Integrated Transporter Elucidation Center
2024 Annual Meeting

Abstract Booklet

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P01

Differential Regulation of Placental OATP Transporters at Low Oxygen Concentrations

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The placenta is a dynamic tissue that begins as a hypoxic tissue with oxygen levels around 3% until blood flow increases and oxygen concentrations rise to 8% during the second and third trimesters. However, pregnancies with complications can decrease blood flow, resulting in reduced oxygen concentrations later in gestation. Organic anion transporting polypeptide (OATP) transporters are responsible for the cellular uptake of endobiotics and xenobiotics in the placenta. In this study, we sought to determine whether varying oxygen concentrations alter the mRNA expression of OATPs in a human immortalized trophoblast cell line. For this purpose, JAR choriocarcinoma cells were exposed to 20%, 8%, and 3% oxygen levels for 24 hours and OATP mRNAs were quantified by qPCR. To confirm that hypoxic responses were activated, downstream targets of hypoxia inducible factor-1α (HIF-1α) including glucose transporter 1 (GLUT1), heme oxygenase 1 (HO1), and vascular endothelial growth factor receptor 1 (VEGFR1) were quantified. Compared to atmospheric oxygen levels (20%), GLUT1 mRNAs were increased 4-fold and 8-fold in trophoblasts exposed to 8% and 3% oxygen, respectively. Similarly, VEGFR1 mRNA levels were up-regulated by 3-fold at 8% oxygen and HO-1 mRNA levels was increased by 2-fold at 3% oxygen. OATP mRNA levels were differentially regulated. Compared to 20% oxygen exposure, trophoblasts exposed to 3% oxygen exhibited a 50% decrease in OATP4A1 mRNAs. OATP1B3 decreased 1-fold from 8% to 3% oxygen. Conversely, OATP2B1 mRNAs increased 10- and 20-fold in trophoblasts exposed to 8% and 3% oxygen, respectively. OATP3A1 mRNAs were also up-regulated by 3-fold at 3% oxygen. These data suggest that OATPs are differentially regulated under low oxygen concentrations and may impact uptake of OATP substrates, including drugs and nutrients in the placenta.

P02

Inhibition of Organic Anion Polypeptide Transporter 2b1 (Oatp2b1) as a therapeutic mechanism to decrease Perfluoroalkyl substance (PFAS) exposure

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PFAS are per- and polyfluoroalkyl substances, a class of synthetic compounds, utilized in consumer goods and industrial applications, for their resistance to degradation and amphiphilic properties. Humans are exposed to PFAS through drinking water, air, diet, and neonatal exposure. A concern for highly exposed populations is that some PFAS have substantial absorption from the gastrointestinal tract (GIT) and only a small portion is excreted. Organic anion transporting polypeptide 2B1 (OATP2B1) expressed at the apical membrane of enterocytes and basolateral membrane of hepatocytes may contribute to PFAS absorptions and enterohepatic recirculation in humans. In previous studies, PFAS substrate activity was evaluated against human hepatic OATP2B1, and in the presence of OATP inhibitor rifamycin SV. 6:2FTS and PFBS had an uptake ratio of 2.30 and 2.45 respectively, and rifamycin sv inhibited the uptake of both PFAS significantly. It is not clear whether OATP2B1 participates in GIT-mediated PFAS uptake or if uptake is mediated predominantly via passive permeability. Thus, the hypothesis tested was that administration of an Oatp inhibitor, rifampicin, can inhibit Oatp2b1 in the GIT, reducing or delaying PFAS absorption. Mice orally received 20mg/kg rifampicin or 0.5%Tween20/PBS, and 30 minutes later received a 0.1mg/kg PFAS cocktail (0.1mg/kg PFOS, PFOA, PFBS, PFHxS, 6:2FTS at equal ratios). To assess Oatp2b1 in the liver, mice received an IV tail injection of 20mg/kg rifampicin or 0.9% saline, immediately followed by a 0.1mg/kg PFAS cocktail. Plasma and tissues were collected 2 hours or 15 minutes respectively, after PFAS administration, snap frozen, homogenized, and processed for LC/MS analysis using QuECheERS. Oral administration of rifampicin reduced plasma PFBS and PFHxS concentrations to 65.5% of controls, and reduced liver PFOS 47.5%, PFOA 47.6%, 6:2FTS 48.1%, PFBS 63.6% and PFHxS 63.4% of the controls. IV rifampicin did not impact PFAS plasma or liver concentrations. These findings suggest that inhibition of Oatp2b1 may be a critical mechanism to interfere with PFAS absorption via the GIT.

