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Skeletal muscle cell death induced by physical agents

Michela Battistelli[1], Sara Salucci[1], Valentina Baldassarri[1], Sabrina Burattini[1], Elisabetta Falcieri[1,2]

[1] Dep. of Earth, Life and Environmental Sciences, Urbino University "Carlo Bo"61029 Urbino (PU)
[2] IGM, CNR, Istituti Ortopedici Rizzoli, 40100 Bologna (Bo); Italy.

Apoptosis plays a pivotal role in the deletion of unwanted, damaged, or infected cells in multicellular organisms, as well as in development and tissue homeostasis, cell differentiation, and proliferation. In skeletal muscle cells it is unique for several reasons. First, skeletal muscle fibre is multinucleated. So muscle cell death is correlated to a loss of gene expression within the local myonuclear domain, potentially leading to muscle atrophy. In addition, skeletal muscle is a plastic tissue capable of changing its mitochondrial content and/or composition in response to chronic alterations in muscle use or disuse (Siu et al., 2009). Most of the research evidenced that many of the external apoptotic stimuli activate signaling pathways that converge on the mitochondria, determining cell death (Adhietty et al., 2008). Physical triggers such as UVB (D'Emilio et al., 2010), hyperthermia (Lee et al., 2011) and hypothermia (Pizanis et al., 2011) induced cell death by mitochondrial pathways in various cell types. In addition also low pH usually induced DNA damage in other cell lines (Xiao et al., 2003). The aim of this work is to investigate in vitro skeletal muscle cell death appearing after exposure to physical triggers, by means of TUNEL reaction, analysed at confocal microscope, and of electron microscopy.

C2C12 myoblasts and myotubes, grown as previously reported (D'Emilio et al., 2010), were exposed to UV-B (312nm) for 30 min, hyperthermia 45°C for 1h and hypothermia (2-6°C) and low pH (5) for 4h. All treatments were followed by 2h recovery.

Control cell evidentiated a good morphology and appeared negative to TUNEL reaction. UVB - treated sample presented nuclear features suggest apoptosis both at electron and confocal microscopy and in undifferentiated and differentiated conditions. Hyperthermia induced both apoptosis and necrosis with cell rounding and a certain positivity to TUNEL reaction both in myoblasts and myotubes. After hypothermia apoptosis was observed in some cells, but the majority appeared similar to the control, so evidentiating a scarce response. Cells treated with low had swollen nuclei, sometimes showing a thin film of condensed chromatin, and occasionally TUNEL-positive. In all conditions cytoplasm vacuolisaton and autophagic vacuole increase appeared.

These findings suggest that skeletal muscle cells seem to be sensitive to physical agents induced cell death.

Keywords: UVB, hyperthermia, hypothermia, pH, C2C12