

MOLECULAR BASIS OF OOCYTE-PARACRINE SIGNALLING THAT PROMOTES MOUSE GRANULOSA CELL PROLIFERATION

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Oocytes regulate follicle growth and development by secreting paracrine growth factors that act on granulosa cells (GC). We have recently determined that growth differentiation factor-9 (GDF-9) accounts for ~50% of the total mitogenic activity of oocytes, the remaining portion is as yet uncharacterised. This study was conducted to identify the receptor/signalling system utilised by oocytes to promote GC proliferation. We used an established oocyte-secreted mitogen bioassay, where denuded oocytes are co-cultured with primed-mouse mural GC. In this system, oocytes, GDF-9, TGF- β 1 and activin-A all promoted GC DNA synthesis in a dose-dependent manner, but bone-morphogenetic protein-6 (BMP-6) and BMP-7 did not. The type-II receptor for GDF-9 is BMPRII and using real-time RT-PCR, cumulus cells (CC) and mural GC were found to express equivalent levels of BMPRII mRNA. We tested the capacity of the receptor ectodomain (ECD) to neutralise oocyte-stimulated mural GC proliferation. The BMPRII ECD antagonised both oocyte and GDF-9 bioactivity in a dose-dependent manner, completely abolishing activity of both mitogens at 1 μ g/mL. The BMPRII ECD did not antagonise TGF- β and partially antagonised activin-A bioactivity, demonstrating its specificity. The TGF β R-II ECD, activin R-II ECD and activin R-IIB ECD all failed to neutralise oocyte- or GDF-9-stimulated GC DNA synthesis, whereas they did antagonise the activity of their respective ligands. The BMPRII ECD also completely antagonised oocyte-stimulated CC DNA synthesis. Using this oocyte-factor bioassay with mural GC transfected with Smad luciferase reporter constructs, we found that oocytes, GDF-9 and TGF- β (but not BMP-6) activated the Smad2/3 pathway. Consistent with this, oocytes and GDF-9 led to phosphorylation of GC Smad2 molecules as detected by Western blot. Conversely the Smad1/5/8 pathway was activated by BMP-6, but not by GDF-9, TGF- β nor surprisingly by oocytes. This study provides evidence that BMPRII is a key receptor for transmitting the paracrine actions of oocytes in GC. However, oocyte-secreted factors do not activate the BMP intracellular signalling pathway but rather the TGF- β /activin intracellular pathway.