P03

Ultrafine particle exposure impairs placental metabolism in a sex-dependent manner

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Inhalation of ultrafine particulate matter during pregnancy has been associated with fetal growth restriction (FGR). FGR is a developmental condition linked to various disease states including respiratory distress, immunological incompetence, adverse neurodevelopment, and in some cases can be the cause of mortality. These findings demonstrate that pregnant populations contribute to the burden of disease caused by particulate air pollution. In these studies, we interrogated the nutrient transport capacity of the placenta as a mechanism for compromised fetal growth, which is heavily influenced by placental metabolism. Glucose, the primary fuel source for fetal growth is both transported to the fetus and metabolized by the placenta for energy. Therefore, in this study we examine placental metabolism at the glycolytic and mitochondrial level. Titanium dioxide nanoparticles (nano-TiO_2) have been used as a surrogate particle for particulate matter in occupational and domestic exposure studies. Our laboratory uses a rodent model to recapitulate the development of FGR via particulate matter exposure. Pregnant Sprague-Dawley rats were exposed to nano-TiO_2 aerosols from gestational day (GD) 6 to 19 via whole-body inhalation. On GD 20 we assessed litter characteristics and used a novel approach to assess placental metabolism by generating precision-cut placenta slices to be evaluated with a Seahorse XF96 Analyzer. Our data suggests a reduction in extracellular acidification rates (ECAR) in exposed placentas compared to control. However, this observation was driven by female placentas showing a 25% decrease in ECAR with gestational exposure to particulate matter in response to oligomycin stimulation, compared to control placentas ($p=0.041$). Furthermore, exposure was associated with a significant increase in glycolytic ATP production compared to control placentas ($p=0.039$). Additional sex-differences were observed with male placentas having a lower spare respiratory capacity than female placentas, indicating that female placentas are more equipped to respond to an increase in energy demand. These data suggest that nano-TiO_2 exposure will cause sexually dimorphic responses in placental metabolism. These outcomes may identify sex-dependent impairments in placental health after particulate matter exposure, warranting a closer examination of the role sex in placental pathophysiology, adverse fetal development, and the developmental onset of adult disease.

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P04

Emerging Impact of Mycoestrogens on Birth and Placental Outcomes and the Protective Role of the ABCG2 Transporter Variant

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Background: Zearalenone (ZEN) is an estrogenic mycotoxin ('mycoestrogen') that contaminates global grain crops leading to detectable concentrations of ZEN and its metabolites, including the synthetic version alpha-zearalanol (ZER), in human populations. Despite in vitro and in vivo animal evidence of endocrine disruption by ZEN, there has been limited investigation in humans. **Objectives:** To examine markers of fetal growth following prenatal exposure to ZEN and evaluate the role of the placental efflux transporter BCRP/ABCG2 in protecting against ZEN's potential fetoplacental toxicity. **Methods:** Placentas were collected from participants (n= 271) in the UPSIDE cohort (Rochester, NY, USA). Placental ZEN and its metabolites were analyzed using HPLC-MS. Birth and placental weights were obtained from medical records and direct measurement, respectively; fetoplacental weight ratio (FPR) was calculated by dividing birthweight by placental weight. Covariate-adjusted generalized linear regression models were used to examine ZEN, ZER, and total mycoestrogens (sum of ZEN, ZER, and their metabolites) in relation to infant and placental size, FPR; we additionally stratified models by infant sex and ABCG2 C421A (Q141K) genotype. **Results:** Mycoestrogens were detected in 84% of placentas (median ZEN: 0.010 ng/g) and total mycoestrogens were associated with lower FPR (-0.20, 95%CI: -0.2,-0.08), particularly in female infants (-0.31, 95%CI:-0.52,-0.09). Associations with birthweight were inverse and overall nonsignificant. Among the 17% of participants with the reduced function 421A ABCG2 variant (AA or AC), total mycoestrogens were associated with lower birthweight (-113.5g, 95%CI: -226.5, -0.50) whereas, in wild-type individuals, total mycoestrogens were associated with higher placental weight (9.9, 95% CI: 0.57, 19.2) and reduced FPR (-0.19, 95%CI: -0.33,-0.05). **Discussion:** Results from this first epidemiological study of prenatal mycoestrogen exposure and perinatal health suggest that mycoestrogens may reduce placental efficiency, resulting in lower birthweight, particularly in female and ABCG2 421A infants.

