Program for 2011 ANZPRA Satellite meeting

8:00-16:40, Friday 7th October 2011

The Sebel Cairns Hotel, Cairns, Queensland

Major Aims:

(1) Create a forum for ANZPRA members to network, exchange ideas and set up collaborations

(2) Provide an opportunity for ANZPRA members including ECRs to showcase their research

(3) Update ANZPRA members on key advances emerging in the field around the globe

Registration: 08:00-08:30

Welcome by ANZPRA president: 08:30-08:35

Session 1: 8:35-10:20

Preeclampsia: emerging animal models, mechanisms and detection

Chairs: Claire Roberts and Larry Chamley

1. **Lentiviral transduction & placenta-specific gene manipulation to study PE in a mouse model**
   Masahito Ikawa Research Institute for Microbial Diseases, Osaka University, Japan
   8:35-9:05

2. **MMP-14 cleaves endoglin to produce soluble endoglin: a new therapeutic target to treat severe preeclampsia**
   Stephen Tong Department of Obstetrics and Gynaecology, Mercy Hospital for Women University of Melbourne, Australia
   9:05-9:30

3. **HtrA3 is a potential biomarker for early detection of preeclampsia**
   Guiying Nie Prince Henry's Institute of Medical Research, Melbourne, Australia
   9:30-9:55

4. **Circulating microRNAs as potential predictive biomarkers for pre-eclampsia**
   Wee-Ching Kong Robinson Institute, University of Adelaide, Adelaide, Australia
   9:55-10:20

Coffee Break: 10:20-10:50

Session 2: 10:50-12:30

Trophoblast shedding, decidual regulation and trophoblast invasion

Chairs: Padma Murthi and Brendan Waddell

1. **Trophoblast deportation**

   Qi Chen Auckland University, New Zealand

   10:50-11:15

2. **Epithelial-mesenchymal transition in first trimester trophoblasts**

   Amanda Highet Robinson Institute University of Adelaide, Adelaide, Australia

   11:15-11:40

3. **Decidual regulation of trophoblast invasion**

   Harmeet Singh Prince Henry's Institute of Medical Research, Melbourne, Australia

   11:40-12:05

4. **Regulators of trophoblast invasion at the maternal-fetal interface**

   Rosemary Keogh The Royal Women's Hospital, Melbourne, Australia

   12:05-12:30

Lunch break: 12:30 – 13:30

Session 3: 13:30-15:10

What's hot in placenta research

Chairs: Eva Dimitriadis and Peter Mark

1. **How the placenta makes pregnant women vulnerable to influenza (Flu)?**
   Jorge Tolosa University of Newcastle, Newcastle, Australia
   13:30-13:55

2. **Decidual-trophoblast interactions - identifying critical factors regulating EVT function**
   Ellen Menkhorst Prince Henry's Institute of Medical Research, Melbourne, Australia
   13:55-14:20

3. **Molecular mechanisms of progesterone action in human placental development**
   Padma Murthi The Royal Women's Hospital, Melbourne, Australia
   14:20-14:45

4. **Circadian variation in clock gene expression in the placenta**
   Brendan Waddell University of Western Australia, Perth, Australia
   14:45-15:10

Coffee Break: 15:10 -15:30

Session 4: 15:30-16:30

New regulators and mechanisms of placentation

Chairs: Guiying Nie and Jeff Keelan

1. **The role of stem cells in normal and pathological placentae**
   Bill Kalionis The Royal Women's Hospital, Melbourne, Australia
   15:30-16:00

2. **Epigenetic control of placentation**
   Richard Saffery Murdoch Childrens Research Institute, Melbourne, Australia
   16:00-16:30

ABSTRACTS

Lentiviral transduction & placenta-specific gene manipulation to study PE in a mouse model

Masahito Ikawa
Research Institute for Microbial Diseases, Osaka University, Osaka, 5650871 Japan

