of more subtle changes as may be associated with maternal environmental exposures (stress, diet etc.) are more challenging. Accounting for confounders such as gestational age and cell composition variation is crucial in these studies and an improved understanding of the effect of such factors on DNA methylation is needed.

Pamela Hoodless discussed the application of RNA-Seq to placental biology. RNA-Seq is rapidly becoming the method of choice for gene expression analysis due to the accuracy and the wide dynamic range of the data generated. RNA-Seq can provide a wealth of knowledge, such as gene expression levels, differential expression, alternative splice forms and novel transcripts, not easily obtained by microarrays. The recent availability of tools for data

processing and analysis of sequencing data generated for RNA libraries has made RNA-Seq accessible to non-bioinformaticians.

Leonardo Ermini discussed the use of mass spectrometry in the imaging of placental metabolites. Recent development of imaging mass spectrometry (IMS) has created a new generation of mass spectrometers suitable for the direct analysis of metabolites in tissues. Traditional matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) uses a liquid substrate. However, in IMS analyte ions are produced directly from a tissue slice coated with MALDI matrix, and sequential mass spectra are acquired by passing the laser beam across the tissue surface. The distribution as well as the composition of many metabolic compounds of interest can be determined in a single experiment. Moreover, the Maldi IMS leaves the tissue intact for further histological analysis thereby facilitating the direct correlation of the metabolic MS data with the morphological features of the tissue.

Finally, Carlos Salomon reported on the burgeoning field of placental exosomes – membrane-bound nanoparticles that are released from placental cells (e.g. cytotrophoblast and placental mesenchymal stem cells) and the placental syncytium. The available data, are consistent with the hypothesis that placental exosomes play a significant role in cell-to-cell communication (as a carrier of specific molecules) within the placenta; at the maternal–fetal interface and systemically. The molecular cargo of exosomes includes, signaling proteins, receptors, ion channels, peptides, microRNA and RNA. Due to their structural stability and ease of isolation from high abundance and interfering factors, exosomes represent a significant target for the application of "omics" approaches.

1.3. Conclusions

Advances in platform technologies are providing placentologists with richer datasets that define placental development and response to environmental challenges, and are identifying new pathways of cellular communication. It is anticipated that such data will ultimately compel a revision of placental and pregnancy disease taxonomy.

2. Bioinformatics tools for gene expression analysis

Chairs: Brian Cox and Douglas Kniss

Speakers: William Ackerman, Brian Cox, Joseph Huang, Douglas Kniss, Yoel Sadovsky

2.1. Outline

The advent of high-content gene expression tools, including microarrays, next generation RNA-Seq, nanostring, ion torrent and other platforms offers researchers the ability to evaluate large datasets for systems biology analysis of complex biological pathways. Normalization, standardization, statistical assessment, pathway analysis, and the enormous volume of high-throughput data make accurate and precise evaluation a non-trivial challenge in modern biology. This workshop aimed to highlight practical,

real-time experiences from messenger RNA and microRNA expression experiments. In addition, a hands-on exercise in the use of several web-based informatics platforms was conducted to demonstrate a typical gene expression analysis workflow.

2.2. Summary

William Ackerman discussed the application of nanostring, RNA-Seq and other high-dimensional platforms. Increasingly, RNA-Seq and the NanoString nCounter system are being adopted for high-dimensional transcriptional profiling. These platforms both offer certain advantages over traditional microarrays, including increased sensitivity, expanded dynamic range, information regarding absolute transcript expression levels and reduced signal-to-noise ratio. Expanding use of these technologies has led to the development of a host of novel bioinformatics and biostatistics algorithms for differential expression analyses; at present, however, there is little consensus for a single preferred method.

Brian Cox shared his insights into bioinformatics workflows. Many software packages exist for the processing of microarray data, from array normalization and value extraction to gene set enrichment calculation. However, it can be cumbersome to reformat data amongst these different platforms and rerunning a process is time-consuming. For these reasons the statistical and graphical scripting language of R (<http://www.r-project.org/>) combined with the available packages from Bioconductor (<http://www.bioconductor.org/>) offers a one-stop shop for processing of microarray data as well as most other data types. The utility of learning the R language was explained and demystified in an attempt to remove the fear barrier to learning programming languages. An example workflow that covers power analysis to determine sample size, array processing, quality control, de-noising and exploratory analysis was presented.

Joseph Huang discussed mRNA gene expression data. The birth of microarray analysis in the '90s initiated the high-throughput systems biology era. This technology provides researchers with opportunities to evaluate global changes in gene expression and pinpoint specific gene regulatory pathways. The principle of array design, workflow of sample preparation, data analysis and interpretation of results were all discussed.