P05

Prenatal Exposure to Cadmium alters Placental Morphology, Vasculature Development, and Macrophage Subpopulations in Mice

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Objective: The environmental metal cadmium (Cd) accumulates in the placenta during pregnancy and is associated with fetal growth restriction and preterm birth in rodents and humans. In the placenta, Cd generates oxidative stress which may be important in disrupting placenta development and macrophage responses. We sought to determine whether prenatal exposure of mice to $CdCl_2$ alters placenta development and enrichment of macrophage populations. **Methods:** Pregnant C57BL/6Crl mice (n=9-10/group) received distilled water with $CdCl_2$ (0, 5, or 50 ppm) *ad libitum* from gestational day (GD) 7-17. Tissues were collected on GD17 for ICP/MS quantification of Cd concentrations, histomorphologic assessment, and immunohistochemistry. **Results:** Exposure to $CdCl_2$ did not affect fetal or placental weight or size, or number of resorptions. Histological analysis of the placentas demonstrated a significant decrease in junctional zone area, with no change in labyrinth zone size, in placentas of dams treated with 50 ppm $CdCl_2$. Compared to vehicle-treated mice, the area of maternal and fetal blood vessels was decreased in placentas exposed to 5 and 50 ppm $CdCl_2$. Immunohistochemical staining for the fetal endothelial surface marker CD34 was significantly increased in the placentas of fetuses exposed to 50 ppm $CdCl_2$. Further, the number of macrophages that stained positive for the marker F4/80 was increased in both the labyrinth and junctional zones of placentas exposed to 50 ppm $CdCl_2$. Conversely, the number of macrophages that stained positive for Iba1, a protein involved in phagocytosis, was decreased in the labyrinth zones of placentas exposed to 50 ppm $CdCl_2$.**Conclusion:** In the absence of overt fetoplacental toxicity, exposure to $CdCl_2$ during gestation altered development of placental zones and vasculature in mice as well as shifted the abundance of macrophage subpopulations. This research is supported by R01ES029275, T32ES007148, F31ES032319, P30ES005022, UC2HD113039, and Grover Fellowship.

P06

Predictive Models for ABC Transporter Inhibition and Drug Efflux: Data Collection, Model Development, and Application

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ATP-binding cassette (ABC) transporters are membrane proteins that efflux a wide range of compounds from the cell, including drugs, pollutants, and endogenous substances. In recent years, multiple computational modeling studies have used machine learning to predict inhibition and substrate binding for ABC transporters. However, many of these previous studies, namely those that use classification models, often rely on small datasets from past modeling studies with limited applicability. In this study, we manually evaluated 22,275 bioactivity records for the ABC transporters MDR1, BCRP, MRP1, and MRP2 from 875 sources in the ChEMBL database. In combination with data supplemented from PubChem and Metrabase, this effort resulted in eight datasets, comprising a combined total of 9,043 chemicals with one or more inhibition or substrate activities for these four transporters. Using different combinations of four machine learning algorithms and three chemical descriptors, 12 quantitative structure-activity relationship (QSAR) models were developed for each of the eight datasets, resulting in 96 individual models that predict outcomes for inhibition or substrate binding. The models demonstrated good performance during five-fold cross-validation, achieving an average correct classification rate (CCR) of 0.754 for the inhibition models and 0.733 for the substrate binding models. The models were also validated with data sets from DrugBank containing drugs with known substrates or inhibitors for the four transporters, demonstrating an average sensitivity of 0.65. We further analyzed how the models' predictions for efflux transporter activity might influence blood-brain barrier (BBB) permeability. The analysis showed that predictions for MDR1 and BCRP substrate binding helped distinguish compounds with low BBB permeability from those with high BBB permeability. This study provides a large, curated repository of annotated inhibitor and substrate data for public use, alongside applicable models for predicting inhibition and efflux across MDR1, BCRP, MRP1, and MRP2 that can be further used for modeling more complex drug bioactivities, such as BBB permeability.

