Interaction of cumulus cells and granulosa cells during luteinization based on analysis of transcriptome

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Significant changes of gene expression induce various cellular functions in ovarian follicular cells undergoing luteinization. We previously reported a transcriptome change in mouse granulosa cells (GCs) undergoing luteinization. However, there are no reports on the transcriptome change in mouse cumulus cells (CCs). In this study, we identified up-and down-regulated genes and their associated cellular functions using RNA-sequencing data of CCs and GCs undergoing luteinization. It has been reported that interactions from GCs to CCs are involved in the regulation of cellular functions in CCs during luteinization. However, the comprehensive interactions between CCs and GCs, especially from CCs to GCs, remain unclear. Therefore we also investigated the interactions between these cell types using RNA-sequencing data.Three-week-old mice were intraperitoneally injected with 4 units of equine chorionic gonadotropin, followed by 5 units of human chorionic gonadotropin (hCG) 48 hours later to induce luteinization. The ovaries were obtained before hCG (0), and 4 and 12 hours after hCG injection to collect cumulus and granulosa cells, and RNA-sequencing was performed. The up-and down-regulated genes during the luteinization were identified in CCs and GCs, respectively, and were subjected to Gene Ontology (GO) analysis. A total of 8,888 genes showed significant changes in CCs during luteinization. Among them, 5,302 genes were up-regulated and were associated with cellular functions in glucose metabolism, reactive oxygen species (ROS) metabolism, and activation of the MAPK cascade. Glucose metabolism in CCs contributes to the oocyte maturation. ROS metabolism reflects its role in protecting the oocyte from excess ROS during the ovulatory process. The MAPK cascade contributes to cumulus-oocyte complex(COC) expansion. In granulosa cells, 11,799 genes showed significant changes. Among them, 4,254 genes were up-regulated and were associated with cellular functions in angiogenesis, ovulation, and steroidogenesis. These functions contribute to the transformation of granulosa cells into a vascular-rich corpus luteum, primarily producing progesterone. To determine the activated interactions between GCs and CCs during luteinization, interactome analysis was performed. At 4 hours after hCG injection, twenty-two interactions were identified as the activated pathways acting from CCs towards GCs. They included Mif-CD74_CXCR4 pathway and, Fgf2-Fgfr3 pathway, which is associated with ovulation and angiogenesis. Thirty-nine interactions were identified, acting from GCs to CCs. They included Nrg1-ITGAV_ITGB3 pathway, which is associated with oocyte maturation. In addition, there were 78 bidirectional interactions, including

EGFR pathway, which is a key pathway for luteinization in both cells.4 to 12 hours after hCG injection, twenty interactions were identified, acting from CCs toward GCs. They included Wnt-FZD4_LRP5 pathway, which is associated with steroidogenesis. Twenty-one interactions were identified, acting from GCs toward CCs. They included Wnt5a-Fzd8 pathway, which is associated with a COC expansion. In addition, there were 25 bidirectional interactions associated with lipid metabolism.

We revealed the transcription changes and functional alterations in CCs and GCs undergoing luteinization. The cellular functions associated with oocyte protection and COC expansion are activated in CCs whereas steroid genesis and ovulatory, angiogenesis function are activated in GCs. These cellular functions might be regulated through the interactions between CCs and GCs.