Skeletal muscle fiber apoptosis: from ultrastructure to biochemistry

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Myotubes are cell syncytia formed, *in vivo* and *in vitro*, by myoblast fusion generating single multinucleated structures which progressively become muscle fibers, after peculiar morpho-functional changes. Many studies have tried to highlight apoptosis in syncytia, which seems to possess a certain territorial discontinuity, probably still correlated to the cells initially forming them (1).

Skeletal muscle apoptosis is not yet a well understood phenomenon, in particular in the case of differentiated cells, where each myonucleus regulates the gene products in a finite fiber volume (2) and individual myonuclear apoptosis, as well as complete cell death, can occur (1). Moreover, the recently described role of apoptosis in a number of muscle pathologies (3, 4, 5, 6) prompted us to investigate this phenomenon, by means of a multidisciplinary approach.

In this study we have analyzed muscle cell death *in vitro,* utilizing C2C12 myoblasts and myotubes (7), undergoing UVB irradiation. Cells were analysed by scanning and transmission electron microscopy (SEM, TEM) DNA gel electrophoresis, caspase activity Western blot, as well as caspase-9 and -3 inhibitor effects, evaluated by means of SEM.

SEM shows the typical membrane blebbing (A, B), while TEM reveals the characteristic chromatin condensation (C) and a peculiar behavior of differentiated fibers, consistently showing apoptotic and non-apoptotic nuclei in the same myotube (D) (8).

Gel electrophoresis never shows oligonucleosomal DNA fragmentation (F), while caspase-9 and -3 cleavage, and, consequently, the activation of the caspase cascade, is demonstrated by Western blot (E). Moreover, a decrease of apoptotic cell number appears after caspase-9 and -3 inhibitor treatment (G), so highlighting the underlying mechanism.

All these results indicate that UVB irradiation induces apoptosis both in myoblasts and in myotubes and that DNA fragmentation, at least the nucleosomic type, does not occur. Nevertheless, characteristic ultrastructural chromatin changes appear, closely comparably to those of more common apoptotic models (9).

Caspase activation cascade takes place and caspase- 9 and -3 inhibitor treatment demonstrates that both intrinsic and extrinsic apoptotic pathways are present.

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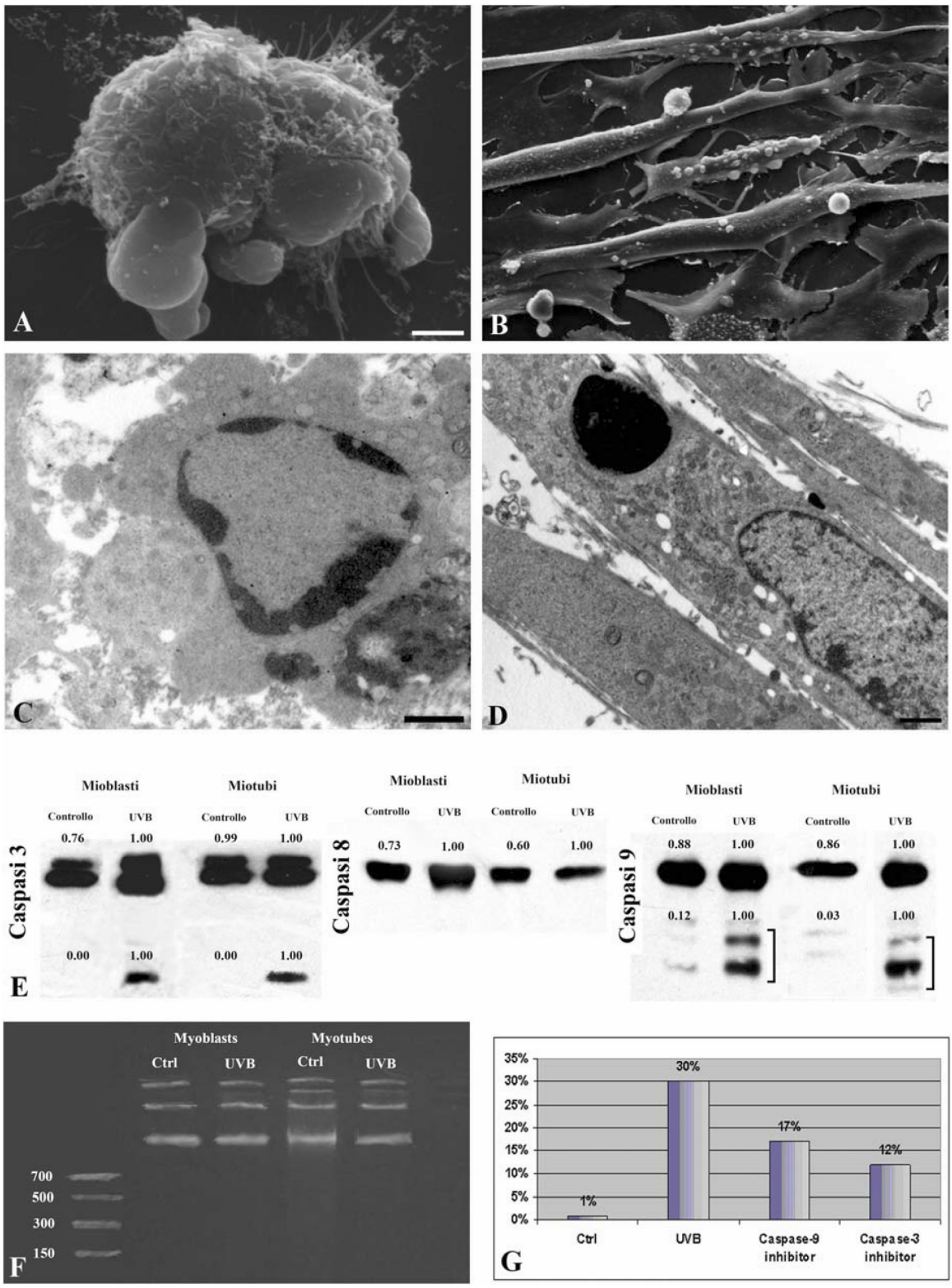

Figure 1. SEM (A, B) and TEM (C, D) of UVB-treated C2C12 myoblasts (A, C) and myotubes (B, D): surface blebs (A, B), chromatin margination (C) and micronuclei (D) are apoptotic markers, even if in the absence of DNA cleavage (F). Caspase activity (E) and its modulation (G) confirm apoptotic response.

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