Three-dimensional multilayered tissue: a model to assess the effect of vascular endothelial growth factors upon cultured lymphatic endothelial cells

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Vascular endothelial growth factors (VEGFs) promote lymphangiogenesis during physiological and pathological condition. Among the VEGF family members, VEGF-C plays a key role in promoting lymphangiogenesis. Recent studies have shown that experimental animal models reveal the potent effect of VEGF-C in several pathological conditions such as tumor progression. Therefore, at the very beginning, we isolated cultured LEC from human skin to show the molecular mechanism of VEGF-C-mediated lymphangiogenesis. However, cultured LEC showed a considerable response to highly-concentrated recombinant VEGF-C in mono-layered culture condition. Therefore, in the present study, we decided to generate a novel three-dimensional culture system that gives rise to the biological effect of recombinant VEGF-C to cultured LEC. Initially, normal human dermal fibroblasts were coated by extracellular matrices such as fibronectin, and assembled by four layers. Thereafter, cultured LEC were seeded on the fibroblasts, and incubated for 48 hours in the presence or absence of recombinant VEGF-C at 50 ng/ml. The three dimensional tissues showed that cultured LEC develop highly organized networks in the presence of VEGF-C as compared with controls. Our results indicate, for the first time, that physiological lymphatic vessel growth is induced by VEGF-C in the three dimensional tissue, and that our novel culture system may clarify the biological mechanism of lymphangiogenesis in physiological and pathological condition.