Ageing affects morpho-functional features of skeletal muscle cell nuclei

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Introduction:

Ageing is associated with a progressive decline of muscle mass, strength and quality, a condition overall known as sarcopenia. These age-related changes have been reported even among healthy, physically active subjects and the rate of muscle loss in humans has been estimated to range 1-2% per year past the age of 50; therefore, sarcopenia represents a powerful risk factor for frailty, loss of independence and physical disability in elderly. The contributing mechanism(s) leading to sarcopenia remain to be fully elucidated; they are probably multifactorial, including among others, denervation and reinnervation of motor units [1], decline in anabolic hormone concentrations [2], decrement in microvascular function and exercice tolerance [3], loss of satellite cells [4], loss of myonuclei through apoptotic mechanisms [5] (although the role of apoptosis in sarcopenic muscle fibre loss is still debated [6]). Despite the extensive literature on sarcopenia, no data on the morpho-functional features of myonuclei of sarcopenic skeletal muscles have been reported so far. In this study we have investigated, by combining morphometry and immunocytochemistry at light and electron microscopy, the fine structure of myonuclei as well as the distribution and amount of factors involved in RNA processing in skeletal muscles of adult and old rats. We chose the quadriceps and biceps muscles, which mostly contain fast type II fibres and are therefore largely affected by sarcopenia [7].

Materials and Methods:

Two adult (9 months) and two old (28 months) male Wistar rats were anaesthetized and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Quadriceps and biceps muscle samples were embedded either in paraffin wax or in LRWhite resin. For light microscopy analyses, sections from paraffin-embedded samples were treated with a mouse monoclonal antibody recognizing the heavy chain of skeletal fast fibre myosin (clone MY-32, Sigma-Aldrich), then revealed with an Alexa 488 conjugated antibody (Molecular Probes, Invitrogen). The sections were counterstained for DNA with Hoechst 33258, to detect apoptotic nuclei based on chromatin morphology. Morphometrical evaluation of fibre crosssectional area was performed by using the software Image J (NIH). For electron microscopy analyses, ultrathin sections were treated with mouse monoclonal antibodies directed against RNA polymerase II (Research Diagnostic Inc.), (Sm)snRNPs (Abcam) and the non-snRNP splicing factor SC-35 (Sigma-Aldrich); with a rabbit polyclonal antibody against DNA/RNA hybrid molecules [8], specifically occurring in transcriptional sites, and with a chicken polyclonal antibody against the cleavage stimulation factors CstF [9]. Moreover, in situ hybridization for polyadenylated RNA was carried out by using a biotinylated oligo d(T) (Sigma-Aldrich). All probes were revealed by gold-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories). To selectively reveal RNP

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constituents, the sections were bleached by the EDTA method. The software Image J was also used to estimate several nuclear parameters (nuclear and nucleolar area, chromatin percentage, perichromatin granule density, size of nucleolar components) and the labelling density (gold grains/square μm). The means ± standard error of the mean (SE) values were calculated for each series of data and statistical comparisons were performed by one-way ANOVA test ($P \leq 0.05$).

Results and Conclusions:

The analyses performed at light microscopy showed a marked reduction in size of both fast and slow myofibres in both quadriceps and biceps of old rats in comparison to adult ones, proving advanced sarcopenia. However, no difference in the apoptotic index was found between old and adult animals in both muscles, suggesting that, if apoptosis plays a role in the loss of myonuclei, this would happen in earlier phases of the sarcopenic process. The ultrastructural analysis revealed that the myonuclei of both quadriceps and biceps muscles from old rats showed significantly smaller size than in adult animals, with a higher percentage of condensed chromatin and a higher density of perichromatin granules. In addition, the nucleolar surface occupied by fibrillar centres showed lower values in old rats, whereas the dense fibrillar component (where rRNA transcription takes place) and the granular component (the site of ribosome subunits accumulation) did not change. It is known that condensed chromatin is transcriptionally inactive and that the nucleolar components undergo quantitative modifications depending on the rRNA transcriptional rate [10]. Moreover, perichromatin granules, involved in intranuclear storage and transport of already spliced mRNA, generally accumulate when an alteration of pre-mRNA processing and/or an impairment of intranuclear or nucleus-to-cytoplasmic transport occur [11]. Accordingly, the immunocytochemical results demonstrated that, although no difference in the intranuclear distribution of transcription, splicing and cleavage factors occurred between adult and old rats, the myonuclei of sarcopenic fibres contained significantly lower amounts of polymerase II, DNA/RNA hybrid molecules (no difference was found in the nucleoli) and snRNPs, suggesting a decreased pre-mRNA transcription and splicing activity; conversely, cleavage factors and polyadenylated RNA were more abundant in old rats, indicating an intranuclear accumulation of mature mRNA. In conclusion, our data suggest that, during ageing, in myonuclei a decrease in pre-mRNA transcription occurs in parallel to alterations of its processing and intranuclear transport, whereas the nucleoli seem to be only slightly affected.These data are consistent with the hypothesis that muscle atrophy is not related to nuclear loss but to metabolic imbalance [6], as well as with previous observations on hepatocyte nuclei of old rats [12] which indicate that such modifications in nuclear activity may be a general phenomenon related to the ageing process.

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