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Generation of spheroids from human primary myofibroblasts: an experimental system to study myofibroblasts deactivation

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Fibroblasts represent a heterogeneous cell population, that in adult body maintains the homeostasis of the extracellular matrix (ECM) and can acquire an immunoregulatory phenotype. Indeed, activated fibroblasts produce large amounts of cyclooxygenase-2 (COX-2) and proinflammatory cytokines (1). The activation of fibroblasts is represented by their differentiation into myofibroblasts. This process, either in wound healing or cancer tissue, is associated with the expression of alpha-smooth muscle actin (alpha-SMA), increased levels of growth factors and ECM-degrading proteases (2). Moreover, myofibroblasts form clusters in wound healing process and hypertrophic scars. In particular, cell clusters of hypertrophic scars are represented by nodules of myofibroblasts (3). It is known that human dermal fibroblasts established from neonatal foreskin, and forced in vitro to form clusters named spheroids, are activated to produce massive amounts of COX-2, prostaglandins and proinflammatory cytokines: this process leads to a programmed necrosis, designated "nemosis" (1). In the present study we generated spheroids from human primary myofibroblasts of skin, to evaluate necrotic, inflammation and activation markers during myofibroblasts clustering. Western blotting analysis, showing low levels of COX-2 and a significant decrease of alpha-SMA in protein extracts of spheroids, led to hypothesize that myofibroblasts have undergone a deactivation process within spheroids. This hypothesis is confirmed by cytostatic effect exerted by spheroids conditioned medium on both normal and cancer cell lines, by confocal immunofluorescence analysis of connexin 43 and immunohistochemical evaluation of proliferation marker Ki-67. This work could represent an experimental model to study myofibroblasts deactivation and highlights an alternative process regulating the turnover of myofibroblasts.

References

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Keywords
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