RGD-Modified Dextran Hydrogel Promotes Follicle Growth and Theca Cell Migration Through Integrins αvβ3/αvβ5 in Three-Dimensional Culture

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Due to ovarian damage that might result from chemotherapy and/or radiation treatment, cancer patients run the risk of losing their fertility. In vitro follicle culture is a promising technology to preserve the fertility of cancer patients. We recently established a three-dimensional (3-D) ovarian tissue culture system supported by a biochemically defined dextran hydrogel. To functionalize the dextran hydrogel, extracellular matrix-derived triple Arg-Gly-Asp (RGD) peptide was supplemented in the gel. Murine 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. Next, we determined the RGD-binding target integrins expressed in the murine ovary. In mammals, the family of integrins is composed of 24 αβ subtypes, and 8 integrin subtypes recognize and bind to the RGD motif. Using qPCR and immunohistochemical analyses, we found that integrins αvβ3 and αvβ5 are the main targets of RGD in the immature ovary. To examine the effect of RGD-integrin interaction on follicle development, we used Cilengitide (Ci), an inhibitor for integrins αvβ3/αvβ5. Ovarian tissues were cultured in dextran hydrogels under three different conditions: RGD−, RGD+, and RGD+Ci. In the RGD+ condition, follicle development and theca cell differentiation were significantly enhanced in the ovarian tissues. However, in the presence of Ci (RGD+Ci), RGD-induced follicle differentiation was dramatically suppressed, similar to the RGD– condition. Next, we examined the direct effect of RGD on ovarian interstitial cells. The aggregates of ovarian interstitial cells were 3-D cultured under the three conditions. The RGD+ condition significantly enhanced cell migration and the expression levels of theca cell marker genes in the aggregates. The RGD-induced cell activity was totally suppressed in the RGD+Ci condition, similar to those in the RGD− condition. To examine theca layer formation, interstitial cell aggregates were isolated from transgenic *Gli1ERcre/td tomato (tdT)* mice. Since *Gli1* is a known marker for theca cells, tamoxifen was used to inject *Gli1ERcre/tdT* pups. This caused cre-mediated recombination and the expression of tdT fluorescence in cells that expressed *Gli1* and all their offspring. The transgenic-derived cell aggregates were co-cultured with

the wild-type (WT) secondary follicles under the three conditions. In the RGD+ aggregates, tdT-positive cells migrated to form the cell layers around follicles. In contrast, cell migration and cell layer formation were absent in both RGD+Ci and RGD– aggregates co-cultured with follicles. In conclusion, these results suggest that RGD peptide promotes follicle growth and theca cell differentiation via binding integrins αvβ3/αvβ5 expressed in the ovarian interstitial compartment in our 3-D culture system.