

Fibroblast nemosis induces endothelial tubulogenesis and promotes wound healing

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OBJECTIVES: We recently identified a novel type of cellular reactivity in three dimensional spheroid cultures of human fibroblasts. This novel phenomenon was associated with massive induction of cyclooxygenase-2 (COX-2) and hepatocyte growth factor/scatter factor (HGF/SF) expression. We subsequently named this new type of fibroblast cell-cell contact-induced reactivity nemosis. Since both COX-2-derived prostaglandins and HGF/SF have been characterized to promote neoangiogenesis, we evaluated the mitogenic, motogenic, and morphogenic effects of nemosis on the human microvascular endothelial cell line HMVEC-1.

MATERIALS AND METHODS: Fibroblast cell-cell contacts were induced in a three-dimensional culture arrangement, and corresponding control monolayer cultures were grown in standard cell culture dishes. For tubulogenesis-assay, endothelial cells were grown in collagen lattice and were treated with nemosis-derived conditioned medium (nemosis-CM) or corresponding monolayer-derived control-CM.

RESULTS: Endothelial cells formed significant tubular structures at 48 hours after stimulation with nemosis-CM, whereas in cells stimulated with control-CM no tubulus formation was observed. Nemosis-CM also caused 8.9-fold greater stimulation on endothelial cell proliferation as compared to control-CM at 24 to 40 hours of stimulation (nemosis-CM: 29.25 ± 1.2 - $33.79 \pm 0.60 \times 10^4$ cells vs. control-CM: 25.31 ± 0.63 - $25.82 \pm 1.09 \times 10^4$ cells, $p < 0.05$ at 24 hours, $p < 0.001$ at 40 hours). A standardized wound-healing assay was used to assess endothelial cell migration. Cells migrating to the wound from the border were calculated. Nemosis-CM induced marked 15-fold endothelial cell migration as compared to control-CM (23.33 ± 5.58 vs. 1.50 ± 0.27 cells/field, $p < 0.001$).

CONCLUSIONS: Taken together, our results show that nemosis-derived soluble mediators can induce powerful endothelial cell proliferation, migration, and tubulogenesis driving the formation of new vasculature. Fibroblasts are easily obtained from skin in an autologous setting, and they may thus present a unique therapeutic cell population to be used alone or in combination with other cell-based transplantation therapies for the promotion of cellular responses.