Lentiviral vectors developed from human immunodeficiency virus (HIV) are well-known for their potential of stable transgene expression by integrating a transgene into the host genome. Here we introduce the current applications of the lentiviral vectors in the study of placental functions in vivo. When we first transduced zona free fertilized eggs with EGFP expressing lentiviral vectors, about 30% of the treated embryos developed to term and about 70% of them were transgenic. Next, when we transduced zona free blastocyst, trophoblast specific transgene incorporation followed by placenta specific transgene expression was obtained. The transgenic efficiency was 100% (81/81) after 5 hrs of transduction at 1 x 10^8 infectious unit/ml. With this method, we complemented placental defects and embryonic lethality in Ets2, Erk2, and p38a KO mice (Nat Biotechnol. 2007 Feb;25(2):233-7). We also reported that placenta specific gene knockout by expressing Cre recombinase combined with integrase defective lentiviral vectors (Genesis. 2009 Dec;47(12):793-8). In the current study, we developed the preecalmptic model mice by placenta specific expression of anti-angiogenic factor, sFLT1. Preeclampsia is a relatively common pregnancy related disorder and both maternal and fetal lives will be endangered if it proceeds unabated. We will introduce our preeclampsia model mice and discuss about the low dose statins as the potential of preeclampsia treatment (Proc Natl Acad Sci U S A. 2011 Jan 25;108(4):1451-5).

MMP-14 expression is increased in preeclamptic placentas and mediates release of soluble endoglin

[1]Tu'uhevaha Kaitu'u-Lino*, [1]Kirsten Palmer*, [1]Clare Whitehead, [2]Elizabeth Williams, [3]Martha Lappas, [1]Stephen Tong (* These authors contributed equally)

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Soluble endoglin (sEng) is an anti-angiogenic protein released from the placenta into the maternal circulation in preeclampsia. It is thought to play a major role in causing endothelial dysfunction and maternal end organ damage. The mechanism of sEng cleavage and release from placenta is unknown. Recent work in an over-expression system identified matrix metalloproteinase (MMP-14; MT1-MMP) as the protease that cleaves endoglin to produce sEng. Thus, we hypothesized MMP-14 may be responsible for placental release of sEng in preeclampsia. We investigated whether 1) MMP-14 is upregulated in preeclamptic placentas, 2) interacts with endoglin, and 3) whether its inhibition decreases sEng release from placental tissue *in vitro* and *in vivo*. We found both endoglin and MMP-14 were localized to the syncytiotrophoblast and significantly ($p \leq 0.05$) elevated in placentas from severe preterm preeclampsia compared to gestationally-matched controls. Proximity ligation assay showed protein-protein interaction between endogenous endoglin and MMP-14 at the syncytiotrophoblast membrane surface of preeclamptic placentas. In syncytialised BeWo cells (models syncytiotrophoblast), we demonstrated MMP-14 inhibition by either small molecules (GM6001) or siRNA decreased sEng release *in vitro* ($p \leq 0.05$). Administering GM6001 to NOD/SCID mice bearing BeWo xenografts decreased circulating sEng levels in mouse serum ($p \leq 0.05$). Therefore, we conclude MMP-14 is upregulated in preeclamptic placentas, interacts with endoglin and mediates release of sEng from placental tissue. Blocking MMP-14 cleavage of endoglin may be a potential therapeutic strategy to decrease circulating sEng, quenching maternal disease in severe preeclampsia.

HtrA3 is a potential biomarker for early detection of preeclampsia

Guiying Nie

Prince Henry's Institute of Medical Research, Clayton VIC 3168, Australia

Preeclampsia is a serious disorder of human pregnancy affecting 5-10% of all pregnant women worldwide. The pathogenic origin of preeclampsia is defective placental development during early pregnancy. Owing to the lack of reliable early detection biomarkers, preeclampsia is not diagnosed until later in pregnancy and premature delivery remains the sole effective therapy. HtrA3 is a recently cloned serine protease with high expression during placentation in the mouse, rhesus monkey and human. We established that in women, placental HtrA3 protein was maximally produced in the 1st trimester then dramatically down-regulated, especially in the syncytiotrophoblasts. HtrA3 was secreted into the maternal circulation with a serum profile reflecting placental production. Importantly, maternal serum HtrA3 levels around the time of the placental oxygen switch significantly differed between women who subsequently experienced normal or preeclamptic pregnancies. Our study provides experimental evidence and molecular rationales that abnormal maternal serum HtrA3 protein levels during early pregnancy may predict preeclampsia. Our results suggest a new avenue for monitoring early placentation abnormalities using a serum biomarker, with important implications in pre-symptomatic screening and early diagnosis of preeclampsia. We have now developed and validated a sensitive and high throughput assay capable of detecting HtrA3 in serum at pM levels. Future studies will screen a large number of appropriate serum samples to determine the assay's sensitivity and specificity in predicting preeclampsia in women during early pregnancy for clinical applications.

