Objectives: Preeclampsia (PE) is a heterogeneous disorder of pregnancy, with no robust biomarkers or effective treatments. We hypothesize that this heterogeneity is due to the existence of multiple subclasses of PE driven by different molecular pathways. In support of this hypothesis, we recently identified five clusters of placentas within a large microarray data set, of which three (clusters 1, 2, and 3) contained clinically distinct subgroups of PE samples. Next, our objective was to investigate gene regulatory mechanisms behind these sub-pathologies.
Methods: A total of 43 of our samples from transcriptional clusters 1, 2, and 3 were assessed by Infinium Human Methylation 450K arrays. Relationships between gene expression and epigenetic data were determined using the limma and ChAMP libraries in R, and the HumanMine database.
Results: In comparison to transcriptional cluster 1 placentas, the healthiest samples in our data set, lower methylation was associated with upregulated expression of genes involved in epithelial differentiation and glycolysis in cluster 2, including the known PE markers FLT1, LEP, and INHBA. Additionally, a significant downregulation of electron transport and ATP synthesis genes were identified in cluster 2 samples without an increase in methylation. Cluster 3 placentas revealed increased expression of genes associated with defense/immune response, for which corresponding epigenetic changes were confirmed. However, we also identified a large number of immune-associated upregulated genes in cluster 3 that could not be explained by methylation, some of which are not normally expressed by the placenta.
Conclusion: Thus far, we have established epigenetic mechanisms behind the development of "canonical" PE in cluster 2 and "immunological" PE in cluster 3. Currently, we are exploring alternative explanations for the changes in gene expression with poor correlation to methylation, such as knockdown by microRNAs of respiration-related genes in cluster 2, and possible infiltration of maternal immune cells in cluster 3.

P2.66
ACTIVATION OF CHOLINERGIC ANTI-INFLAMMATORY PATHWAY BY NICOTINE AMELIORATES LIPOPOLYSACCHARIDE-INDUCED PREECLAMPSIA-LIKE SYMPTOMS IN PREGNANT RATS

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Objectives: Preeclampsia (PE) exerts more intense systemic inflammatory response than normal pregnancy. Recently, the role of cholinergic anti-inflammatory pathway (CAP) in regulation of inflammation has been extensively studied. The aim of this study was to investigate the effect of nicotine, a selective cholinergic agonist, on lipopolysaccharide (LPS)-induced preeclampsia-like symptoms in pregnant rats and the underlying molecular mechanism.
Methods: Rats were administered LPS (1.0 μg/kg) via tail vein injection on gestational day 14to induce preeclampsia like symptoms. Nicotine (1.0mg/kg/d) and α-bungarotoxin (1.0μg/kg/d) were injected subcutaneously from gestational day 14 to 19 of pregnancy into rats, respectively. Systolic blood pressure (SBP), urinary albumin excretion and pregnancy outcomes were recorded. On gestational day 20, serum and placentas were collected for cytokines levels measurements using Luminex.
The mRNA and protein expression of α7 nicotinic acetylcholine receptor (α7nAChR) were determined by Real time-PCR and Western blot analysis. Immunohistochemistry was used to determine the activation of nuclear factor-κB (NF-κB) in placentas.
Results: LPS treatment significantly increased SBP and urinary albumin excretion, and decreased fetal weights compared with control group, while nicotine treatment reversed these changes. Nicotine treatment decreased the levels of LPS-induced pro-inflammatory cytokines in serum and placenta. Nicotine significantly increased the expression of α7nAChR and attenuated NF-κB p65 activation in placenta in LPS-induced preeclampsia. Meanwhile, these protective effects of nicotine were abolished by cholinergic antagonist α-bungarotoxin in preeclampsia rats.
Conclusion: Our findings suggest that activation of α7nAChR by nicotine attenuate preeclampsia-like symptoms and this protective effects is likely due to inhibition of inflammation through NF-κB p65 pathway.

P2.67
DECIDUAL NK CELLS FACILITATE THE INTERACTION BETWEEN TROPHOBLASTIC AND ENDOTHELIAL CELLS VIA PRODUCING VEGF-C AND HGF

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Objectives: Accumulating evidences suggest the tight interactions between trophoblast and dNK cells, which plays essential roles in modulating trophoblast differentiation. However, the underlying mechanism remains to be explored in-depth.
Methods: The condition media were collected from the $CD56^{+}CD16^{-}CD3^{-}$ dNK cells that were isolated and purified from human decidual tissues at early pregnancy. The primary cultured cytotrophoblast cells (CTBs) or human trophoblast cell line, HTR8/SVneo cells, were co-cultured with human umbilical vein endothelial cells (HUVECs) in a 3-dimensional Matrigel scaffold, and the process of tube-structure formation was dynamically monitored with live cell imaging. The invasion ability of the trophoblastic cells were analyzed by transwell invasion assay.
Results: Treatment of HTR8/SVneo cells or the primary CTBs with dNK-CM could remarkably promote trophoblast cell invasion and tube formation ability with HUVECs. The expression of the epithelial cell marker E-cadherin was reduced, and the expressions of NCAM, VE-cadherin and integrinβ1, which represent features of endothelial phenotype, were significantly promoted in HTR8/SVneo cells upon the treatment of dNK-CM. Antibody blocking experiments revealed that dNK cells promoted trophoblast cell invasion through the production of IL-8 and HGF, while induced trophoblast cell differentiation towards endothelial phenotype through producing VEGF-C and HGF.
Conclusion: We developed a three-dimensional model to mimic and dynamically recording the process of blood vessel remodeling, and identified the different roles of dNK-derived factors in modulating EVT cell differentiation. dNK cells could promote iEVTs differentiation through the production of IL-8 and HGF, whereas direct enEVTs properties through the production of VEGF-C and HGF.

P2.68
GLUT1-DOWN-REGULATION LEADS TO A "PREMATURE SENESCENCE" IN PREECLAMPTIC SYNCYTIOTROPHOBLASTS

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Introduction: Cell senescence is known to maintain permanent cell cycle arrest in damaged cells, as a response to certain types of stress. Premature senescence in placental syncytiotrophoblast can offer a link among diverse placental pathologies including those associated with stress or placental insufficiency, such as preeclampsia. Our hypothesis sustain that the down-regulation of GLUT1 might lead to a "premature senescence" in the pre-eclamptic syncytiotrophoblast.
Methods: Several senescence markers (p16, p21, p27, and p53) were first evaluated on mRNA and protein levels in preterm, normal term and pre-eclamptic placental tissues. Using a cytokine array, a variety of senescence-associated secretory phenotype (SASP) proteins secreted by senescent cells, including pro-inflammatory cytokines, were analyzed.
Results: We saw no differences in placental mRNA and protein expression of p16, p21 and p27. However, we detected a higher p53 protein expression in preterm and preelamptic placentae. An increased secretion of cytokines associated with SASP, such as CXCL1, CD54, IL-1ra and PAI-1 was revealed in preeclamptic placentae compared to normal term placentae.