Abstract title

Cytoskeleton and exosome trafficking proteins in endometrial cells interact with trophoblast genes during secretory phase

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Study question:
Which proteins and molecular mechanisms are involved in epithelial endometrial and trophoblastic cells crosstalk during the secretory phase?

Summary answer:
Extracellular proteins of epithelial endometrial cells related to cytoskeleton organization and exosome trafficking potentially interact with trophoblast during implantation.

What is known already:
Endometrium is receptive for blastocyst for a limited period during mid-secretory phase coinciding with the maximal differentiation state of epithelial endometrial cells (EECs). In this phase a complex and not well-understood crosstalk between EECs and trophoblast cells (TCs) is established for initiating embryo implantation. Recent studies provided the EECs proteome during secretory phase (Hood et al 2015) and TCs transcriptome derived from blastocysts (Yan et al 2013) that enable the study of their putative and functional protein interactions. The identification and characterization of these biomolecular interactions will provide new insights into cellular mechanism during embryo implantation.

Study design, size, duration:
Here we show an integrative approach to study and characterize the biomolecular interactions between expressed genes in EECs and TCs respectively. This analysis was carried out using network methods on the human interactome (Rolland et al. 2014) in combination with 1.216 proteins from EECs mass spectrometry proteomic data during secretory phase (Hood et al 2015) and 14.230 transcripts from TCs (RNAseq, Yan et al 2013).

Participants/materials, setting, methods:
Several network methods were used for characterising the resulting subnetwork of the human interactome based on subsets of genes expressed in TCs and EECs. Different biocomputational tools were used for data integration and network building (Bioconductor) and for network representation and data curation (Cytoscape). The resulting cluster of genes from EECs physically interacting to TCs genes were used for functional enrichment analysis (GProfiler) and for detecting cellular mechanisms overrepresented during the early embryo-maternal interaction.

Main results and the role of chance:
A Protein-Protein Interaction (PPI) network was built based on the direct interaction between EECs and TCs genes. The resulting subnetwork contains a total of 91 and 33 genes of TCs and EECs respectively. These represent the interactome between both cell types based on putative functional and direct PPIs. Subsequent functional analysis of resulting clusters extracted from the curated network revealed an overrepresentation of biological processes such as; cytoskeleton organization (13 genes), Fructose metabolism (3 genes) and Intermediate filament organization (3 genes). Cellular components containing the proteins that could be establishing these interactions were associated with extracellular regions such as exosomes that involved a cluster of 17 genes, cytoskeleton intermediate filament (7 genes) and the supramolecular complex fibres (12 genes).
The network analysis showed two EECs proteins KRT13 and KRT15 that are part of the cytoskeleton and were connected with a high number of genes of TCs, 28 and 45 respectively, other cytoskeleton related genes were GFAP (9), KRT6A (7), EMD (7), PPL(6), KRT5 and DES (1). We also found an adhesion protein, VTN, connected to several other proteins.

Limitations, reasons for caution:
The present study is based on published data and represents an approach to explore features of the interactome between expressed EECs proteins and TCs genes. The absence of a functionally validated protein representation of TCs makes to lose accuracy for the establishment of validated *in vivo* PPIs

Wider implications of the findings:
EECs proteins that potentially interact with TCs have been revealed and put light over specific biological process during implantation. Our findings suggest a representative role of cytoskeleton and intermediate filament organization and also of exosome in the extracellular context, where could have a direct contact with the implanting blastocyst.

Trial registration number:
N/A

COI
No conflict of interest

Keywords
Interactome
Epithelial endometrial cell
Trophoblast
Cytoskeleton
Intermediate filament