Yoel Sadovsky discussed microRNA gene expression data. The expression of at least half of the human genes is thought to be regulated by microRNA (miRNAs), non-coding RNA molecules, acting through mRNA degradation or inhibition of protein translation. Most miRNAs regulate gene networks by preventing stochastic gene activation and thereby maintaining homeostasis. A rigorous approach for analysis of miRNA function was discussed.

2.3. Conclusions

The workshop provided both theoretical and practical approaches to large-scale gene expression dataset analysis. Of particular note was the discussion of computational tools necessary for sophisticated gene expression analysis using public domain software applications (e.g. R-scripting language) and that current web-based software packages to analyze potential miRNA target genes are subject to several shortcomings due to the simplicity of the prediction algorithms based upon assumptions about the molecular interactions of a given miRNA and its putative targets. This workshop was an excellent complement to the 'omics' workshop presented by Drs. Rice and Post (see Section 1).

3. Planning and coordination of a Placenta Research Network

Chairs: Sally Collins and Stacy Zamudio

3.1. Outline

The crucial, likely causal role played by the placenta in many pregnancy pathologies is being increasingly recognized. Exploring the role of the placenta in these pathologies presents a number of challenges. One is the limited numbers of clinical cases available through individual institutions, a problem resolved through development of consortia. Frequently however, these groupings are formed around specific pathologies, limiting the amount and type of data applicable to other pathologies. Another challenge is the type and standardization of data parameters and differences in data gathering and analytical platforms between institutions. To overcome these challenges the development of a Placenta Research Network was proposed. It is envisaged that participant institutions and clinicians/scientists will work jointly to determine the role of the placenta in pregnancy pathologies and to use placental structural and functional analyses for diagnostic purposes, clinical management, and generation of basic science questions of translational importance.

The aim of the workshop was to facilitate discussion focusing on a number of related goals addressing the feasibility of a Placenta Research Network.

Participants: A core of participants interested in discussing Network possibilities was formed in advance and attended the workshop, in person or via Skype. The email addresses of all participants have been collated and will form the core of the network.

3.2. Results

There was substantial support for the idea of a Placental Research Network (PRN) from the attendees. Several individuals who already have bio-banks, long-term projects following measures of placental function/morphology/pregnancy outcomes in relation to specific disorders, spoke on their experience and needs. There was agreement that we could do several things to facilitate development of a PRN.

- 1) Develop a listing of the resources of network participants and beyond, including tissue/blood samples (leaders: Brian Cox, Donald Chaffin, Lee Adamson, Stephen Tong). Examples included Global Alliance to Prevent Prematurity and Stillbirth (GAPPS, Donald Chaffin), Global Pregnancy CoLaboratory (CoLab, Annetine Staff), PRE-eclampsia Eclampsia Monitoring Prevention and Treatment (PRE-EMPT, Jim Roberts), European Working Group on Abnormally Invasive Placenta (EW-AIP, Sally Collins), and the Placental Health Project (John Kingdom), each of which has websites that could be linked to the PRN.
- 2) Assemble reviewed and verified protocols for sample collection and processing, and other placental procedures, making such protocols and examples of their use available on the PRN (leaders: Yoel Sadovsky, Nick Illsley). Examples included isolation of RNA from placental samples (UCSD, Louise Laurent), and soon to be published protocols in Placenta (e.g. sampling for morphometric analysis).
- 3) Develop a group of participating perinatal pathologists for the purpose of standardizing parameters related to placental diagnoses, structures etc. The example given was of the enormous variation in measurement of placental weight globally and from institution to institution (leaders: Stacy Zamudio, Fusun Gundogan).
- 4) Construct a layered (minimum, medium, maximum) set of standardized imaging and clinical data collection protocols (leaders: Sally Collins, John Kingdom, Tullia Todros).
- 5) Develop a website for the assembling and dissemination of the material described in 1–4 above, as well as a place to find like-

minded people for collaborative purposes (leaders: Brian Cox, Yoel Sadovsky, John Kingdom).

A major long-term goal is funding. The idea was put forward that we could use an extant network focused on a rare placental pathology, placenta accreta, as an example and an organizing tool for developing the network. The website <http://ew-aip.org/> presently focuses on clinical (including ultrasound) data acquisition, pathological analysis, and surgical methods. It lacks cellular/molecular analysis, but this could be easily remedied. This network could serve as a model for a broader network when seeking to obtain funding. Other, similar ideas for experimental protocols which can build on the advantages of a network without duplicating other efforts, and remembering the limitations of a small, untested organization, are welcome.

