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No correlation of increase in size of ribonuclear inclusions to type II fibre atrophy in myotonic dystrophy type 2 over time

R. Cardani [1]; R. Perbellini [2]; E. Mancinelli [1]; G. Meola [2]
[1] *University of Milan, Department of Biomolecular Sciences and Biotechnologies, Milan, Italy;* [2] *University of Milan, IRCSS Istituto Policlinico San Donato, Department of Neurology, Milan, Italy*

Myotonic dystrophy type 2 (DM2) is an autosomal dominant neuromuscular disorder characterized by a complex multisystemic phenotype. The genetic basis of the disease is a large expansion of a CCTG repeat located in intron 1 of the ZNF9 gene. Mutant transcripts are retained in muscle nuclei producing ribonuclear inclusions (RIs), which can alter the functions of alternative splicing regulators leading to misregulated splicing events that explain many symptoms of DM2, such as myotonia and insulin resistance. Nevertheless the cause of skeletal muscle degeneration, which is a cardinal feature of the disease, remains still unknown. The aim of this work is to study if RIs and the subsequent nuclear sequestration of MBNL1, a splicing factor, are involved in DM2 muscle wasting. Since expanded DM2 alleles show somatic instability which increases in length over time, we have correlated the degree of muscle fibre atrophy with the number of RIs or with the dimension of RIs and MBNL1 nuclear foci. Muscle sections from 4 DM2 patients who underwent 2 biopsies of biceps brachii at different years of age have been analysed. An increase in fluorescence intensity and area of both RIs and MBNL1 foci is observable between the first and the second biopsy in each patient examined. Nevertheless this increase is not always accompanied by an increase in the number of RIs or in the degree of type II fibre atrophy, indicating that the number and the dimensions of RIs and MBNL1 foci do not seem to be indicators of the severity of muscle wasting. Moreover, the increase in fluorescence intensity and area of both RIs and MBNL1 foci in the second biopsy, seems indicate an increase in expansions size and in MBNL1 sequestration over the time. Indeed, a relationship between repeats and length and dimension of foci seems exist, since in a patient with 82 CCTG repeats RIs and MBNL1 foci appear to be significantly smaller then those observed in a patient with 1000 repeats.

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In vitro study of DM1 primary myotubes

E. Loro [1]; A. Botta [2]; C. Catalli [2]; V. Romeo [1]; F. Rinaldi [2]; C. Angelini [1]; L. Vergani [1]
[1] *University of Padova, Department of Neurosciences, Padova, Italy;* [2] *University of Roma, Department of Genetics, Roma, Italy*

Myotonic Dystrophy is the most common form of adult muscular dystrophy, with a prevalence of 1/8000. Type 1, accounting for the 98% of total cases, is caused by a $(CTG)_n$ triplet expansion in 3′-untranslated region of DMPK gene. In muscle tissue myotonia and insulin resistance have been associated to altered splicing of chloride channel-1 (ClC-1) and insulin receptor (IR) mRNAs, due to pleiotropic effect of mutant DMPK. An inhibition of differentiation in DM1 myoblasts, maybe due to anomalous interactions between mutant RNA and many key myogenic factors, was supposed to be the primary determinant of muscle wasting and weakness. Primary human cell lines from adult-onset and congenital DM1 patients, with $(CTG)_n$ ranging from 180 to 1850, were established and differentiated into polynucleated myotubes. In vitro muscle maturation of DM1 primary cells was monitored by morphological and molecular analysis in undifferentiated and 4/10 days differentiated (aneural) myotubes. The increased myogenic markers expression was correlated with the splicing pattern of IR and MBNL1 genes in myoblasts, aneural myotubes and DM1 mature muscle. Further maturation was reached by innervation with rat embryo spinal-cord (mature myotubes). Myotubes were tested by FISH and immunohistochemistry. We observed that differentiation and innervation of DM1 primary myoblasts were unaffected by the CTG expansion and that both aneural and mature myotubes expressed pathological hallmarks of DM1 muscle. DM1 myotubes were compared with controls for apoptosis susceptibility by TUNEL analysis and for hydrogen peroxide release by the fluorogenic indicator Amplex red assay to test whether such parameters were influenced by the number of repeats in differentiated myotubes.

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P16 triggers premature senescence of congenital DM1 myoblasts

G.S. Butler-Browne; A. Bigot; E. Gasnier; V. Mouly; D. Furling
UMRS787 Inserm/UPMC/Institut de Myologie, Groupe Myologie, Paris, France

Myotonic Dystrophy type I (DM1) is caused by a CTG expansion in the 3′-UTR of the DMPK gene and is characterized by progressive muscle weakness and wasting. Large CTG repeats affect the differentiation program and we have showed that the proliferative capacity of the cDM1 myoblasts was significantly reduced when compared to non-affected cells. DM1 myoblasts have not exhausted their proliferative capacity but have a premature replicative arrest. Analysis of several markers suggests that a mechanism of premature senescence triggers this early arrest. We found that an early accumulation of the cdk inhibitor p16 is associated with this phenotype in DM1 cells. We show that an inactivation of p16 activity in DM1 myoblasts was able to inhibit premature senescence and to restore proliferative capacity: DM1 cells overexpressing CdK4 that binds and inhibits p16, can make the same number of division as control cells. Our results also indicate that the accelerated telomere shortening measured in DM1 satellite cells may not contribute to the aberrant induction of p16. We propose that deregulation of the mitotic clock is a consequence of a stress related to the amplified CTG repeat that promotes premature senescence mediating by a p16-dependent mechanism in DM1 muscle cell precursors. The mechanism of p16 regulation in the DM1 cells is currently under investigation in order to determine how the CTG mutation interferes with the p16 pathway.

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Defective mRNA in myotonic dystrophy accumulates at the periphery of nuclear splicing speckles

I. Holt [1]; S. Mittal [2]; D. Furling [3]; G.S. Butler-Browne [3]; J.D. Brook [2]; G.E. Morris [1]
[1] *RJAH Orthopaedic Hospital, Wolfson CIND, Oswestry, UK;* [2] *University of Nottingham, Institute of Genetics, Nottingham, UK;* [3] *INSERM-Universite Paris 6, UMRS 787, Institute of Myology, Paris, France*

Nuclear speckles are storage sites for small nuclear RNPs and other splicing factors. Current ideas about the role of speckles suggest that some pre-mRNAs are processed at the speckle periphery before being exported as mRNA. In myotonic dystrophy type 1 (DM1), the export of mutant DMPK mRNA is prevented by the presence of expanded CUG repeats which accumulate in nuclear foci. We show that these foci accumulate at the periphery of nuclear speckles. In myotonic dystrophy type 2 (DM2), mRNA from the mutant ZNF9 gene is exported normally, because the expanded CCUG repeats are removed during splicing. We show that the nuclear foci formed by DM2 intronic repeats are widely dispersed in the nucleoplasm and not associated with either nuclear speckles or exosomes. We hypothesize that the expanded CUG repeats in DMPK mRNA are blocking a stage in its export pathway that would normally