Circulating microRNAs as potential predictive biomarkers for preeclampsia

Wee-Ching Kong, Claire T. Roberts, Julie A. Owens
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Preeclampsia is a leading cause of maternal and fetal mortality and although its exact origins are unknown, it is believed that impaired early establishment of the placenta is a key contributor. It has recently been shown that syncytiotrophoblasts can release microRNAs (miRNAs) into the circulation [1]. miRNAs are short, non-coding RNAs that post-transcriptionally regulate translation. Their circulating levels are altered in pathologies such as cancer [2] and are also altered during pregnancy [3]. Thus, the current study aimed to determine if circulating miRNAs could be used as a predictive biomarker of preeclampsia. Plasma samples from 12 healthy women and 12 women of matched BMI who later developed severe preeclampsia were collected at 15 weeks gestation. Plasma miRNAs were extracted and profiled using Taqman® MicroRNA array cards. Predicted mRNA targets of miRNAs were found using miRecords database [4] and highly represented molecular networks involving targets were identified using Ingenuity® Pathways Analysis (www.ingenuity.com). Plasma concentrations of 9 miRNAs (mir-148a, 30c, 491-5p, 28-5p, 483-5p, 330-3p, 140-3p, 636, 331-3p) were decreased in preeclamptic mothers compared to BMI-matched healthy mothers ($p<0.05$). Many predicted targets of these miRNAs are involved in the molecular mechanisms of cancer, a process which has many parallels with trophoblast migration in the early establishment of the placenta. These findings suggest plasma miRNAs have potential as an early predictive test for preeclampsia, to enable development of preventative measures to try and limit severity of symptoms and improve neonatal outcomes.

1. Luo SS et al 2009 Biol Reprod, 81, 717-29; 2. Brase JC et al 2010 Mol Cancer, 9, 306;
3. Miura K et al 2010 Clin Chem, 56, 1767-71; 4. Xiao F et al 2009 Nucl Acids Res, 37, D105-10.

Trophoblast deportation and maternal vascular response

Q Chen, P Stone, L Chamley
Department of Obstetrics & Gynaecology, The University of Auckland.

Preeclampsia is characterized by systemic maternal endothelial dysfunction and inflammation prior to the onset of clinical symptoms. It is thought that a factor released from the placenta triggers the maternal endothelial response. . In normal pregnancy 100,000 syncytial knots are estimated to be shed into the maternal circulation daily as a result of apoptotic death in the syncytiotrophoblast. Syncytial knots deported to the lungs where they lodge in the pulmonary capillaries. The number and the nature of syncytial knots is altered in preeclamptic pregnancies. We have shown that phagocytosis of necrotic, but not apoptotic syncytial knots by endothelial cells results in their activation accompanied by secretion of IL-6 and TGFβ1. These cytokines are reported to be elevated in the sera of women with preeclampsia their production by pulmonary endothelial cells could lead to activation of vascular beds distal to the lungs. Treating peripheral blood mononuclear cells (PBMNCs) with conditioned medium from endothelial cells that had phagocytosed necrotic syncytial knots resulted in proliferation of the PBMNCs suggesting that phagocytosis of necrotic syncytial knots by endothelial cells may also subsequently lead indirectly to immune activation similar to the heightened inflammatory response seen in preeclampsia. Recent we demonstrated that phagocytosis of apoptotic syncytial knots resulted in the suppression of the ability of endothelial cells to be activated by potent activators, suggesting that the release of apoptotic syncytial knots from the placenta during normal pregnancy may be a mechanism by which the fetus attempts to protect the maternal vasculature from activation.

Epithelial-mesenchymal transition in first trimester trophoblasts

Amanda Highet, Claire Roberts
Research Centre for Reproductive Health, Robinson Institute, University of Adelaide.

