Investigating the enzymatic properties of endometrial DPPIV in the molecular adhesive pathways at implantation using a co-culture model.

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Introduction

In the UK, 1 out of 6 couples are infertile and 10% of them suffer from recurrent implantation failures. The clinical success rate has been stagnant at 25-30% per cycle for a few decades. The knowledge of molecular events at the embryo implantation remains limited, hindering further increase in clinical pregnancy success rates.

Identifying epithelial endometrial candidates from published differentially expressed genomics datasets of human endometrial biopsies sets priorities for in vitro assays using a model of embryo implantation.

A transmembrane receptor, Dipeptidyl peptidase-IV (DPPIV), was one of the identified endometrial targets involved in cell-cell adhesion at Implantation (Shimomura, Y. *et al.,* 2006). Complementing the interesting function complexity, DPPIV contains an enzymatic domain which we identified to be active, *in vitro*, in Ishikawa cell lysates by a series of DPPIV inhibitor concentrations and aim to further investigate.

Aims of the project

The project was designed to identify new insight into the adhesive pathway at Implantation at the embryo-maternal interface by investigating the role of DPPIV enzymatic function in relation to fibronectin using Diprotin A.

Project aims:

1. Localise DPPIV in endometrial Ishikawa cells and attachment sites
2. Determine the impact of the inhibition of DPPIV enzymatic function on endometrial cell adhesion
3. Identify the role of DPPIV enzymatic function in an *in-vitro* model of implantation

Description of work

Cell lines The human endometrial epithelial adenocarcinoma Ishikawa cell line and trophoblastic choriocarcinoma BeWo cell line were bought from the European Collection of Authenticated Cell Cultures (ECACC, UK). Both cell lines were cultured in DMEM:F12 medium (10% Fetal Bovine Serum and Glutamax) at 37 °C and 5% CO2.

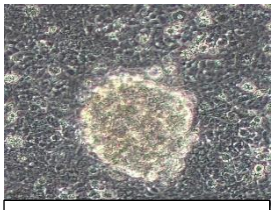

Figure 1: BeWo spheroids attached onto a monolayer of Ishikawa cells. Phase contrast light microscopy

Spheroids generation In 25 μL drop of complete cell culture medium, 3,000 cells of BeWo cell lines were plated onto the lid of a sterile petri-dish. A total of five Petri dishes with 30 drops per Petri lid were incubated at 37 °C and 5% CO2 for 72 hours.

Co-culture assay One hour before the co-culture assay, half of the well were pre-treated with 50μg/ mL of Diprotein A, inhibitor of DPPIV. BCECF-AM-Fluorescent spheroids were resuspended again in 1% BSA serum-free culture medium, to be finally co-cultured onto the Ishikawa monolayer (8 spheres per well) at 5% CO2 and 37 °C. Spheroid attachment was assessed after 1h of co-culture and capturing phase-contrast images using Inverted Microscope.

Assessment of results and outcomes of studentship

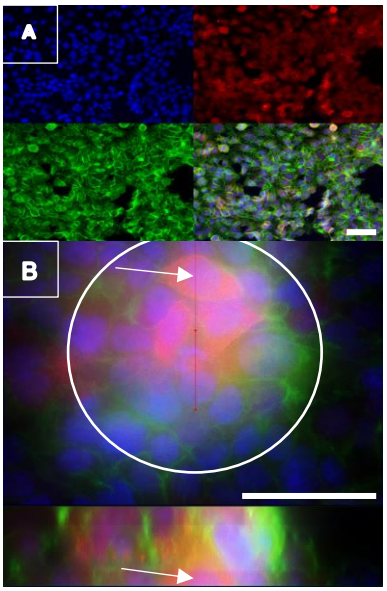

Figure 2: Localisation of endometrial DPPIV in Ishikawa cells and at BeWo spheroids attachment
A= Immunocytochemistry of PFA-fixed Ishikawa cells using DAPI (nucleus, blue), an anti-DPPIV antibody (red), and phalloidin (actin filaments, green).
B= Z-stack image of Ishikawa monolayer and spheroid co-culture using an anti-DPPIV antibody (red), DAPI (blue), and phalloidin (green), with transverse cut seen below. Circled area of z-stack shows the site of attachment between spheroid and endometrial cell monolayer. Arrows show highly DPPIV-positive endometrial

