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CLINICAL PROTEOMICS IN ASSESSMENT OF THE PLACENTA

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Proteomics is the study of expressed proteins which emerged to complement genomic research. The major advantage of proteomics is it investigates directly the functional molecules (proteins), not the source code. Proteomics holds the promise of providing more direct insight into true mechanisms of human diseases. Because placenta is a key pathophysiological participant in several major obstetrical syndromes such as preterm labor and preeclampsia (PE), identification of relevant biomarkers can profoundly impact on prediction of fetal outcome and treatment efficacy. For the last several years our group pioneered a complex series of experiments aimed to better understand the pathophysiology of preterm labor and PE.
We first concentrated in understanding the relationships between histological chorioamnionitis, funisitis and presence in amniotic fluid of proteomic biomarkers characteristic of inflammation (DEFENSIN-1 and -2, and CALGRANULIN C and A). Amniotic fluid profiles were generated from 139 women with singleton pregnancies admitted with signs or symptoms of preterm birth and enrolled prospectively. Proteomic analysis of the amniotic fluid provides an opportunity for early recognition of histological chorioamnionitis and targeted treatment of the fetus, in utero, prior to clinical manifestation of the disease.
We further performed comprehensive proteomics profiling of the urine of women with hypertensive disorders during pregnancy (n=272), including women with severe or superimposed PE. We found that a panel of proteomic biomarkers, non-random fragments of SERPINA-1 and ALBUMIN, can accurately predict and diagnose PE and discriminate this condition from other hypertensive proteinuric diseases in pregnancy. De-novo sequencing in MS/MS mode followed by validation experiments in the urine and placenta identified presence and localization of the biomarkers. In the placenta SERPINA-1 was sequestered within the fetal space. Our results provided insight into a novel pathological mechanism of PE which may offer new therapeutic opportunities in the future.
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THE CHEMOKINE SCAVENGER RECEPTOR D6 PROTECTS THE FETUS FROM INFLAMMATION-INDUCED ABORTION

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Chemokines play a key role during inflammatory reactions, driving the leukocyte recruitment process through the activation of specific 7-TM receptors. A set of chemokine receptors structurally unable to elicit conventional signalling responses and cell migration after ligand engagement has also been described. These 'silent' (i.e. non-signalling) chemokine receptors include D6, a high affinity receptor for inflammatory CC chemokines that cycles constitutively and targets the ligand to degradation. D6 is mainly expressed by lymphatic endothelial cells in several tissues, and in vivo evidence demonstrates its non-redundant role in the control of the local inflammatory response. In this study we demonstrated that D6 is also expressed on invading extravillous trophoblasts and on the apical side of syncytiotrophoblast cells, at the very interface between maternal blood and fetus. In vitro experiments using trophoblast cell lines indicate that D6 does not support chemokine transcytosis and efficiently removes inflammatory chemokines from the milieu. The role of placental D6 was investigated in two different models of inflammation-driven abortion. When compared with WT littermates, pregnant $D6^{-/-}$ mice exposed to LPS or antiphospholipid autoantibodies showed higher levels of inflammatory CC chemokines and increased leukocyte infiltrate in placenta. This exacerbated inflammatory reactions caused an increased fetal loss rate in $D6^{-/-}$, which could be reverted in animals treated with a combination of anti inflammatory CC chemokine monoclonal antibodies. Thus, D6 is a chemokine scavenger receptor strategically located at the fetal−maternal interface to dampen placental inflammation. The chemokine system is a prime target for the development of new therapeutic strategies for diverse disorders. This evidence raises the possibility that blocking strategies targeting inflammatory CC chemokines may also be effective in fetal loss prevention.

