Distinct Transcriptional and Epigenomic Programs Define Hofbauer Cells in Term Placenta

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In Vitro Approach to study HBC and TB interactions

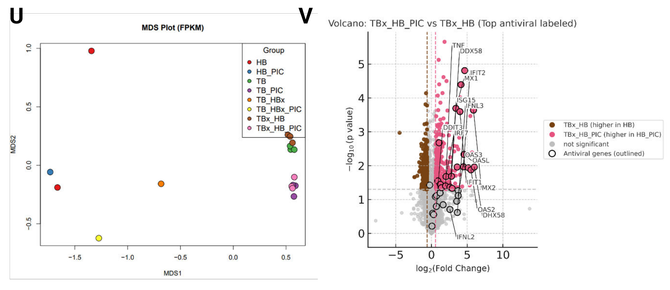
The biological relevance of animal models is limited by the inter-species differences regarding the placental structure and gestational physiology.
Co-culture experiments with HBCs and TBs can provide a novel approach to model intercellular communication.
Output data is consists of:

- transepithelial electrical resistance (TEER) measurements – to monitor barrier integrity of cellular monolayers
- bulk-RNA sequencing – to compare the gene expression of cells in monocultures and transwell co-culture
- measurement of cytokine, hormone production and other signaling molecules – to characterize communication

1. Seeded ~96k TB on transwell (12-well, 0.4um pore) coated with 2% Matrigel (Day 0)
2. 30uL droplet containing ~40k HB seeded on bottom of transwell – 2hr incubation (Day 4)
3. Treatment added & experiment continued for 3 days, cells imaged, and media exchanged every day (Day 5)
4. Collected apical and basolateral media separately for Luminex (Day 7)
5. Cells washed and collected in TRIzol for RNA seq (Day 7)

(U) Multidimensional scaling (MDS) plot of transcriptome profiles across trophoblast and Hofbauer cell cultures.

(V) Volcano plot showing differential gene expression in trophoblasts co-cultured with Hofbauer cells, with or without Poly I:C stimulation.



Conclusions

- HBCs represent a transcriptionally distinct macrophage population with a unique chromatin landscape enriched for RFX and NR4A transcription factor motifs.
- HBCs display an uncomitted macrophage activation profile, expressing both M1- and M2-associated genes, suggesting context-dependent plasticity rather than polarized phenotypes.
- Comparative ATAC-seq and RNA-seq analyses revealed that SREBP-driven lipid metabolism and nuclear receptor signaling contribute to HBC-specific immune and metabolic functions.
- Establishing an in vitro co-culture model of human trophoblasts and HBCs enables physiologically relevant study of fetal–maternal cell communication.
- Poly I:C stimulation of the co-culture induces robust antiviral and interferon-responsive transcriptional programs in trophoblasts, alongside selective cytokine and adhesion molecule secretion detectable in both apical and basolateral media.
- Our approach demonstrates that HBC–trophoblast interactions modulate trophoblast immune function and barrier properties during antiviral challenge.
- The combined transcriptomic, chromatin accessibility, and Luminex data provide a comprehensive molecular framework for understanding how HBCs and trophoblasts jointly shape placental immunity and homeostasis.
- Further methodological refinements are needed as important limitations have risen.