Result 1: DPPIV was identified in Ishikawa cells and at BeWo spheroid attachment site

Immunocytochemistry (ICC) using an anti-DPPIV antibody of Ishikawa cells showed DPPIV to be detected at the lateral and apical cell membrane (Fig. 2A). In confluent monolayer of endometrial cells, positive cells were more present but still heterogenous (not shown). IgG from mouse serum and secondary antibody alone were used as controls for ICC. From preliminary observations, highly DPPIV-positive region displays in the endometrial cell membrane where spheroids attachment took place (Fig. 2B).

Result 2: Inhibition of DPPIV using Diprotin A significantly decreased endometrial cell attachment

Using endometrial single cells, the PolyL-lysine treatment was not as high as expected (expiry to be investigated). However, the fibronectin coating (DPPIV ligand) induced further endometrial cell attachment compared to BSA (basal level).

The specific endometrial attachment to Fibronectin coat was fully inhibited in presence of 50μg/ mL of Diprotein A, back to unspecific basal cell attachment level (BSA).

Diprotein A, enzymatic inhibitor of DPPIV, inhibited endometrial cell attachment to DPPIV ligand, linking both separate enzymatic and adhesive functions of DPPIV.

Result 3: Attachment trophoblast spheroids was impaired by the inhibition of endometrial DPPIV

In supplement to the single endometrial cell attachment assay, the treatment 50μg/ mL of Diprotin A of endometrial cells was tested in the coculture. From preliminary data, after 1h of co-culture, approx. 40% of spheroids in treated monolayers were not attached. Since BeWo cells do not express DPPIV, only endometrial DPPIV was inhibited and was responsible for the lack of attachment.

Outcomes

In this study, combined with its subcellular localisation at spheroids attachment sites, we demonstrated that the inhibition of the enzymatic activity of DPPIV had a negative impact on the DPPIV-specific binding to fibronectin in Ishikawa cells, leading to the preliminary data on the impairment of the trophoblast/endometrial attachment *in-vitro.* These data set the scene for the investigation of DPPIV enzymatic activity and its prescribed inhibitors at implantation.

With these outcomes, Monica presented her results at the PABS Research Seminar (see figure 3). Moreover, these results were combined with the lab results into an abstract which has been accepted at Fertility 2023 conference, January, Belfast.

Figure 3: Image of Monica presenting her results at the PABS research seminar.

Future directions in which the project could be taken

Sitagliptin and vildagliptin, currently prescribed DPPIV inhibitors, could be used to investigate potential *in-vivo* inhibition at embryo implantation. To determine whether the inhibition of DPPIV has an impact on the adhesion, the co-culture model used could be carried out to complement the initial dataset with Diprotin A.

In addition to the inhibition, a siRNA-mediated knockdown of DPPIV was successfully carried out to decrease the expression of DPPIV at the cell surface membrane. However, this technique has not yet been tested in our in-vitro co-culture model.

Value of studentship to the student and to the lab

Working in Berneau's lab team, I have developed many lab skills such as cell culture, spheroids generation, and fluorescence microscopy. I have also enriched many transferable skills such as time management: an essential skill in the lab due to the timed experiments I was carrying out. Due to these experiments, I was also able to develop my independence and organisational skills, making decisions regarding the project, planning tasks, and prioritising appropriately. I also improved my scientific communication skills in weekly meetings with the Berneau lab team.

Completing this project has allowed me to experience life in the lab as a researcher and has reinforced my decision on pursuing academic research as a career.

The studentship was very valuable to the labs as my data complemented the ongoing research in Berneau's lab team, moreover, these data were included in an international conference poster.

Figure 4: Image of Monica pipetting spheroids into the co-culture assay and an image of Dr Stéphane Berneau and Monica Smithies in the lab.

References

Shimomura, Y. et al. (2006) Possible involvement of crosstalk cell-adhesion mechanism by endometrial CD26/dipeptidyl peptidase IV and embryonal fibronectin in human blastocyst implantation. Molecular human reproduction. [Online] 12 (8), 491–495.