26. BONE MORPHOGENIC PROTEIN RECEPTOR-II IS A KEY RECEPTOR FOR TRANSMITTING THE ACTIONS OF OOCYTE-SECRETED FACTORS AND GROWTH DIFFERENTIATION FACTOR-9 IN GRANULOSA CELLS

L.J. Ritter[1], D.G. Mottershead[2], L. Jeffery[2], N. Kaivo-Oja[2], S. Scott[1], O. Ritvos[2], R.B. Gilchrist[1]

[1]Reproductive Medicine Unit, Department of Obstetrics and Gynaecology, University of Adelaide, Australia. [2]Programme for Developmental and Reproductive Biology, Biomedicum Helsinki, and Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Finland.

Oocytes regulate ovarian follicle growth and development by secreting paracrine growth factors that act on granulosa cells. Little is known about the identity of these oocyte factors or the receptor system(s) they use. We have recently determined that growth differentiation factor-9 (GDF-9) accounts for ~1/2 of the total mitogenic activity of oocytes [1]. The present study was conducted to examine the receptor and intra-cellular signalling system utilised by oocytes to promote granulosa cell proliferation. We used an established oocyte-secreted mitogen bioassay, where denuded oocytes co-cultured with primed-mouse mural granulosa cells (MGC) promote cell proliferation in a dose-dependent manner [2]. At sub-maximal doses of mGDF-9, additional co-culture with oocytes had an additive effect on MGC ^{3}H-thymidine incorporation. However, at a saturating dose of 80 ng/mL mGDF-9, GDF-9 + oocyte additivity was lost, suggesting total oocyte mitogenic activity may function through the GDF-9 signalling system. Consistent with oocyte secretion of bioactive GDF-9, oocytes led to phosphorylation of granulosa cell Smad2 intracellular signalling molecules as detected by Western blot. Because it is known that the type-II receptor for GDF-9 is the bone-morphogenic protein receptor-II (BMPR-II), we tested the capacity of the receptor ectodomain (BMPR-II ECD; R&D Systems) to neutralise oocyte mitogenic activity. The BMPR-II ECD antagonised both oocyte and mGDF-9 bioactivity in a dose-dependent manner, completely abolishing activity of both mitogens at 1 μg/mL. The antagonistic actions of the BMPR-II ECD were specific, having no effect on bioactivity of the closely related TGF-β1 and partially antagonising activin-A. This study provides evidence that BMPR-II is a key receptor in transmitting oocyte-secreted factors in granulosa cells, and that the bioactivity of oocytes not accounted for by GDF-9 is likely to be due to a closely related molecule utilising this receptor.

[1] Gilchrist RB *et al.* (2003) *Reprod. Fertil. Dev.* Suppl. **15**, Abs. 93. [2] Gilchrist RB *et al.* (2001) *Dev. Biol.* **240**, 289–298.