Skeletal muscle features in myotonic dystrophy and sarcopenia: do similar nuclear mechanisms lead to skeletal muscle wasting?

M. Malatesta

Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie, Sezione di Anatomia e Istologia, Università di Verona, Italy

Abstract

In the cell nucleus, the gene primary transcripts undergo molecular processing to generate mature RNAs, which are finally exported to the cytoplasm. These mRNA maturation events are chronologically and spatially ordered, and mostly occur on distinct ribonucleoprotein (RNP)-containing structures. Defects in the mRNA maturation pathways have been demonstrated in myotonic dystrophy type 1 (DM1) and type 2 (DM2) whose characteristic multisystemic features are caused by the expansion of two distinct nucleotide sequences: (CTG)n in the DMPK gene on chromosome 19q13 in DM1, and (CCTG)n in the ZNF9 gene on chromosome 3q21 in DM2. By combining biomolecular and cytochemical techniques, it has been shown that the basic mechanisms of DMs reside in the accumulation of CUG- or CCUG-containing transcripts in intranuclear *foci* where several RNA-binding proteins necessary for the physiological processing of pre-mRNA are sequestered. Moreover, a nucleoplasmic accumulation of splicing and cleavage factors has been found in DMs. This suggests that the dystrophic phenotype could depend on a general alteration of the pre-mRNA post-transcriptional pathway. Interestingly, the accumulation of pre-mRNA processing factors in the myonuclei of DM1 and DM2 patients is reminiscent of the nuclear alterations typical of sarcopenia, *i.e.*, the loss of muscle mass and function which physiologically occurs during ageing. Consistently, in an *in vitro* study, we observed that satellite-cell-derived DM2 myoblasts show cell senescence alterations and impairment of the pre-mRNA maturation pathways earlier than the myoblasts from healthy patient. These results suggest possible common cellular mechanisms responsible for skeletal muscle wasting in sarcopenia and in myotonic dystrophy.

Skeletal muscle features in myotonic dystrophy

In recent years, the role of mutations in non-protein-coding regions has come to light in the pathogenesis of different neuromuscular diseases,[1,2] so that the terms *toxic RNA* and *spliceopathy* have been increasingly referred to pathological conditions in which accumulation of mutant RNAs results in a deleterious gain-of-function deregulating transcript processing and protein synthesis in multiple metabolic pathways.

One of the best studied examples of RNA-dominant disease is myotonic dystrophy (DM), an autosomal dominant disorder characterised by a variety of multisystemic features including muscular dystrophy with increased number of centrally located or clumped nuclei in muscle fibres,[3] myotonia (muscle hyperexcitability), dilated cardiomyopathy, cardiac conduction defects,[4] insulin-resistance, cataracts,[5] and disease-specific serological abnormalities.[6,7] Two forms of DM are presently known: the more severe DM1-Steinert's disease (OMIM 160900), caused by an expanded (CTG)n nucleotide sequence in the 3' untranslated region of the Dystrophia Myotonic Protein Kinase (DMPK) gene (OMIM 605377) on chromosome 19q13,[8-10] and the milder form DM2 (OMIM 602688), caused by the expansion of the tetranucleotidic repeat (CCTG)n in the first intron of the Zinc Finger Protein (ZNF)-9 gene (OMIM 116955) on chromosome 3q21.[11]

In the cell nucleus, the gene primary transcripts undergo molecular processing to generate mature RNAs, which are finally exported to the cytoplasm: these mRNA maturation events are chronologically and spatially ordered, and mostly occur on distinct RNP-containing structures.[12] By combining biomolecular techniques with the analysis *in situ* of the nuclear organization and molecular composition, it has been demonstrated that the expanded-CUG- or CCUG-containing transcripts, in DM1 and DM2 cells respectively, are retained in the cell nucleus, and accumulate in the form of RNP-containing focal aggregates.[13] These nuclear *foci* specifically sequester the alternative splicing regulators CUG-binding protein 1 (CUGBP1) and muscle-blind-like 1 (MBLN1) protein,[14-16] which are necessary for the physiological processing of pre-mRNA, especially for contractile protein synthesis.[17] These focal aggregates are considered as a biomolecular feature of DMs, and have been detected in several adult tissues as well as in cultured cells from DM patients.[13,18-23] These *foci* also sequester hnRNPs and snRNPs, *i.e.* splicing factors involved in the early phases of the pre-mRNA processing,[24] thus strengthening the hypothesis that the multifactorial phenotype of dystrophic patients may result from a more general alteration of the pre-mRNA post-transcriptional pathway.