Yoel Sadovsky, American editor of Placenta, reported that the journal would be interested in supporting the network and was already developing a series of papers addressing specific protocols, something which could contribute to (2) above. Moreover it may be possible to develop a PRN website in collaboration with Placenta as a host.

Additional discussion items of importance were multicenter ethical issues related to Individual consent forms, Institutional Review Boards (IRBs), cross-border collaboration, and use of a common Materials Transfer Agreement (MTA). The PRN will encourage institutions to link to the NIH global MTA, which many international institutions already accept, rendering it much easier to transfer samples between collaborating institutions.

PRN contacts: Stacy Zamudio (stacy.zamudio@gmail.com), Nick Illsley (Nillsley@HackensackUMC.org) and Sally Collins (sally.collins@obs-gyn.ox.ac.uk).

4. Placental imaging: insights into current and future modalities

Chairs: Andrée Gruslin and Tullia Todros

Speakers: Lee Adamson, Andrée Gruslin, Carolyn Salafia, John Sled, Tullia Todros

4.1. Outline

Placental imaging is being increasingly used to help inform patient care. Several types of imaging techniques, which provide different information, are available to the clinician. These include ultrasound based technology (2D and 3D sonography and Doppler) and MRI based technology. The workshop aimed to examine the use of 2D and 3D sonography in examination of normal and pathological placental growth and development; how Doppler and color flow analysis can help in diagnoses of placental abnormalities and evaluation of vascularization; how MRI is used in the evaluation of invasive placental disease (accreta); and how MRI is used to evaluate placental function/perfusion.

4.2. Summary

Tullia Todros gave an overview of ultrasound techniques in placental biology. Ultrasound techniques enable investigation of normal and abnormal development of the human placenta throughout pregnancy, for both morphology and localization. Ultrasound classification of placental morphological changes has been proposed as predictive of fetal lung maturity and maternal–fetal outcomes. Placental localization is useful to establish the distance between the inferior margin and the internal uterine os (opening to the cervix). The prevalence of low lying placenta

decreases from 20 weeks of gestational age to term due to “placental migration”. Different hypotheses have been suggested to explain this: the development of the lower uterine segment, a “dynamic placentation”, trophotropism. Ultrasound is also useful in the assessment of placenta praevia and associated complication accretism (absence of retroplacental sonolucent zone, abnormal placental lacunae with turbulent flow, discontinuity of vascular arch, thinning of the myometrium). In addition, ultrasound can detect tumors which appear as cystic changes of placenta. Differential diagnosis includes molar pregnancies and placental mesenchymal dysplasia: both situations require complex clinical management.

Andrée Gruslin discussed the use of Doppler in placental biology. Doppler interrogation of the uteroplacental and fetal circulation can provide important information regarding the physiology of placental development, the pathophysiology of placenta mediated diseases and the health of the developing fetus. Abnormalities in Doppler indices of particular vessels such as the uterine artery can also provide insight into risks related to pre-eclampsia and fetal growth restriction. Novel investigations of intraplacental Doppler (including spiral arteries) may help further our understanding of such syndromes and improve our identification of women at risk.

Carolyn Salafia discussed imaging of the placenta after delivery. Imaging the placenta after birth poses many challenges. Methods were presented for imaging the whole delivered placenta shape and the potential for topographical analysis of placenta growth, extracting data from the surface chorionic vascular networks or arteries and veins and the resulting variables that can be calculated. In addition, methods for automating these procedures were discussed.

John Sled gave an overview of MRI and its applications in the placenta. The practical aspects of MRI and standard imaging protocols were reviewed. In addition, specific research oriented techniques for measuring tissue structure, blood flow, perfusion, blood volume and oxygenation were discussed. Human and mouse studies were described along with future directions for this technology.

Lee Adamson discussed imaging the placenta in animal models. Placental imaging applied to animal models is providing new insights into the genetic and environmental factors important in placental development. Vascular corrosion casting and micro-computed tomography are being used to generate 3-dimensional images of blood spaces in post mortem specimens whereas micro-ultrasound is used to generate 2-dimensional placental images and color Doppler blood flow visualization *in vivo*. Placental imaging provides important qualitative evaluation of placental phenotypes. In addition, quantitative analysis of Doppler blood velocity *in vivo* and feto–placental vascularity by micro-computed tomography permits statistical comparisons between experimental and control groups.

4.3. Conclusions

Imaging techniques such as ultrasound, Doppler and MRI have provided highly relevant non-invasive tools in the evaluation of placental anatomy and function both in the human and in animal models. This has furthered our understanding of placental physiology, including growth and development and also of certain elements involved in placenta mediated diseases.