P07

Transporter-Mediated Uptake of the Microcystin-LR Toxin in Human Placenta Cells at Different Oxygen Concentrations

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Objectives: With global warming and waterway eutrophication, cyanobacterial harmful algal blooms are increasing in incidence and intensity, releasing toxins including microcystin-LR (MC-LR). While MC-LR is a well-established hepatotoxin and neurotoxin, there is growing interest in evaluating its female reproductive toxicities. We sought to evaluate the ability of MC-LR to enter and accumulate in trophoblasts at different oxygen concentrations. **Methods:** Intracellular accumulation of MC-LR (0.1, 1, and 10 μM) over 3 hrs was evaluated in immortalized human cytotrophoblasts (JAR and BeWo cells) and extravillous trophoblasts (HTR8/SVneo) cells using western blotting. Moreover, the function of OATP transporters in JAR cells was determined by pre-incubating cells with cyclosporin A (10 μM), a general OATP inhibitor, prior to uptake of the OATP substrate fluorescein (1 μM) or MC-LR (1 μM) for up to 40 min. The impact of oxygen concentration on MC-LR uptake was tested by incubating JAR cells under 3, 8, and 20% O_2 for 24 hrs, then exposing to MC-LR (1 μM) for 3 hrs. **Results:** Concentration-dependent increases in MC-LR bound proteins (37 kDa) were observed in all three trophoblast cell lines. In addition, inhibition of OATP function in JAR cells using cyclosporin A caused a 57% reduction in MC-LR uptake. Compared to 20% O_2, MC-LR uptake was decreased by 52% and 72% in JAR cells exposed to 3% and 8% O_2 respectively. **Conclusions:** Microcystin-LR enters human trophoblasts by active OATP transporters in a concentration-dependent manner. This uptake is reduced at lower oxygen concentrations which are observed early in pregnancies and during pathological conditions. Ongoing research aims to identify specific OATP isoforms involved in MC-LR uptake into the placenta and the impact of microcystins on key placental functions.
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P08

P-glycoprotein Transporter Expression on Placental Cell- and Size-Specific Extracellular Vesicles Measured by Nanoscale Multiplex High-Resolution Flow Cytometry

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Objectives: Circulating extracellular vesicles (EVs) have surface markers that represent placental cell-specific phenotypes. In turn, placental EVs may provide blood-based biomarkers that reflect placental health and relative risk for later pregnancy complications. Since P-glycoprotein (P-gp) plays an important role in maternal-fetal transport, we hypothesized that variance in placental cell-specific EV expression of P-gp in maternal blood may be a potential biomarker for integrity of the placental barrier. **Methods:** As a proof-of-principle study, we tested culture media from BeWo cells and P-gp expressing HEK cells collected after 72 hours as positive controls for P-gp positive EVs. We employed a highly sensitive and specific nanoscale flow cytometry approach to multiplex antibodies on EVs in control media, stained PBS negative controls, and first trimester plasma samples (11 weeks) for PLAP, HLA-C, P-gp, and EV tetraspanins. Sample volume was standardized with 200nm bead PBS buffer to dilute test samples with results in triplicate reported as nanocounts/1000 beads. **Results:** Media from BeWo cells showed abundant PLAP+/HLA-C- (syncytiotrophoblast, STB) EVs with fewer numbers of HLA-C+ (extravillous trophoblast, EVT) EVs. First trimester plasma samples were similarly enriched with predominantly STB EVs, but also contained 10-fold greater concentrations of EVT EVs compared with BeWo cells. Co-expression of P-gp on these EVs was more abundant in BeWo than HEK cells. Moreover, P-gp was detected on EVs from both EVTs and STBs in first trimester plasma. Potentially significant was the observation that placental STB and EVT EVs from a woman who later developed severe preeclampsia did not express P-gp. **Conclusion:** This pilot study demonstrates the ability to image and quantitate the transport protein P-gp on placental cell-specific EVs. Early data suggest there may be variance related to early onset preeclampsia, which now warrants further study.
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P09

