Poster Number 11

Cell-Specific Asymmetric Regulation of the P-glycoprotein/NHERF-1 Scaffolding Axis at the Human Feto-Maternal Interface

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Background: The feto-maternal interface (FMi) limits fetal exposure to maternal xenobiotics, and the ATP-binding cassette efflux transporter P-glycoprotein (P-gp/ABCB1) performs much of this barrier function. The PDZ-domain scaffolding protein NHERF-1 (SLC9A3R1) has been linked to the regulation of P-gp expression and efflux, but whether this dependence is uniform across the cell types of the FMi, how it changes during inflammation, and the structural basis of the NHERF-1/P-gp interaction remain undefined.

Methods: We examined the P-gp/NHERF-1 relationship in maternal decidual stromal cells (DEC) and fetal chorion trophoblast cells (CTC) using western blot, a fluorometric multidrug-resistance efflux assay, siRNA knockdown of ABCB1 and SLC9A3R1, and cytokine and ATP measurements under lipopolysaccharide (LPS) challenge. The NHERF-1/P-gp interface was modeled by HADDOCK docking with 100-ns molecular dynamics simulations. Two humanized organ-on-chip platforms: a chorio-decidua interface chip (CDi-OOC) and a fetal membrane-placenta FMi chip (FMi-PLA-OOC) were used to test tacrolimus disposition, quantified by LC-MS/MS.

Results: P-gp's dependence on NHERF-1 was cell-type specific. NHERF-1 knockdown (~60–70%) reduced P-gp efflux activity and drove intracellular substrate accumulation in DEC ($P < 0.05$–0.001) but not in CTC. LPS suppressed P-gp ($P < 0.05$) while inducing NHERF-1 and phospho-ERK (both $P < 0.001$) in DEC, a paradoxical uncoupling of pump from scaffold, whereas CTC coordinately preserved all three components ($P < 0.05$–0.001). Structural modeling identified two binding interfaces mediated by the PDZ1 and PDZ2 domains, with PDZ1 forming the stronger, more stable interaction ($\Delta G = -16.4$ kcal/mol). In the CDi-OOC, LPS reproduced these protein-level changes and a DEC-specific cytokine response (IL-6, IL-8, TNF-α; $P < 0.001$) without significantly altering ABCB1 or SLC9A3R1 transcript levels. LC-MS/MS quantification in the FMi-PLA-OOC showed that tacrolimus transport was most impaired at the maternal decidua during both LPS exposure and P-gp knockdown.

Conclusions: These results define a cell-type-dependent P-gp/NHERF-1 scaffolding axis that protects the fetus from xenobiotics and is selectively remodeled by inflammation on the maternal side. NHERF-1 emerges as a tractable molecular target for restoring transporter function in inflammation-associated pregnancy complications, with implications for pregnancy pharmacotherapy and adverse pregnancy outcomes.