

Spheroids from human primary skin myofibroblasts as experimental system for myofibroblast deactivation studies

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Myofibroblasts are activated fibroblasts, involved in tissue repair and cancer, characterized by *de novo* expression of alpha smooth muscle actin (α -SMA), increased secretion of growth factors and immunoregulatory phenotype [1].

At the end of wound healing myofibroblasts undergo apoptotic cell death, whereas *in vitro* they are also subjected to a programmed necrosis-like cell death, termed nemosis, associated with cyclooxygenase-2 (COX-2) expression induction and inflammatory response [1,2]. Moreover, fibroblasts form clusters during wound healing, fibrotic states and tumorigenesis.

In this investigation, we produced and analysed clusters such as spheroids from human primary cutaneous myofibroblasts to evaluate apoptotic or necrotic cell death, inflammation and activation markers during myofibroblasts clustering. The spheroids formation does not induce apoptosis, necrotic cell death and COX-2 protein induction. The significant decrease of α -SMA in protein extracts of spheroids, the anti-migratory effect of spheroid-conditioned medium on normal cell lines and the absence of proliferation marker Ki-67 after 72 h of three-dimensional culture indicated that myofibroblasts undergo a deactivation process within spheroids. The cells of spheroids, reverted to adhesion growth, preserve their proliferation capability and are able to reacquire a myofibroblastic phenotype. Furthermore, the spontaneous formation of clusters and spheroids on plastic and glass substrates suggests that aggregates formation could be a physiological feature of cutaneous myofibroblasts.

This study represents an experimental model to analyse myofibroblasts deactivation and indicates that fibroblast clusters could be a cell reservoir regulating tissue turnover.

References

[1] Öhlund et al. (2014) Fibroblast heterogeneity in the cancer wound. *J Exp Med*; 211: 1503-1523.

[2] Vaheri et al. (2009) Nemosis, a novel way of fibroblast activation, in inflammation and cancer. *Exp Cell Res*; 315: 1633-1638.

Keywords

Myofibroblasts, spheroids