In early development, epithelial cells switch between epithelial and mesenchymal forms to give rise to diverse cell types. This differentiation process is called epithelial-mesenchymal transition (EMT). The role of EMT in the differentiation of placental trophoblast cells into invasive cytotrophoblasts is unclear. We hypothesise that trophoblasts undergo EMT when stimulated by low oxygen and insulin-like growth factor-II (IGF-II) in the early first trimester placenta environment. The first trimester trophoblast cell line, HTR8/SVneo, and choriocarcinoma cell line, JEG-3, were cultured in serum reduced media containing 25nM IGF-II or Leu^{27}IGF-II (IGF2 receptor specific), in 1%, 5% and 20% oxygen for 24h. The cells were labelled with fluorescent antibodies against E-cadherin (down-regulated in EMT), snail (transcription factor up-regulated during EMT) and vimentin (marker of mesenchymal cells). RNA was extracted from HTR8/SVneo and JEG-3 cells cultured in parallel for analysis of E- and VE-cadherins (*CDH1, CDH5*), *snai1* and *vimentin* expression. HTR8/SVneo did not express surface E-cadherin but treatment with IGF-II and Leu^{27}IGF-II increased vimentin expression, especially in low oxygen. JEG-3 cells expressed high surface E-cadherin but no vimentin or snail, and were unaffected by IGF-II treatment and oxygen concentration. Preliminary gene expression data showed *CDH5* and *CDH1* mRNA expression in HTR8/SVneo was decreased by IGF-II, consistent with EMT. *Snai1* was expressed in HTR8/SVneo in all conditions but was also decreased by IGF treatment, suggesting that it is not involved in IGF-II-induced transition in HTR8/SVneo. Down-regulation of cadherins and increased vimentin expression after IGF-II treatment in HTR8/SVneo suggest they are capable of EMT-like differentiation.

Decidual regulation of trophoblast invasion

Harmeet Singh [1], Shin-ichi Makino [2,3], Yaeta Endo [2], and Guiying Nie [1]

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Controlled trophoblast invasion into the maternal decidua (interstitial invasion) is important for placental development and function. Abnormalities in the invasion process may lead to pregnancy complications. Decidua secretes many factors to regulate trophoblast invasion. Serine protease HtrA3 is highly expressed in the decidual cells in the late secretory phase of the menstrual cycle and throughout pregnancy. It is highly expressed in first trimester in most trophoblast cell types, but not in the invading interstitial trophoblast. HtrA3 and its family members are down-regulated in a number of cancers and are proposed as tumor suppressors. We hypothesized that HtrA3 is an inhibitor of trophoblast invasion and HtrA3 secreted by decidual cells regulates trophoblast invasion.

Human endometrial stromal cells (HESC) were decidualised with estradiol, medroxyprogesterone acetate and cyclic-AMP. With decidualization, an increase in HtrA3 mRNA and protein expression was observed. HtrA3 was also detected by western blotting in the conditioned media (CM) of decidualised HESC, confirming its secretory nature. For functional studies, wild type and protease inactive mutant HtrA3 were produced using wheat germ cell-free technology. The mutant has negligible protease activity and significantly inhibited the wild type protease activity, supporting its dominant-negative inhibition and utility as a specific inhibitor of the wild type protein. CM of decidualised HESC suppressed invasion of trophoblast HTR-8 cells, whereas inhibition of HtrA3 in the decidual HESC CM by exogeneous addition of HtrA3 mutant increased trophoblast HTR-8 cell invasion. Results support our hypothesis that HtrA3 is tightly regulated during decidualization and decidual HtrA3 negatively regulates trophobalst invasion.

Regulators of trophoblast invasion at the maternal-fetal interface

Rosemary J. Keogh, Simon E. Chau, May H. Wong, Jessie Z-J. Chen and Shaun P. Brennecke.

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An essential event for fetal growth and development is the remodelling of the maternal spiral arteries in the uterine wall to increase blood flow to the placenta and fetus, thus enable fetal growth and development. Trophoblast instigate the remodelling of the vessel structure by migrating away from the placenta and invading the maternal spiral arterioles of the uterine wall. Not only does the remodelling process occur during a defined time frame, but it also proceeds in a defined manner. Trophoblast movement is directed predominantly into the maternal uterine spiral arterioles and not veins and is limited in extent to only penetrate into the first third of the myometrium before ceasing. Trophoblast proliferation, migration and invasion are regulated by the complex interplay of growth factors, cytokines, endocrine factors, oxygen concentrations and haemodynamics at the maternal-fetal interface. These act both temporally and spatially to initially promote and then limit the extent of trophoblast invasion. Shallow or incomplete trophoblast invasion with limited vessel remodelling has been associated with complications of human pregnancy such as pre-eclampsia and fetal growth restriction. We have investigated the roles of two pathways in regulating trophoblast function; chemokines (cytokines) and pregnancy hormones (endocrine). Gaining detailed knowledge of the factors that control trophoblast invasion will help to identify defects in these regulatory pathways that contribute to the pathogenesis of such pregnancy complications.

This work has been supported by a grant-in-aid from the Heart Foundation (G 10M 5185).

