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Targeted SLC and ABC Proteome Quantification in Human Placentas

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Quantification of SLC and ABC transporters in healthy and pathological term placentas, as well as commonly used human placental cell lines, is important for understanding maternal-fetal barrier function and nutrient exchange. Here we use quantitative targeted proteomics (QTAP) by microLC-MS/MS to identify and quantify transporter enrichment across cell lines and healthy human term placentas. High mRNA expression from placenta trophoblasts guided selection of SLC and ABC proteins for quantifying. Proteotypic peptides were selected by in silico analysis that included use of R code. The peptides were searched for in placental tissue (n=4) and three human placental cell lines (n=6; JAR, BeWo, and HTR-8/SVneo) using an M-Class Acquity LC coupled to a SCIEX Triple Quadrupole 7500. Cells from the cell lines had been cultured for 48 hours and membrane proteins extracted by differential detergent fractionation. Trypsin digestion incorporating stable isotope labeled peptides for quantification was followed by SPE cleanup and MRM microLC-MS/MS analysis. Some transport proteins (NET, 1.07±0.41 pmol/mg protein; OAT4, 2.46±0.88 pmol/mg protein) were detected in the tissue membrane digests but not the three placental cell lines. LAT1 and CD98 showed higher levels in cytotrophoblast-like cell lines (JAR, 64.20±7.61 and 94.94±14.92 pmol/mg protein, respectively; BeWo, 39.76±13.86 and 69.01±22.04 pmol/mg protein, respectively) compared to the extravillous trophoblast cell line (HTR-8/SVneo, 12.30±2.44 and 20.52±3.28 pmol/mg protein, respectively) and placental tissue (4.86±1.54 and 12.65±3.92 pmol/mg protein, respectively). These data are useful for informing pharmacokinetic modeling and predicting maternal-fetal drug disposition. Further studies will include assessing effects of maternal and gestational disease on transporter enrichment. Supported by NIH UC2HD113039 and S10OD032350.