Title:
RAPH1, a gene regulated by progesterone affects trophoblast migration and invasion

Authors:
Lauren Sundheimer, Bora Lee, Yayu Lin, Rosemarie DiPentino, Tejal Ghadiali, Erica Wang, John Williams III, Margareta D Pisarska

Cedars-Sinai Medical Center, Los Angeles, CA, UCLA School of Medicine, Los Angeles CA

Introduction:
Pregnancies conceived using in-vitro fertilization (IVF) are associated with higher rates of adverse outcomes related to placental dysfunction compared to spontaneous conceptions. This may be due to abnormal placentation and dysfunctional trophoblast migration and invasion that results from an altered hormonal milieu, which may persist past discontinuation of exogenous hormones, as recently reported by our lab. We identified RAPH1, a gene that encodes adapter proteins which function in migration, to be highly expressed in first trimester chorionic villi and contains progesterone response elements in the promoter. Therefore, we set out to determine if RAPH1 is regulated by progesterone and determine if it plays a role in trophoblast migration/invasion.

Methods:
To determine if RAPH1 is regulated by progesterone, HTR8/SVneo cells were treated with progesterone (1,10, 20, 100 uM) for 48 hours. qRT-PCR was performed to determine the effects of progesterone on RAPH1 expression. To assess the role of RAPH1 in trophoblast function, HTR8/SVneo cells were transfected with RAPH1 siRNA to create a transient knock down cell lines. Transwell Migration and Matrigel Invasion assays were performed to characterize cell function. BrdU Cell Proliferation and Annexin V Apoptosis assays were also performed. Statistical analysis was performed using a one-way ANOVA followed by Dunnet's multiple comparison tests.

Results:
Significant increases in RAPH1 gene expression were seen following progesterone treatment of HTR8/SVneo cells at 20 and 100 uM concentrations ($p=0.0014$ and <0.001). Transfection of HTR8/SVneo cells with RAPH1 siRNA resulted in an average of 63% ($p=0.036$) knockdown of RAPH1 expression compared to cells transfected with scrambled siRNA. RAPH1 knock down cells demonstrated a 63% ($p=0.0002$) and 86.7% ($p=0.017$) decrease in migration and invasion, respectively, when normalized to scrambled siRNA-transfected control cells. There was no difference in proliferation ($p=0.78$) or apoptosis ($p=0.97$) between the two groups.

Conclusion:
Low RAPH1 expression may lead to decreased migration and invasion. In the presence of progesterone and increased RAPH1 expression, migration and invasion may be enhanced, leading to aberrant placental invasion and adverse outcomes in pregnancies conceived with IVF.

Funding:
NICHD (R01 HD074368)