How the placenta makes pregnant women vulnerable to influenza (Flu)

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As in previous influenza epidemics and pandemics, pregnant women infected with 2009 pandemic influenza A (H1N1) appeared to have an increased risk of developing more severe disease and were disproportionately represented among hospitalizations, ICU admissions, and deaths (50% of the pregnant women infected worldwide were hospitalized, of whom 23% were admitted to an ICU and 8% died). Pregnancy requires adaptations of the maternal immune system, both locally and systemically to prevent the rejection of the semi-allogeneic fetus. Although the underlying mechanisms are poorly understood, these changes are likely to explain the increased susceptibility to influenza and other viral infections observed during pregnancy. Recent investigations have shown that the placenta produces and secretes immunosuppressive retroviral envelope proteins and immunomodulating exosomes that may explain the inhibition of innate and adaptive cell-mediated immune responses characteristic of pregnancy. Therefore, to explain the susceptibility of pregnant women to influenza, we hypothesise that the human endogenous retroviral envelope protein Syncytin-1 and human placental exosomes suppress maternal innate and adaptive cell mediated immune responses to influenza viruses by altering the function of peripheral blood mononuclear cells and the function of respiratory epithelial cells. We have shown that pregnant women had evidence of impaired antiviral immune responses to influenza virus compared to healthy non-pregnant women, as measured by the release of IFN-α and IFN-γ by PBMCs following exposure to influenza virus. We have also shown that Syncytin-1 has immunosuppressive properties and that Syncytin-1 by its self is able to reduce the antiviral response to PHA and rhinovirus in PBMCs from non-pregnant women.

Decidual-trophoblast interactions – identifying critical factors regulating EVT function

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Extravillous trophoblast (EVT) must adhere, migrate and invade through the decidua to form a functional placenta. Abnormal decidualization of endometrial stromal cells (ESC) leads to unregulated trophoblast invasion and pregnancy failure in mice. Recent evidence in humans suggests that preeclampsia is associated with impaired decidualization. The mechanisms by which decidual cells interact with EVT remain largely unknown. We hypothesise that decidual cells interact with EVT to regulate invasion and that the extent of decidualization is critical for the regulation of EVT function. We aimed to identify EVT proteins regulated by decidualized and non-decidualized ESC secreted factors.

Primary human ESC were decidualized with cAMP, estradiol 17β and medroxyprogesterone acetate. ESC conditioned media (CM) was collected from Days (D) 0-2 (non-decidualized) and D12-14 (decidualized) of treatment. Prolactin (decidual marker), was measured by ELISA. Cytotrophoblast were isolated from 1st trimester placenta and cultured on MatrigelTM to induce the EVT phenotype. EVT were treated with ESC CM and secreted proteins <30kD were purified using size exclusion affinity particles (SEAN) and identified by mass spectrometry. Identified protein were validated by immunohistochemistry (cellular localization) and Western blot (regulation by ESC CM).

Decidualized/non-decidualized secreted factors differentially regulated EVT proteins. More than 40% of EVT-secreted proteins were previously unknown in the placenta and of the known proteins, >60% have previously been shown to be dysregulated in preeclampsia or IUGR, suggesting that this method is ideal for identifying novel, EVT-expressed factors with potential for diagnostics or therapeutics. Identified proteins with important functional roles in EVT invasion will be discussed. In conclusion, adequate decidualization may be critical for appropriate EVT function and therefore placental development in humans.

Molecular mechanisms of progesterone action in human placental development

Padma Murthi
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Fetal growth restriction (FGR) is a significant pregnancy disorder affecting 3-5% of all human pregnancies. In most FGR cases, the cause is unknown but there is a strong association with abnormal placental development and function. In these so-called idiopathic FGR cases, placental trophoblasts display increased apoptosis associated with reduced placental growth during the second trimester of pregnancy, the period of maximum fetal growth. Overall aim of our study is to identify a key molecular mechanism controlling placental development, which, when aberrant, leads to FGR. Progesterone (P) is critical for the establishment and successful maintenance of pregnancy. The effects of P depend on the availability of progesterone receptors (PRs) called PR-A and PR-B. We hypothesised that increased ratio of progesterone receptors in FGR placentae may contribute to abnormal trophoblast function. PRA:PRB ratio was assessed by real-time PCR and immunoblotting in placentae from FGR and gestation-matched controls (n=5). Functional role of PR in trophoblast was assessed using BeWo cells and RNAi technology. Increased ratio of PRA:PRB mRNA (0.26 ± 0.009 FGR vs. 0.02 ± 0.1 control, $p<0.05$ n= 5) and protein (2.5 ± 1.2, FGR vs 1.2 ± 0.6, control, n=5, $p<0.5$) was observed in FGR-affected placentae compared with control. PRB inactivation demonstrated a significant in markers of trophoblast differentiation, 3BHSD, DLX3 and increased markers of apoptosis such as p53. These studies provide potential role for progesterone receptors in feto-placental growth in FGR.

