SRF Report: Determining the effect of extra-villous trophoblast cells on spiral artery remodelling: what is the role of MMP10?

Supervisor: Professor Judith Cartwright

Background

In a healthy human pregnancy, cells of the placenta called extravillous trophoblasts (EVT) invade into the decidua and interact with the endothelial cells (EC) and vascular smooth muscle cells (VSMC) of the maternal spiral arteries (SA) increasing the blood supply to the developing fetus. Trophoblast cells invade either directly into the lumen of the SA (endovascular) or in to the decidua (interstitial) in a process known as spiral artery remodelling. Early in the 1st trimester, EVT cells are thought to stimulate the loss of ECs and VSMCs through processes such as apoptosis, dedifferentiation and cell migration but these mechanisms are not clearly understood. Problems that arise in this remodelling process are associated with pathologies such as pre-eclampsia and fetal growth restriction. When EVT conditioned media (or trophoblast conditioned media -TCM) was incubated within a vascular spheroid model for 24 hours, a subsequent microarray of spheroid RNA showed that 101 genes including the matrix metalloproteinase (MMP) 10 were significantly up/down regulated (Wallace et al 2013). This project explores the role of MMP10.

Aim

The aim of this project was to investigate how EC secretion of MMP10 is regulated by EVT.

Methods

Experiments were carried out using the human endothelial line SGHEC-7, derived from the umbilical vein, SGHEC-7. Having determined the appropriate cell density, cells were stimulated with trophoblast conditioned medium (TCM), IL1β or PMA. The release of MMP10 by the endothelial cell cultures was determined by ELISA using an R&D Systems DuoSet ELISA kit. To determine the contribution made by IL1β present in TCM on MMP10 secretion, TCM was first incubated with either an IL1β neutralising antibody or a non-specific IgG control and the secretion of MMP10 determined after 48h. The cell monolayer for each experiment was then frozen and used for subsequent protein determination (Bradford Assay).

Results

Stimulated MMP10 secretion by SGHEC7 cells

A dose-dependent increase in MMP10 production was observed when SGHEC-7 cells were stimulated with both TCM and IL1β. This reached significance at a concentration of 100ng/ml and 5ng/ml respectively.

The effect of inhibiting IL1β present in TCM on MMP10 secretion

These results show an increase in the amount of MMP10 detected when TCM is added. It also shows a clear decrease in the amount of MMP10 produced when the TCM was added in the presence of an IL1β neutralising antibody (and only a very slight decrease when IgG was added to the TCM).

Intracellular signalling pathways involved in MMP10 secretion by SGHEC7 cells

A screen of signalling pathway intermediates indicated that activation of PKC could increase MMP10 secretion. However blocking PKC activity in TCM stimulated ECs, had little or no effect on MMP10 secretion, indicating it may not be that important in this context. Preliminary experiments inhibiting both mitogen activated protein kinase and AKT pathways had no effect.

Conclusion

TCM stimulates MMP10 secretion by ECs. Preliminary data indicates blocking IL1β in TCM reduces the secretion of MMP10 by EC. It would be interesting to further investigate the potential role of IL1β in TCM induced MMP10 production and whether or not any other cytokines or growth factors within TCM contribute to MMP10 secretion. A different line of investigation that may be interesting may involve measuring the presence of MMP10 directly from serum samples taken from patients with pregnancy complications such as pre-eclampsia.

Acknowledgements

I would like to thank Professor Judith Cartwright for supporting my application to this scheme and the Society of Reproduction and Fertility for awarding me the vacation scholarship. I am very grateful to Sandy Ashford for helping me throughout my time working in the lab and Professor Guy Whitley for encouraging me to apply.

References

1. Wallace AE, Cartwright JE, Begum R, Laing K, Thilaganathan and Whitley GS. Trophoblast-Induced changes in C-X-C Motif Chemokine 10 Expression Contribute to Vascular Smooth Muscle Cell Dedifferentiation. During Spiral Artery Remodelling. Arteriosclerosis, Thrombosis, and Vascular Biology. 2013;33:e93-e101
2. Whitley GS & Cartwright JE. Cellular and Molecular Regulation of Spiral Artery Remodelling: Lessons from the Cardiovascular Field. Placenta. 2010;31:465-474