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Expression of bone morphogenetic protein receptors in bovine oviductal epithelial cells: Evidence of autocrine BMP signaling

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ABSTRACT

Members of the transforming growth factor beta (TGF-β) family, including bone morphogenetic proteins (BMPs), are expressed in the epithelial cells of the mammalian oviduct. These signaling molecules play important roles in development and tissue homeostasis; however, little is known about their function in the mammalian oviduct. In the present study, RT-qPCR was used to analyze the mRNA abundance of BMP type I (BMPR1A, BMPR1B, ACVR1) and type II receptors (BMPR2, ACVR2A, ACVR2B) in the bovine oviduct epithelial cells (BOEC) isolated from ampulla and isthmus at both the follicular (FP) and the luteal (LP) phase of the estrous cycle. Results indicate that mRNAs for all the BMP receptors studied are expressed in the BOEC. Significant mRNA abundance differences were observed for both BMPR1B and ACVR2B when comparing both the ampulla and isthmus regions with the greater abundance at the isthmus. When both FP and LP samples were compared, ACVR2B mRNA showed greater abundance during the LP, with significant differences in the isthmus region. These variations highlight differences between the isthmus and ampulla regions of the oviduct. By means of wound healing assays on BOEC primary cultures, exogenous recombinant human BMP5 induced a significant increase in wound healing at 24 h. The observed changes at the mRNA abundance of components of the signaling pathway and the BMP5 effect on oviductal epithelial cells suggest a possible autocrine role for the BMP pathway that could affect epithelial cell functions necessary for normal physiology and reproductive success in BOEC homeostasis.

1. Introduction

Bone Morphogenetic Proteins (BMPs) are extracellular multifunctional proteins that belong to the Transforming Growth Factor beta (TGF-β) superfamily (Bragdon et al., 2011). Like other TGF-βs, BMPs regulate various processes of a large variety of cell types in embryogenesis, development and adult tissue homeostasis (Wang et al., 2014). Bone Morphogenetic Proteins mediate their cellular effects by binding to type I (ACVRL1, ACVR1, BMPR1A, and BMPR1B) and type II (BMPR2, ACVR2A, and ACVR2B) serine/threonine kinase receptors, forming heterotetrameric complexes of two dimers of type I and type II receptors (Miyazono et al., 2005). Upon receptors activation, a signal transduction cascade is initiated by phosphorylation of downstream BMP-Smad effector proteins; non-

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Smad specific signal transduction pathways such as MAPK/PI3K/Akt can also be activated by BMPs (Wang et al., 2014).

A number of studies have reported the importance of BMPs as regulators of mammalian reproduction and fertility events (Shimasaki et al., 2004). Bone Morphogenetic Proteins 2, 4, and 7 mRNAs were detected in mouse oviduct (Tanwar and McFarlane, 2011); Bone Morphogenetic Proteins 2, 3, 4, 7, 10, and 15 mRNAs were found to be expressed in bovine oviduct epithelial cells (BOEC) throughout the estrous cycle at both ampulla and isthmus, and BMP5 mRNA is specifically expressed in isthmus BOEC (E.V. García et al., 2014). Large-scale transcriptional analyses in bovine, porcine and human oviducts also contributed to show the presence of BMPs and BMP signaling transcripts (Almiñana et al., 2014; Bauersachs et al., 2004; Hess et al., 2013; Mondéjar et al., 2012). These reports suggest physiological roles for BMPs in the mammalian oviduct that have not been extensively explored.

As BMP pathways are active in oocytes and embryos, studies showing the effects of BMPs on *in vitro* embryo development suggest paracrine functions in the regulation of embryo development for oviduct-secreted BMPs (García et al., 2015; La Rosa et al., 2011). On the other hand, transgenic and knockout mice generated to study TGF-β members display reproductive pathologies, highlighting the importance of TGF-β in maintaining reproductive homeostasis (Li et al., 2011; Pangas et al., 2008; Rodriguez et al., 2016; Tian et al., 2010); therefore, oviduct-produced BMPs might act as both paracrine and autocrine factors in regulating oviduct reproductive events.

The mammalian oviduct plays an important role in gamete transport, fertilization and early embryo development (Ghersevich et al., 2015; Maillo et al., 2016). The extent to which TGF-β/BMP signaling in the mammalian oviduct contributes to fertility and organ homeostasis needs to be explored thoroughly. The aim of the present work was to evaluate if BMPs produced by oviductal epithelial cells can act as autocrine factors that could affect cellular functions necessary for normal physiology and reproductive success.