Circadian variation in clock gene expression in the placenta

Brendan J Waddell, Michaela Wharfe and Peter J. Mark
School of Anatomy & Human Biology, The University of Western Australia

Rhythmic expression of clock genes drives circadian variation in physiological processes both centrally and within peripheral tissues. The aims of this study were to determine if the two zones of the rat placenta (junctional and labyrinth) differentially express clock genes and, if so, whether these exhibit circadian patterns. Rats were sampled from day 21 of pregnancy (term = day 23) and at diestrus. Adult liver, fetal liver, and placental zones were collected at 0800, 1400, 2000 and 0200 h. Both placental zones expressed all clock genes, with zonal differences clearly evident for each. While placental expression of *Bmal1*, *Per1* and *Per2* varied with time of day (25-50% increases), circadian variation was less robust than in adult liver (4-20-fold changes). Clock gene expression in maternal liver changed with pregnancy; expression of *Per1*, *Cry1* and *Cry2* were lower in pregnancy (by 40-50%) whereas *Per3* expression increased (2-fold) and *Clock* expression no longer varied with time of day. In fetal liver, all clock genes were expressed, but only *Per2*, *Per3* and *Cry1* varied with time of day suggestive of an immature clock. Moreover, placental and fetal liver clock profiles were less robust and not synchronous with those in maternal liver. Maternal, but not fetal, corticosterone levels showed clear circadian variation. In conclusion, our data show that the rat placenta expresses canonical clock genes in a zone-specific manner but with relatively little circadian variation. Moreover, pregnancy alters the circadian expression of clock genes in maternal liver, possibly contributing to maternal physiological adaptations.

The role of stem cells in normal and pathological placentae

B Kalionis[1], B Alsowayan[1], G Kusuma[1], S Qin[1], RA Pace[1], N Castrechini[1], P Murthi[1], M Abumaree[2], M Al Jumah[2], SP Brennecke[2].

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The placenta is an abundant, non-invasive and ethically acceptable source of mesenchymal stem cells (MSCs, also referred to as mesenchymal stromal cells). Other reproductive tissues associated with the placenta (e.g. umbilical cord, decidua) have been found to contain MSCs, whilst the fetal membranes contain both MSCs and an epithelial stem cell type. MSCs are being used in preclinical trails to assess their potential for use in regenerative medicine with some success. However, the role of MSCs in their natural microenvironment (i.e. the niche) within the placenta, and their potential role in placental pathologies are poorly understood. We have provided evidence by immunohistochemistry and immunofluorescence that the stem cell niche in the placenta villi, and in placenta-associated tissues, is perivascular. More recently, we have developed *ex vivo* perfusion methods to better understand the stem cell niche. Finally, we have combined explant tissue culture, cell culture methods with gene expression, FACS analysis and stem cell functional assays to show that MSCs are abnormal in placental pathologies.

Evidence for widespread changes in placental promoter methylation in response to increasing gestational age and environmental/stochastic factors

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Due to its varied roles at the feto-maternal interface, the placenta is subject to many environmental exposures that can potentially alter its epigenetic profile. Previous studies have reported gene expression differences in placenta over gestation, as well as inter-individual variation in expression of some genes. However, the factors contributing to this variation in gene expression remain poorly understood. In this study, we performed a genome-wide DNA methylation analysis of gene promoters in placenta tissue from three pregnancy trimesters. We identified large-scale differences in global DNA methylation levels between each trimester, with an overall progressive increase in average methylation during gestation. The most differentially methylated genes included many immune regulators, reflecting the change in placental immuno-modulation as pregnancy progresses. We also detected increased inter-individual variation in the third trimester relative to first and second, supporting an accumulation of environmentally induced (or stochastic) changes in DNA methylation pattern. These highly variable genes were enriched for those involved in amino acid and other metabolic pathways, potentially reflecting the adaptation of the human placenta to different environments. The identification of cellular pathways subject to drift in response to environmental influences provide a basis for future studies examining the role of specific environmental factors on DNA methylation pattern and placenta-associated adverse pregnancy outcomes.