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Analysis of human primary fibroblast spheroids

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The organization and physiological functions of multicellular organisms depend also on the presence of connective tissues (1). Fibroblasts are versatile connective tissue cells and represent a heterogeneous cell population (2). They maintain the homeostasis of the extracellular matrix but can also acquire an immunoregulatory phenotype (2).

Moreover, fibroblasts can form multicellular aggregates when activated in *vivo* to myofibroblasts, during wound repair and in fibrotic states (1).

It is known that established human dermal fibroblasts, when forced to form multicellular aggregates named spheroids, are activated to produce massive amounts of COX-2, prostaglandins and proinflammatory cytokines: this process terminates in a programmed necrosis, designated as nemosis (2).

In the present study we analysed spheroids of human primary fibroblasts from skin. To this aim we formed spheroids adapting the hanging-drops and agarose-coated U-bottom well plates methods.

Immunohistochemical analysis of spheroids, collected at different times, detected the presence of vimentin, a myofibroblast marker. Moreover, hematoxylin and eosin staining showed very negligible areas of necrosis. Lactate dehydrogenase (LDH) release, associated with loss of membrane integrity, was estimated in conditioned media from fibroblasts grown as spheroids or monolayers. Marginal levels of LDH activity were detected in conditioned media from spheroids and monolayers, although after 96 h an increase of LDH release, more evident in monolayers media, was measured. Western blotting analysis of spheroid extracts showed the absence of COX-2 and the presence of a-smooth muscle actin, a marker of myofibroblast differentiation. Hence, very low levels of LDH activity associated with the absence of COX-2 demonstrate that human primary fibroblasts from skin, cultured as spheroids, don't undergo nemosis. Furthermore, TUNEL staining of sectioned spheroids showed very few apoptotic cells, positive for DNA breaks.

Our study highlights new aspects about fibroblasts biology and interactions.

References

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Keywords

Fibroblasts; spheroids; COX-2.