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PROFILING TOTAL AND ALLELE-SPECIFIC DNA METHYLATION IN HUMAN PLACENTAS

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Objectives: Epigenetic regulation of gene dosage is documented as a mechanism of controlling placental and fetal growth. For example, the phenotypes of mice under- or overexpressing imprinted genes and molecular data from human syndromes of overgrowth and growth restriction show that a major role of imprinted genes is to act as feto-placental growth rheostats. Understanding the full extent of epigenetic regulation in human placenta will be important for a complete understanding of intrauterine growth restriction. We have developed a new approach, called methylation-sensitive SNP array analysis (MSNP), for profiling net DNA methylation and allele-specific DNA methylation (ASM) in human tissues.
Methods: MSNP is a simple adaptation of Affymetrix SNP arrays for profiling DNA methylation; we have profiled DNA methylation at 40K informative markers (SNP-tagged loci) distributed across the human genome, assessing patterns of net and allele-specific DNA methylation in human placentas, peripheral blood leukocytes (PBL), fibroblasts and several types of tumors, including acute myeloid leukemias and complete hydatidiform moles.
Results: The MSNP method has revealed tissue-specific patterns of net DNA methylation, which clearly distinguish normal placenta and hydatidiform moles from non-placental tissues, and has also uncovered new examples of loci with ASM, some showing ASM in placenta but not in PBL, and others showing the opposite pattern. We are currently assessing these loci for whether the ASM is imprinted or non-imprinted. For the loci with non-imprinted ASM we are asking whether the allelic asymmetry may depend on the local DNA sequence.
Conclusions: MSNP is an effective method for profiling net and allele-specific DNA methylation in human tissues. This approach is beginning to reveal clear differences in epigenetic marks in human placenta compared to other human tissues, and is also uncovering novel examples of loci with ASM, some of which are imprinted and others non-imprinted. Data from MSNP will lead to a more complete understanding of the role of DNA methylation in human placental biology.

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ACCUMULATION OF MACROPHAGES IN PLACENTA AND ADIPOSE TISSUE OF OBESE WOMEN.

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Chronic low grade systemic inflammation is a hallmark of obesity and diabetes. We have shown previously that expression of cytokines and markers of inflammation are increased in placenta of obese women with GDM. The aim of this study was to examine the contribution of maternal white adipose tissue and placental macrophages to the inflammatory responses of pregnancy.
Methods: Lean (BMI<25) and obese women (BMI>30) were recruited at term elective cesarean deliveries (39±0.4 weeks) with no sign of infection. Abdominal subcutaneous adipose tissue (3-5g) was obtained at the time of laparotomy and placentas were collected at delivery. Fragments of both tissues were embedded in paraffin for immunohistochemistry and snap frozen in liquid N_2 for molecular assays. Macrophages were visualized by labelling tissue sections with the macrophage markers CD68 and CD14. The macrophage areas were quantitated from 10 labeled fields/ section using ImageJ1.30v software. The phenotype of macrophages was characterized by flow cytometry and realtime PCR.
Results: CD68+ macrophages in adipose tissue increased from 7.7+1.1 to 66 +3.2 % ($p<0.001$) in lean (BMI 21.4±8.8) vs. obese (BMI 35.2±6.2) women. The CD68+ area staining the Hofbauer placental resident macrophages also increased with maternal adiposity (892±243 to 2583±247 mm2/section ($p<0.001$). In obese women with GDM, the mean CD68+ area was 4.6 fold larger than in lean controls due to dramatic focal accumulation of macrophages in stem villi ($p<0.05$). Double staining of placental sections showed a differential localization (peripheral vs. central) of CD14+ and CD68+ macrophages within the villous section. Macrophage accumulation in tissue was associated with a 1.8, 2.4 and 1.4 increased in the expression of CD64, CD68 and EMR1 as well as a 2.2 fold increase in TNF-alpha.
Conclusion: Obesity during pregnancy is associated with dramatic macrophage accumulation in adipose tissue and placenta. The concomitant enrichment of maternal circulating macrophages suggest that systemic macrophages represent one common source for tissue infiltration. On-going studies are investigating the possibility that a second population of placental macrophages multiply from pre-existing resident Hofbauer cells.