Title

Lost in transcription: a comparative microarray analysis of the trophoblast stem cell niche in mouse and human

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This study demonstrates that although EVT express three S1P receptor isoforms, S1P predominantly signals through S1PR2 / Gα12/13 to activate Rho and actin stress fibre formation and thereby acts as potent inhibitor of EVT migration. Strategies aimed at shifting the balance of receptor isoform or intracellular pathway activation towards Gαi/Rac, which is known to promote cellular motility in other systems, may provide a mechanism for improving impaired trophoblast migration in compromised pregnancies.

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P2.125.
INHIBITION OF PHOSPHODIESTERASE 4 PROMOTES DIFFERENTIATION AND FUSION OF BEWO CHORIOCARCINOMA CELLS

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Objectives: Trophoblast differentiation and fusion are essential for the growth and maintenance of the syncytiotrophoblast layer of the human placenta. Both processes are regulated by cyclic AMP (cAMP). Cyclic nucleotide phosphodiesterases (PDEs) provide the only way of degrading cAMP and thus are key to the regulation of intracellular cAMP and its subsequent downstream signalling. In this study we assessed the role of PDE4 in trophoblast differentiation and fusion using the BeWo human choriocarcinoma cell line.
Methods: Confluent BeWo cell cultures were treated with or without the specific pan-PDE4 inhibitor rolipram. At 24h and 48h after the start of treatment cells were either fixed in methanol to assess fusion by desmosomal protein staining, or lysed to analyse differentiation and proliferation markers by western blotting. Intracellular cAMP levels were determined using a cAMP ELISA kit.
Results: BeWo cells expressed PDE4A, PDE4B and PDE4D, but not PDE4C. Treatment of cells with the pan-PDE4 inhibitor rolipram resulted in a rapid increase in intracellular cAMP, and this was followed by an increase in the expression of differentiation markers human chorionic gonadotrophin and placental alkaline phosphatase. In addition, rolipram upregulated cell fusion. Finally, rolipram treatment inhibited cell proliferation, as shown by decreased expression of phosphorylated histone-H3.
Conclusion: BeWo cells express multiple PDE4 isoforms and their inhibition leads to increases in intracellular cAMP, differentiation and fusion. These results suggest that PDE4 may have potential as a therapeutic target in pregnancy complications associated with aberrant trophoblast fusion, such as fetal growth restriction and pre-eclampsia. The specific PDE4 isoform(s) involved in these processes remain to be determined.

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P2.126.
ANGIOMOTIN: A NEW PLAYER IN THE REGULATION OF TROPHOBLAST CELL POLARITY

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Objectives: The membrane associated protein Angiomotin (Amot), belongs to the motin family of proteins that is characterized by a C-terminal PDZ-binding domain and two conserved coiled-coil domains that couples with other polarity complex proteins, thereby regulating cell polarity and migration by maintaining tight junctions. We have recently reported that Partitioning defective protein 6 (Par6), a known regulator of cell polarity, is expressed in the human placenta where it regulates trophoblast cell fusion. Par6 elicits its function via its involvement in the TGFβ Smad-independent signaling pathway. Accordingly, this study examines the contribution of TGFβ in regulating Amot expression and its interaction with Par6 in the human placenta.
Methods: The temporal expression of Amot and Par6 was examined in placentae throughout gestation (5-40 wks) by Western Blotting (WB). Amot, Amot\Par6 and Amot\Zo-1 localization were assessed in human choriocarcinoma Jeg3 cells following treatment with TGFβ1/3 (10ng/ml) by immunofluorescence analysis. Subsequently, Amot/Par6 interaction was examined by co-immunoprecipitation across placental development and in Jeg3 cells in the presence and absence of TGFβ1/TGFβ3.
Results: During placental development, Amot and Par6 protein expression as well as their association peaked at around 12 week of gestation. Immunofluorescence analysis showed strong positive signal for Amot at cell boundaries in Jeg3 cells where it co-localized with ZO-1 indicating its presence at tight junctions. Notably, TGFβ3 and to a lesser extent β1, switched Amot expression from the cell boundaries to the cytoplasm where it associated with Par6 and localized to the cytoskeleton. Similarly, immunoprecipitation studies revealed increased association of Amot to Par6 following TGFβ1/3 treatment.
Conclusion: Our data demonstrates for the first time the expression of Amot in the human placenta. TGFβ3-induced association of Amot to Par6 may play a role in regulating tight junction dissolution and migration of trophoblast cells during placental development. (Supported by CIHR)

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P2.127.
LOST IN TRANSCRIPTION: A COMPARATIVE MICROARRAY ANALYSIS OF THE TROPHOBLAST STEM CELL NICHE IN MOUSE AND HUMAN

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Objectives: There have been detailed studies on the developmental potential of mouse trophoblast stem cells (mTSCs), and some of the molecular signals regulating mTSC lineage specification have been discovered. However, comprehensive genomic studies to identify factors critical to the TSC state have not been performed. Moreover, despite attempts by many laboratories, human TSCs have not been successfully isolated. To better define the mTSC state and to determine whether similar cells exist in the human system, we generated comprehensive transcriptomic profiles of mTSCs and human placental samples across gestation.
Methods: Mouse cells were all from the same genetic background (129S1/SvImJ): embryonic stem cells (mESCs) were purchased, while mTSCs, epiblast stem cells (mEpiSCs) and embryonic fibroblasts (MEFs) were derived in our lab. Human placental samples (4-39 weeks) were obtained following IRB approval and informed consent. RNA was profiled using Illumina mouse gene expression microarrays. Data was analyzed using Qlucore and CLICK.
Results: We identified 393 mTSC-specific genes, including well-accepted markers Cdx2, Eomes, Cd40, and Fgfr2, confirming the phenotype of these samples. We identified additional mTSC-specific genes, including Sox21, Dppa1 and Stat1, not previously associated with mTSCs. We also compared the mTSC-specific genes with genes highly expressed in early gestation human placentas and downregulated during the course of gestation, and found 25 common genes, including known mTSC markers, such as ELF5 and ASCL2, as well as novel candidate genes, such as PCSA and P57.
Conclusion: The molecular basis of trophoblast lineage specification is poorly understood, particularly in the human placenta. Our in-depth characterization of mTSCs and comparative analysis with early human placental samples has begun to fill the knowledge gap in this area. This will not only contribute to a better understanding of the molecular determinant of the TSC state, but will also identify differences between the mouse and human systems.

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