The Effects of RGD and Hedgehog Ligands on Theca Cell Differentiation in Three-Dimensional Culture System

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Bacterial-derived dextran is a bioinert, nontoxic, and biocompatible polysaccharide. Therefore, dextran-based hydrogels have been widely used in the biomedical and pharmaceutical fields. We recently established a three-dimensional (3-D) ovarian tissue culture system supported by dextran hydrogel. To mimic the natural environment, we modified dextran hydrogel with Arg-Gly-Asp (RGD) peptide, a cell adhesion motif derived from extracellular matrix (ECM) proteins. The ovarian tissues at 14 days old cultured in the RGD-modified dextran hydrogel significantly promoted antral follicle development and oocyte quality compared with those in dextran hydrogel alone. We also determined that RGD ligand directly stimulated the ovarian interstitial cell population to induce cell migration and steroidogenic gene expression, suggesting RGD peptide contributes to theca cell differentiation in our 3-D culture system. In the ovary, two Hedgehog (Hh) pathway ligands, Indian hedgehog (Ihh) and Desert hedgehog (Dhh), are produced by granulosa cells to regulate theca cell specification and differentiation. To examine the effects of RGD and Ihh ligands on theca cell differentiation, ovarian interstitial cells were isolated from immature murine ovaries and cultured in dextran hydrogels under four different conditions: RGD−/IHH− (R−/I−), (R+/I−), (R−/I+), and (R+/I+). After 7 days of culture, cell migration was observed only in the aggregates cultured with RGD peptide: (R+/I−) and (R+/I+). In particular, cell aggregates in the (R+/I+) condition had expanded areas compared to the (R+/I−) condition. In the presence of IHH, mRNA levels of steroidogenic related genes were dramatically increased in the cell aggregates compared with those cultured without IHH. Furthermore, the combination of RGD and IHH effectively upregulated the gene expression levels in the cell aggregates. Our results demonstrated synergistic effects of RGD and Ihh ligands on theca cell differentiation using the 3-D culture supported by dextran hydrogel.