A bioengineered model of human placental exposure to environmental metals during pregnancy

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Exposure of pregnant women to environmental metals is an environmental health issue associated with various pregnancy complications. Efforts to advance our biological understanding of this problem and mitigate its adverse effects, however, have been challenged by the difficulty of human subject research. Here, we present an alternative approach that leverages the design flexibility, controllability, and scalability of bioengineered human reproductive tissues to enable experimental simulation and in-depth investigation of placental exposure to environmental metals in maternal circulation. Central to this method is an in vitro analog of the maternal-fetal interface and its dynamic tissue-specific environment constructed using primary human placental cells grown in a microengineered device. Using cadmium as a representative toxicant, we demonstrate the proof-of-concept of emulating the human placental barrier subjected to the flow of cadmium-containing maternal blood to show how this model can be used to examine adverse biological responses and impaired tissue function on both the maternal and fetal sides. Moreover, we present a mechanistic study of maternal-to-fetal cadmium transport in this system to reveal that efflux membrane transporters expressed by trophoblasts may play an important protective role against cadmium-induced toxicity. Finally, we describe metabolomic analysis of our microphysiological system to demonstrate the feasibility of discovering metabolic biomarkers that may potentially be useful for detection and monitoring of cadmium-induced placental dysfunction.

P10

Placental Barrier Transport: Identification of Maternal and Infant Determinants of BCRP and MDR1 Transporter Levels

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Objective: The 'placenta barrier' is comprised of membrane transporters including BCRP and MDR1 that actively efflux chemicals from the syncytiotrophoblast back to the maternal circulation. This function allows BCRP and MDR1 to limit the accumulation of drugs and toxicants and prevents transplacental transfer of substrates. In this study, we evaluated relationships between maternal (gestational weight gain, early pregnancy body mass index, smoking, parity, pregnancy complications, age) and infant factors (birth weight, race, transporter genetics) and placental levels of BCRP and MDR1 proteins in a healthy U.S. cohort. **Methods:** Term placentas were collected from healthy participants in the Understanding Pregnancy Signals and Infant Development Study (UPSIDE) birth cohort (Rochester, NY, n=237). BCRP and MDR1 proteins were quantified in frozen villous placenta tissues using quantitative targeted absolute proteomic mass spectrometry. We examined determinants of placental protein concentrations through bivariate analyses and multivariable linear regression models. **Results:** Levels of BCRP and MDR1 proteins in term placentas were moderately correlated (r=0.58, p<0.001). In bivariate analyses, BCRP levels were associated with gestational weight gain, infant race, parity, and the rs2231142 genetic variant. In mutually adjusted models, only parity (β=0.07; 95%CI:0.02,0.11) and the rs2231142 AC/AA genotype (β=-0.09, 95%CI:-0.14,-0.04) showed notable associations with BCRP levels. By comparison, in bivariate analyses, MDR1 levels were associated with parity and more weakly, with birthweight, race, smoking, and the rs1045642 and rs2032582 gene variants. In mutually adjusted models, parity (β=0.11; 95%CI:0.05,0.17), smoking (β=-0.11; 95%CI:-0.21,-0.01), the rs1046642 TT genotype (β=-0.14, 95%CI:-0.24,-0.03), and the rs2032582 TT genotype (β=0.10; 95%CI:-0.02,0.21) were associated with MDR1 levels. **Conclusion:** Identifying maternal and infant factors that contribute to the regulation of BCRP and MDR1 levels in healthy, term placentas is the first step in dissecting the impact of disease and environment on the integrity of the placental barrier.
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P11

Integrated Transporter Elucidation Center: Identifying the SLC and ABC Proteome in Human Placenta