5. Evolutionary approaches to understanding pre-eclampsia: the view from the placenta

Chairs: Anthony M. Carter and Derek E. Wildman

Speakers: Anthony M. Carter, Michael G. Elliot, Michael R. McGowen, Derek E. Wildman, Stacy Zamudio

5.1. Outline

Among primates the depth of trophoblast invasion in humans is matched only in chimpanzees and gorillas. Pre-eclampsia is a human obstetrical syndrome that often has been linked to the depth of trophoblast invasion. This workshop aimed to explore whether placental evolution can be linked to pre-eclampsia using genomic data and information on the placental transcriptome.

5.2. Summary

Anthony Carter noted that interstitial implantation occurred in all great apes but was not always accompanied by deep trophoblast invasion. Gibbons showed a pattern close to that known from macaques and baboons. Gorilla and chimpanzee resembled the human in two respects: there was invasion by interstitial and vascular routes and trophoblast reached the inner myometrium. Shallow invasion was a feature of pre-eclampsia in humans but it was unclear whether this syndrome occurred in the great apes. Evaluation of the genome and placental transcriptome of primates might throw light on the evolution of deep trophoblast invasion and the origin of pre-eclampsia.

Derek Wildman addressed molecular approaches commonly used to elucidate evolutionary aspects of placentation and obstetrical syndromes such as pre-eclampsia. There is a complementary relationship between the evolution of protein coding genes and the evolution of gene regulation. It remained unclear, however, whether either of these processes was more important in the evolution of divergent placenta phenotypes. There were examples of adaptively evolving protein-coding genes that were expressed in the placenta throughout human descent from the time of the last common ancestor of placental mammals. Additionally, humans and other primates possessed a suite of genes uniquely expressed amongst mammalian placentas. Deciphering the meaning of molecular evolutionary changes associated with placentation would require functional validation in addition to inferential studies.

Michael McGowen discussed the evolution of gene expression in the term placenta of therian mammals and the emergence of pre-eclampsia. Understanding gene expression patterns among multiple species could illuminate the molecular basis of placental function and potentially enrich our knowledge of the biology of obstetrical syndromes. RNA-Seq was used to profile the term placenta of 6 therian mammals. Genes that showed significant elevation in expression along the human lineage were enriched in gene ontology categories such as angiogenesis, cell migration and regulation of inflammatory response. Three of these genes (*KISS1*, *ADAM12*, *GPC3*) showed exceptionally high expression in humans;

these were genes implicated in pre-eclampsia and may indicate that the risk of pre-eclampsia recently emerged on the human lineage.

Michael Elliot spoke about the adaptive evolution of co-regulated genes during evolutionary transitions from haemochorial to non-haemochorial placentation and the implications for pre-eclampsia. Genes evolving adaptively during three independent transitions in Euarchontoglires from highly invasive haemochorial to less-invasive epitheliochorial or endotheliochorial placentation were enriched with pre-eclampsia-associated single-nucleotide polymorphisms (SNPs) and genes that were differentially expressed in pre-eclampsia. Analysis of these genes through mapping onto known co-regulation networks and co-expression networks in various human placental tissues, had given new insights into transcription factor and micro-RNA regulatory systems that were targets of selection during transitions in placental invasiveness, and which may also be involved in the pathogenesis of pre-eclampsia.

Stacy Zamudio spoke to the persistence in human populations of pre-eclampsia, a syndrome that should be subject to profound negative natural selection. A key insight was that near term, mild pre-eclampsia was often associated with larger babies. Her data showed that a rise in blood pressure late in pregnancy is a critical factor in increasing oxygen and nutrient delivery to the fetus and placenta and therefore contributes to reproductive success. It was well known that blood volume does not increase in severe pre-eclampsia, hence an increase in blood pressure and consequent vascular and organ system damage may result. "Overshoot" or abnormally early onset of the normal blood pressure changes of pregnancy might account for the persistence of pre-eclampsia in human populations.

5.3. Conclusions

Analysis of gene expression patterns in the placenta of mammals is informative regarding the transition from haemochorial to endotheliochorial placentation and the evolution of deep trophoblast invasion in the human lineage. When the results are interpreted in relation to data bases concerning gene expression pathways and human disease they yield new insights about the origin of pre-eclampsia. It is important, however, not to lose sight of the pathophysiology of what likely is a syndrome unique to human pregnancy. Phenotypic data on placentation needs to be systematized in a way that allows better integration with molecular data bases. A promising tool could be the current MorphoBank project on mammalian fetal membranes, placentation and development.

Conflict of interest statement

None of the authors have any conflict of interest to declare.