2. Materials and methods

2.1. Isolation of bovine oviductal epithelial cells

Genital tracts from young beef cows (*Bos taurus*) were collected at a commercial abattoir and transported to the laboratory for processing within 3 h of slaughter. Only samples from non-pregnant animals with no anatomical abnormalities or defects in their reproductive tracts were included in the study. The stage of the estrous cycle was identified according to previous reports based on the direct examination of ovarian morphology and the presence and viscosity of the mucus in the uterine horns (D.C. Garcia et al., 2014; Ulbrich et al., 2004). Once classified, ipsilateral oviducts to the active ovary (corpus luteum/dominant follicle) were separated. Six oviducts from animals at either the follicular (FP) or the luteal phase (LP) were used to study the mRNA abundance of BMP receptors, ID2, and SMAD6 (n = 3 oviducts per stage). The oviducts were dissected free from surrounding tissues and washed with PBS containing 100 IU/mL of penicillin, 100 mg/mL of streptomycin and 0.25 μg/mL of fungizone (Gibco, Life Technology, Burlington, ON, Canada). The ampulla and isthmus regions were isolated from each oviduct and the BOEC were mechanically dislodged by gently squeezing each region separately with forceps, as described elsewhere (D.C. García et al., 2014; Rottmayer et al., 2006). Then, the collected sheets of BOEC from each region of the oviducts were separated from the rest of the contaminating cells by three rounds of simple sedimentation in 15 mL conical tubes with PBS. Epithelial sheets were obtained and controlled under microscope observation to ensure their being free of cells from the oviductal stroma. Cell viability testing was performed with Trypan blue (Sigma) staining. BOEC were pelleted and immediately used for total RNA isolation or primary cultures.

2.2. Bovine oviductal epithelial cell culture

For primary BOEC cultures, istmus portions of ipsilateral oviducts to the ovary with a dominant follicle were used. Epithelial sheets, obtained as described above, from four different istmus were pooled and cultured for each *in vitro* BMP5 assay (ID2 and SMAD6 mRNAs abundance, and wound healing assay) that consisted of four biological replicates performed in duplicated. The collected sheets of BOEC were transferred to Tissue Culture Medium 199 with Earle's salts (TCM-199) (Gibco, Life Technology, Burlington, ON, Canada) supplemented with 10% fetal bovine serum (FBS) (Internegocios, Buenos Aires, Argentina), 25 mM Hepes (Gibco, Life Technology, Burlington, ON, Canada), 0.2 mM sodium pyruvate, 100 IU/mL of penicillin, 100 μg/mL of streptomycin and 0.25 μg/mL of fungizone (Gibco, Life Technology, Burlington, ON, Canada). The cell suspension was pipetted 5 times through a 21-gauge syringe needle; then, three steps of washing were performed, each followed by 25 min sedimentation in culture medium in the cell culture incubator. Cell viability at seeding was analyzed by Trypan blue (Sigma) staining and microscopic observation of beating cilia. Cells were grown in 25 mm^2 culture flasks (Nunclon, Roskilde, Denmark) in 5 mL TCM-199 supplemented as described earlier (D.C. García et al., 2014). Culture took place at 38.5 °C in an atmosphere of 5% CO_2 in air in 100% humidity. Culture media was replaced every 48 h until the cells reached 70–80% confluency. Isolated epithelial sheets spontaneously form vesicles after 24 h of culture. During media changes vesicles were maintained in culture to allow attachment to the bottom of the plate after they formed a monolayer. After that, BOEC cultures were washed with PBS and trypsinized (0.05% trypsin–EDTA; GIBCO) until single cells appeared. These cells were then plated into 24-well culture plates (Nunclon, Roskilde, Denmark) at a density of 10^4 cells per well and incubated at 38.5 °C in 5% CO_2 in culture medium supplemented with 10% FBS, until cells reached 90% confluency.

For BMP5 assays, the culture medium was replaced by serum-free medium and cultured overnight. BOEC monolayer cultures were treated with 100 ng/mL BMP5 for 24 h. The selected concentration was used previously by other authors (García et al., 2015; Romagnoli et al., 2012). Untreated cells, used as control, were incubated for the same time period as the treated group with an equivalent volume of vehicle, 4 mmol/L HCl in serum-free medium, as described by García et al. (2015). At the specified time, total RNA was isolated from control and treated cells to study the expression pattern of *ID2* and *SMAD6* genes.