Recently, it has been demonstrated that MBNL1 accumulate in the nuclear *foci* during interphase but, at mitosis, the *foci* relocate to the cytoplasm where they undergo degradation, while newly-formed *foci* develop in the nucleus of the daughter cells as a consequence of *de novo* accumulation of expanded RNAs.[25] Therefore, in proliferating cells, the cyclic release from the nucleus of the *foci* and their cytoplasmic degradation would prevent the massive intranuclear sequestration of nuclear factors; on the contrary, in non cycling cells, the nuclear *foci* do not undergo intracellular relocation/degradation and progressively increase in number and size. This dynamic behaviour of nuclear *foci* is compatible with the evidence that in DM patients the most affected organs or tissues are those where non-renewing cells are mainly present, such as the skeletal muscle, heart and the central nervous system, whereas cells from self-renewing tissues (such as skin fibroblasts or layering epithelial cells) are much less affected. Accordingly, measuring intranuclear *foci* in skeletal muscle biopsies taken from patients at different times it has been demonstrated that the MBNL1-containing *foci* actually become larger with increasing patient's age.[25]

In addition to the formation of intranuclear *foci*, DM1 and DM2 cells show an altered distribution of nuclear ribonucleoprotein (RNP)-containing structures and molecular factors responsible for pre-mRNA transcription and maturation. In particular, by means of ultrastructural immunocytochemistry on skeletal muscle biopsies from DM1 and DM2 patients, it has been shown[26,27] that splicing and cleavage factors accumulate in the intranuclear functional sites where they usually localize, sometimes ectopi-

Correspondence: Manuela Malatesta, Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie, Sezione di Anatomia e Istologia, Università degli Studi di Verona, Strada Le Grazie 8, 37134 Verona, Italy.
Tel. +39.045.8027157 - Fax: +39.045.8027163.
E-mail: manuela.malatesta@univr.it

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cally relocating also into nuclear RNP domains where they generally do not occur (*unpublished results*). This accumulation could hamper the functionality of the splicing machinery and slow down the intranuclear molecular trafficking thus reducing the metabolic activity of myonuclei, consistent with recent findings demonstrating a reduced protein synthesis in DM1 and DM2 myoblasts.[28,29]

Skeletal muscle features in sarcopenia

During ageing, the skeletal muscle undergoes a progressive loss of mass, strength and function, in the process known as sarcopenia.[30,31] Sarcopenia affects healthy, physically active subjects: the rate of muscle loss in humans has been estimated to range 1 to 2% per year after age of fifty. Therefore, sarcopenia represents a great risk factor for frailty, loss of independence and physical disability in elderly, since it is associated with decreased functional performance, higher risk of falls and motor function impairment. The mechanisms underlying age-related skeletal muscle wasting and weakness are probably manifold and still remain to be fully elucidated;[30] however, although no specific therapy is presently available to counteract its onset or progress, studies performed on humans and other mammals have stressed the importance of physical exercise as an effective, although still debated, approach to prevent or limit the age-related muscle mass loss.[32-36]

Interestingly, the sarcopenic process is characterised by structural and functional alterations of the skeletal muscle that are reminiscent of myotonic dystrophy. In fact, the aged muscle shows grouped atrophy, fibre size variability and centrally located nuclei.[37] In addition, factors involved in the post-transcriptional processing of pre-mRNA have been found to accumulate not only in the nucleoplasmic RNP-containing structures where they usually locate but also in ectopic nuclear domains.[38-40] This intranuclear accumulation/ delocalization of RNP structures containing splicing and cleavage factors has been found not only in the skeletal muscle but also in other tissues (*e.g.*, liver, brain) of aged mammals.[41-44] Moreover, aged cells undergo malfunctions of the degradation systems both in the cytoplasm[45] and in the nucleus[46,47] with accumulation of crosslinked insoluble molecules (including non-coding RNAs) which hampers the intracellular transport mechanisms. This suggests that in ageing cells the entire production chain of mRNA, from its synthesis to the cytoplasmic export, becomes less efficient, likely contributing to the reduced capability of cells to positively react to metabolic stimuli, which typically occurs in elderly. This loss of responsiveness would have particularly severe effects in skeletal muscles, where a misregulated protein turnover would result in a structural imbalance between muscle protein degradation and the restoring protein synthesis.[48]

Concluding remarks

A recent *in vitro* study[49] reported that satellite-cell-derived myoblasts from DM2 patients show cell-senescence alterations (*e.g.*, cytoplasmic vacuolisation, reduction of the proteosynthetic apparatus, accumulation of heterochromatin and impairment of the pre-mRNA maturation pathways) earlier than the myoblasts from healthy patients; moreover, when grown in a differentiation medium DM2 myoblasts fuse into multinucleated myotubes exhibiting structural defects similar to those observed in senescent myotubes from healthy patients.[50] The early occurrence of senescence-related features in satellite cell-derived myoblasts suggests that satellite cells from DM2 patients have a reduced regeneration capability, which would contribute to the muscular dystrophic phenotype.

The cytochemical and ultrastructural evidence demonstrates that the skeletal muscle of DM patients shares intriguing similarities with the muscle from aged individuals in several nuclear features, especially in the altered nuclear RNP-containing structures involved in pre-mRNA transcription and splicing. This opens interesting perspectives on the role of the RNP nuclear components in the onset of muscle cell dysfunctions and encourages comparative studies aimed at detecting common cellular mechanisms at the basis of skeletal muscle wasting.

Finally, it is worth noting that the analysis *in situ* of the organization and molecular composition of nuclear domains is a powerful tool not only for getting information about the DNA/RNA pathways which govern cellular metabolism, but also for detecting the occurrence of cell dysfunctions related to pathological phenotypes.[51-53]

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