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Objectives: A key challenge confronting the transporter field is the lack of a complete proteomic profile of the SLC and ABC transporters enriched in the placenta. One goal of the Integrated Transporter Elucidation Center is to comprehensively define the SLC and ABC proteome in healthy and diseased human placentas using quantitative targeted proteomics (QTAP). In this study, we prioritized transport proteins of interest in human placental cell lines and healthy placental tissue and identified proteotypic peptides of these target proteins. **Methods:** mRNA data from placenta trophoblasts were used to guide the selection of SLC and ABC proteins for QTAP analysis using microLC-MS/MS. Transport proteins (n=22) with high mRNA expression (> 4 Fragments Per Kilobase of transcript per Million mapped reads [FPKM], placentome database) were selected if they were not already included in our QTAP panels. First, R code was designed to accelerate proteotypic analysis by identifying unique and missed cleavage-free peptides. Second, a placental tissue sample and three human placental cell lines (BeWo, JAR, and HTR-8/SVneo) were examined for the presence of these identified unique peptides using an M-Class Acquity LC coupled to a SCIEX Triple Quadrupole 7500. Human cells were seeded at 1x106 and cultured for 48 hours. Membrane proteins were extracted from each sample by differential detergent fractionation, and extracts were analyzed by (label free) QTAP following trypsin digestion. Three MRMs per peptide were used for response assessment with up to two additional MRMs being used for confirmation. **Results:** R coding eliminated missed cleavages, peptides that were too hydrophilic or too hydrophobic (for chromatography purposes), and non-unique peptides. This approach reduced the large number of candidate peptides (>600) for the 22 selected proteins to ~220 peptides. As examples, OAT4 and OATP4A1 were detected in digested placenta membranes but not membranes from the three cell types. OAT4 (peptide: SVFTSTIVAK) combined peak areas for three MRMs (+2y8, +2y7, +2y6) in the placenta digest was $0.65x10^6$. Four MRMs for OAT4.GCFGISLTCLTIYK could also be detected. OATP4A1 (peptide: YEVELDAGVR) combined peak areas for three MRMs was $0.66x10^6$. Membrane marker Na+/K+ ATPase (peptide: VDNSSLTGESEPQTR) combined peak areas for two MRMs, which are consistently used in our laboratory as endogenous controls, were $1.44x10^6$ for the placenta digest, $3.64x10^6$ for the BeWo digest, 5.05x106 for the JAR digest, and 1.81x106 for the HTR-8/SVneo digest. In accordance with high mRNA expression, peptides were detected in placenta digest for other proteins including LAT4. **Conclusion:** These data support a novel approach to streamline the workflow for quantifying SLC and ABC transport proteins, and are useful in extrapolating in vitro mechanistic studies performed in placental cell lines to human pregnancies.
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Quantitative Targeted Proteomic (QTAP) Analysis of Transporters in Human Placental Cell Lines: Impact of Trypsin Cell Detachment

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Objectives: Human BeWo and JAR cytotrophoblasts and HTR-8/SVneo extravillous trophoblasts are commonly used to study placental functions including hormone production, migration, and maternal-fetal transfer. The purpose of this study was to 1) quantify transport protein concentrations in three human placental cell lines using QTAP and 2) determine whether use of trypsin to remove adherent cells in culture impacts transport protein concentration. **Methods:** Human placental cell lines (BeWo, JAR, HTR-8/SVneo; n=6/group) were seeded in 6 well-plates and grown for 48 hours before removal from the wells with 1) PBS and manual scraping, or 2) trypsin detachment for 4 min. Membrane proteins were extracted using differential detergent fractionation and analyzed by QTAP using nanoLC-MS/MS incorporating stable isotope labeled proteotypic peptides for quantification. **Results:** Glucose transporters (GLUT) 1, 3, and 8 were detected in all three cell lines; GLUT1 and GLUT3 were more highly enriched in BeWo and JAR compared to HTR-8/SVneo cells (**Table 1**). GLUT1 and GLUT3 were over 100-fold higher than GLUT8 in BeWo and JAR cells. The BCRP transporter was highest in BeWo cells compared to JAR and HTR-8/SVneo cells. By comparison, levels of the reduced folate carrier (RFC) were similar across the 3 cell lines. Levels of the placental alkaline phosphatase (PLAP), a marker of syncytiotrophoblast, were lowest in HTR-8/SVneo cells. For most transporters, trypsin detachment did not alter concentrations with a few notable exceptions, including reduced GLUT1 in BeWo cells (2-fold lower with trypsin) and JAR cells (3.6-fold lower with trypsin) and increased PLAP in BeWo cells (3.5-fold higher with trypsin). **Conclusion:** Absolute quantification of placental transporter concentrations in human placental cell lines is critical for studying placental transfer in *in vitro* model systems.
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