The conserved microRNA miR-151 alters the ability of the endometrial epithelium to facilitate implantation

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In order to determine what the regulatory networks are required to facilitate uterine receptivity to implantation in mammals our group has previously identified a core set of microRNAs that arose when placental mammals arose and have been retained in all species studied. As repressors of gene expression and consequentially protein synthesis, miRNA may not only play a role in successful implantation but could also highlight fundamental pathways involved and/or dysregulated in implantation failure. With that in mind we hypothesise that these identified microRNA's hsa-miRNA-151a-3p and hsa-miRNA-151a-5p may function to facilitate implantation in placental mammals.
The aims of these experiments were 1) to identify the potential mechanism by which these miRNAs may facilitate receptivity to implantation in vitro, and 2) to investigated if these miRNAs functionally affected implantation. Human endometrial epithelial (Ishikawa) cells were transfected with 1) control, 2) transfection reagent alone, 3) non-targeting mimic, 4) non-targeting inhibitor, or 5-8) mimic or inhibitor for miR-151a-3p, or -151a-5p, alone for 48 hrs. Protein was isolated from samples (n=3 per group) and subjected to tandem mass tag proteomic analysis to determine how these microRNAs alter the proteome of the cell. In parallel, BeWo spheroids were generated and co-cultured with Ishikawa cells transfected with either 1-8 above and the spheroid attachment rate quantified by allowing spheroids to attach to the endometrial monolayer for 30 minutes and calculating the percentage remaining attached, after washing.

Proteomic analysis of the endometrial monolayers transfected with miRNA-151a-3p and miRNA-151a-5p mimics/inhibitors against relevant controls, displayed a large shift in protein expression. Dysregulation of miRNA-151a-3p was shown to significantly affect 1738 proteins with 33 of these proteins showing dysregulation by both mimic and inhibitor treatment. Similarly, treatment of cells with miRNA-151a-5p altered 1788 proteins, with 80 of these proteins changed by both miRNA 151a-5p mimic and inhibitor. Interestingly, from these 80 proteins, a total of 46 proteins were dysregulated in opposing directions with transfection of miRNA mimics and inhibitors, indicating a strong likelihood of miRNA targeting.
Further DAVID analysis of the proteins significantly affected by our miRNA showed that both miRNAs 151a-3p and 151a-5p regulated cytoskeletal function, microtubule formation, cell division, and mitosis. miRNA 151a-3p regulated proteins involved in; stem cell differentiation, epithelial cell proliferation, actin binding, Wnt pathway signalling, epidermal growth factor stimulated responses and T cell differentiation.

Most interestingly of all, miRNA151a-5p was shown to regulate proteins involved in; *in utero* embryonic development, cellular differentiation embryonic placenta development, lactation and female pregnancy. There was functional effect of these microRNAs on the ability of the epithelial cells to facilitate attachment as inhibition of miRNA-151a-3p increased the percentage of spheroids that attached, whilst increased abundance of of miRNA-151a-5p was showed to increase attachment percentage, when compared to attachment in non-specific miRNA mimic/inhibitor transfected cells. To further develop these findings, we are developing the assay with the addition of a physiological like flow system using the IBIDI µ-Slide VI 0.4 ibiTreat and a more high-throughput assay is also under development using the fluorescent spheroid approach. These data collectively show that miR-151 plays an important role in facilitating implantation in mammals.