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Myotube vs myoblast sensitivity to apoptosis induction by chemical triggers

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Apoptosis is necessary for skeletal muscle tissue development and homeostasis, where it plays a multifaceted role. Muscle cell death increase is associated to disuse, denervation and to several muscle myopathies (Siu et al., Life Sci, 2009), while apoptosis resistance is typical of muscle differentiation (Xiao et al., Apoptosis, 2011)

The aim of this study is to evaluate in vitro apoptotic response in differentiated myotubes exposed to chemical triggers and to compare apoptosis susceptibility to that of proliferant myoblasts. (Salucci et al., Micron, 2010).

C2C12 myotubes, differentiated as previously described (Curci et al, Micron, 2008), were exposed to etoposide (ETO), cisplatin (CP), hydrogen peroxide (H_2O_2) and staurosporine (SP) and apoptosis was evaluated by flow cytometry and transmission electron microscopy. Flow cytometry analysis revealed a certain subdiploid peak after ETO and CP exposure, lower, however, than that evidenced in myoblasts. Differently, DNA cleavage after H_2O_2 or SP treatments, was absent, in contrast with undifferentiated cells. At ultrastructural analysis, characteristic chromatin condensation was observed after ETO and CP treatments, but few apoptotic cells were detected, differently from what evidenced at undifferentiated stage. Moreover, autophagic cell death appeared. Cells exposed to ETO presented a particular behaviour: myonuclei with condensed chromatin coexisted with normal nuclei, in the same myotube, as previously demonstrated in UVB-induced apoptosis (D'Emilio et al., Histol Histopathol, 2010).

On the contrary, and differently from myoblasts, apoptosis was absent in myotubes after H_2O_2 exposure, being necrosis and autophagy the most common cell death processes. After SP treatment all cells showed features of secondary necrosis, a response present also in myoblasts. In addition, autophagic vacuoles were observed, exclusively in differentiated cells.

These results show a certain resistance to apoptosis in myotubes, if compared to myoblasts, that could be associated to an increase in autophagic cell death and in a probable upregulation of anti-apoptotic pathways. Particularly intriguing appears the response to ETO treatment where apoptosis could be correlated to a localized gene expression within each myonuclear domain, suggesting that this process works to selectively eliminate the designated nuclei in multinucleated skeletal muscle fibers.

Keywords: myotubes, apoptosis, chemical triggers, flow cytometry, transmission electron microscopy

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