Structural and functional alterations of the cell nucleus in skeletal muscle wasting: the evidence *in situ*

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Abstract

The histochemical and ultrastructural analysis of the nuclear components involved in RNA transcription and splicing can reveal the occurrence of cellular dysfunctions eventually related to the onset of a pathological phenotype. In recent years, nuclear histochemistry at light and electron microscopy has increasingly been used to investigate the basic mechanisms of skeletal muscle diseases; the *in situ* study of nuclei of myofibres and satellite cells proved to be crucial for understanding the pathogenesis of skeletal muscle wasting in sarcopenia, myotonic dystrophy and laminopathies.

In recent years, histochemistry has become a popular approach to investigate the structural organization and function of skeletal muscle cells,[1-8] being widely used as a diagnostic tool in neuromuscular disorders.[9,10] In particular, the cytochemical analysis of the cell nucleus has been applied more and more frequently for investigating the basic mechanisms of skeletal muscle diseases.

In the cell nucleus, genes are transcribed and the primary transcripts undergo molecular processing which generates mature RNAs to be exported into the cytoplasm. The events leading to the formation of mature RNAs are chronologically and spatially ordered, and they mostly occur on distinct ribonucleoprotein (RNP)-containing structures.[11,12] These nuclear components have specific locations, and this is a necessary prerequisite for the correct processing of nuclear RNAs to occur, so that whenever transcription and/or splicing are altered, the organization, composition, and intranuclear location of RNP-containing structures are also affected.[13-16] As a consequence, the *in situ* analysis of the nuclear organization and molecular composition in muscle cells not only provides information about the DNA/RNA pathways which govern myofibre metabolism, but also may reveal the occurrence of dysfunctions related to the pathological phenotype of diseased skeletal muscle.

Recently, ultrastructural immunocytochemical investigation of cell nuclear components has been applied to study sarcopenia,[17,18] i.e. the age-related condition characterized by the decline of muscle mass, strength and quality, which is responsible for frailty, disability and premature death in elderly.[19] The cellular mechanisms involved in the onset of sarcopenia are probably manifold, and they still remain to be completely elucidated.[20,21] One of the possible causes is the remarkable decline in the efficiency of muscle regeneration, which has been associated with a decrease in the number of satellite cells and/or with the alteration of their proliferation and differentiation potential.[22,23] Consistent with this hypothesis, through the *in situ* analysis of the nuclear RNP-containing structures involved in the different steps of mRNA formation, it has recently been demonstrated that satellite cells of old muscles exhibit a significantly reduced activity of pre-mRNA splicing and cleavage, which hampers their responsiveness to muscle damage.[18] In addition, the cytochemical approach allowed to demonstrate that the entire production chain of mRNA, from its synthesis to the export into the cytoplasm, is impaired in the myonuclei of old muscles:[17] this would likely contribute to the reduced responsiveness of muscle fibres to anabolic stimuli, as it typically occurs in elderly. It may be inferred that many of the structural and functional alterations occurring in old muscles could be the phenotypic expression of a failure in nuclear functions. Accordingly, when the sarcopenic process is prevented by physical activity,[24,25] the cell nuclei of senescent skeletal muscles show the RNP pattern typical of the adult age (personal unpublished results). It is worth noting that in hibernating mammals the muscle mass is maintained even after long periods of inactivity (which may last for months, in some species): in the skeletal muscles of hibernating mammals the nucleus displays "active" characteristics,[26] thus suggesting that preservation of the myofibre/muscle structure implies the maintenance of a correct nuclear functionality.

Defects in the RNA maturation pathways have also been related to diseases leading to muscle dystrophy: in both the myotonic dystrophy type 1 (DM1) and type 2 (DM2) the expansion of two distinct nucleotidic sequences ((CTG)n in the 3' untranslated region of the DMPK gene on chromosome 19q13 in DM1[27-29] and (CCTG)n in the first intron of the ZNF9 gene on chromosome 3q21 in DM2[30,31]) causes pathologies characterized by a variety of multisystemic features including myotonia (muscle hyperexcitability), muscular dystrophy, dilated cardiomyopathy, cardiac conduction defects, cataracts, insulin-resistance, and disease-specific serological abnormalities such as hyperglycemia and gamma-glutamyltransferase elevations, hypotestosteronism, and decreased levels of IgG and IgM immunoglobulins. Combining biomolecular and cytochemical techniques, it has been demonstrated that the basic mechanisms of both DMs reside in the nuclear sequestration of the expanded RNAs: CUG- and CCUG-containing transcripts accumulate in intranuclear foci in DM1 and DM2 cells respectively, and alter the regulation and intranuclear localization of the RNA-binding proteins CUGBP1 and MBLN, which are necessary for the physiological processing of pre-mRNA.[31-37] A recent study based on immunocytochemical analyses at light and electron microscopy[38] has demonstrated that MBNL1-containing foci in DM2 cells also sequester snRNPs and hnRNPs, splicing factors involved in the early phases of transcript processing;[11] this strengthens the hypothesis that the multifactorial phenotype of dystrophic patients could be due to a general alteration of the pre-mRNA post-transcriptional pathway.

Laminopathies represent a family of multisystemic disorders resulting from mutations in the LMNA gene on chromosome 1q21, encoding nuclear lamins A and C. They include several distinct disease phenotypes, many of which characterised by skeletal muscle dystrophy.[39] Also in this case, the combination of biomolecular and cytochemical studies revealed that the basic mechanisms of the pathological features reside in the nucleus, where prelamin A is accumulated, heterochromatin undergoes a severe disorganisation and many heterochromatin-associated proteins show altered properties.[39-42] This defective heterochromatin remodelling affects gene expression thus causing a cascade of epigenetic

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events altering several systems.

The analysis *in situ* of the cell nucleus may therefore represent a decisive approach for understanding the basic mechanisms leading to fibre muscle loss/disorganization, which is crucial for the development of effective interventions to fight physical disability. This type of investigation requires that sufficient amounts of bioptic material are adequately removed and processed in order to obtain reliable results; however, this is scarcely compatible with the surgical needs and, for a long time, this restriction has limited the application of *in situ* techniques to investigate muscle physiopathology. The recent demonstration that routinely frozen biopsies of human skeletal muscle can be successfully processed for morphological and immunocytochemical studies at transmission electron microscopy[43] opens promising perspectives for multiple exploitation of the bioptic muscle samples stored in tissue banks, especially for the study of rare muscle diseases.

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