

500 word abstract (498 words)

Regulation of chorionic girdle differentiation by TGF β signalling pathways

S. Richardson, D. Miller¹, K. Winstanley, S. Cariese, A. Mukherjee, D.F. Antczak¹ and A. M. de Mestre*

Royal Veterinary College, Department Veterinary Basic Science, Royal College Street, London, NW1 0TU, United Kingdom

¹Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

*Corresponding Author

Dr. Amanda de Mestre

Phone +44 0207 121 1906

Fax +44 0207 388 2342

Email: ademestre@rvc.ac.uk

1. Introduction

The chorionic girdle (CG) is a discrete annular structure of the early equine conceptus that gives rise to the endometrial cups. The rapidly proliferating trophoblast cells of the CG differentiate into eCG-secreting binucleate cells beginning at around day 32 of pregnancy. This study investigated the role of TGF β signalling pathways in regulation of terminal differentiation of trophoblast cells of the CG.

2. Materials and methods

Pure chorionic girdle and chorion tissue was isolated from day 30 to 35 conceptuses by established methods. Tissue was snap frozen for either cell lysates or RNA isolation. A 44K gene probe equine expression array was used to compare Type I and Type II serine/threonine kinase receptor expression between Day 34 chorion and CG tissue (see ISER Abstract, Miller et al). Western blotting was used to detect the expression of Total SMAD5, Total SMAD2, phospho-SMAD2 and Tubulin in chorionic girdle or chorion cell lysates using antibodies directed against the human proteins (Cell Signaling, Technology, MA). Day 33 CG trophoblast cells were isolated and cultured using established methods [19, 22]. CG cells were supplemented with 1, 10 or 100 ng/ml human BMP4 or an equivalent volume of PBS/BSA. Media was harvested after 10 days. The concentration of eCG was determined using a PMSG enzyme linked immunoassay (DRG International).

3. Results

In the CG, we observed preferential expression of Type I and II receptors, Bone Morphogenetic Protein Receptor Type 1A (ALK3) and Bone Morphogenetic Protein Receptor 2 (BMPR-II) that bind the ligand BMP4. Total SMAD5 protein was detected in day 32, 34 and 35 CG, but not in day 30 CG or day 30 to 35 chorion. Total SMAD2 and phospho-SMAD2 was detectable in Day 30 and Day 35 chorionic girdle, with higher expression in day 30 chorionic girdle when compared with day 35 tissue. Furthermore, stimulation of cultured CG cells with BMP4 for 10 days resulted in an increase in eCG concentration, consistent with the induction of binucleate cell differentiation.

4. Discussion/conclusion

TGF β superfamily ligands are abundantly expressed at mammalian maternal-fetal interface. Our findings support a role for TGF β signalling in regulation of chorionic girdle differentiation via BMP4 binding to BMPR-II and ALK3 receptors subsequently activating downstream intracellular signals through SMAD5. We also observed a decrease in SMAD2 signalling during chorionic girdle

development. We propose that SMAD “switching” between the SMAD2 and SMAD5 pathways during chorionic girdle development may be critical to the concomitant regulation of trophoblast proliferation and differentiation.

Acknowledgements

This project was supported by RVC Internal Grant Scheme and Wellcome Trust Vacation Scholarship for Veterinary students.