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The intriguing case of motor neuron disease: ALS and SMA come closer

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Abstract

MNDs (motor neuron diseases) form a heterogeneous group of pathologies characterized by the progressive degeneration of motor neurons. More and more genetic factors associated with MND encode proteins that have a function in RNA metabolism, suggesting that disturbed RNA metabolism could be a common underlying problem in several, perhaps all, forms of MND. In the present paper we review recent developments showing a functional link between SMN (survival of motor neuron), the causative factor of SMA (spinal muscular atrophy), and FUS (fused in sarcoma), a genetic factor in ALS (amyotrophic lateral sclerosis). SMN is long known to have a crucial role in the biogenesis and localization of the spliceosomal snRNPs (small nuclear ribonucleoproteins), which are essential assembly modules of the splicing machinery. Now we know that FUS interacts with SMN and pathogenic FUS mutations have a significant effect on snRNP localization. Together with other recently published evidence, this finding potentially links ALS pathogenesis to disturbances in the splicing machinery, and implies that pre-mRNA splicing may be the common weak point in MND, although other steps in mRNA metabolism could also play a role. Certainly, further comparison of the RNA metabolism in different MND will greatly help our understanding of the molecular causes of these devastating diseases.

Introduction

Proximal SMA (spinal muscular atrophy) and ALS (amyotrophic lateral sclerosis) are the most common pathologies in the family of MND (motor neuron diseases) and they are both characterized by progressive degeneration of motor neurons in the anterior horns of the spinal cord, causing progressive muscle weakness and atrophy. SMA occurs in approximately 1 per 6000–10000 newborns, whereas 1–2 people per 100000 develop ALS each year. Although disease onset, genetic causes and the affected neurons vary considerably, SMA and ALS share significant similarities.

The most typical feature of ALS is the progressive death of lower motor neurons, which directly innervate skeletal muscles, and the parallel degeneration of upper corticospinal motor neurons. Most of the ALS patients die within 2–5 years of diagnosis, but the disease is heterogeneous in its clinical presentation, depending on which set of motor neurons is affected first: symptoms may start to appear either at limb muscles or face muscles. Age of onset (on average around

50 years) and disease duration are also quite heterogeneous among ALS patients.

Approximately 10% of ALS cases have an established genetic origin. ALS can be inherited either as an autosomal-dominant or autosomal-recessive trait. A number of ALS loci have been identified and candidates include genes coding for proteins involved in a wide range of cellular processes, from oxidation to axonal transport, RNA processing, vesicular transport and angiogenesis [1]. ALS was generally considered a disease intrinsic to motor neurons. More recently, evidence has accumulated that not only do glial cells contribute to the progression of the disease, but also muscle cells may trigger dysfunction of motor neurons through a dying-back mechanism involving degeneration of axons at the neuromuscular junction [2].

Proximal SMA is the most common genetic cause of infant mortality and is caused, in 95% of all patients, by deletion of the *SMN1* (SMN is survival of motor neuron) gene, whereas the remaining 5% carry small mutations in the same gene. *SMN1* lies within an inverted duplication that also contains a copy of the gene, *SMN2*. *SMN1* and *SMN2* are virtually identical and encode the same SMN protein. A single C > T transition in exon 7, however, causes predominant skipping of the exon: ~90% of *SMN2* mRNAs lack exon 7 and do not encode a functional protein. The clinical severity of SMA ranges from respiratory distress at birth associated with short life expectancy (SMA1) to onset after 10 years of age and a normal life expectancy (SMA4) [3], and is inversely related to the copy number of *SMN2* genes [4], because the

Key words: alternative splicing, amyotrophic lateral sclerosis (ALS), fused in sarcoma (FUS) motor neuron disease, RNA metabolism, spinal muscular atrophy (SMA), survival of motor neuron (SMN).

Abbreviations used: ALS, amyotrophic lateral sclerosis; AS, alternative splicing; CNS, central nervous system; FUS, fused in sarcoma; hnRNP, heterogeneous nuclear ribonucleoprotein; LSm, like-Sm; MND, motor neuron disease(s); mRNP, messenger ribonucleoprotein; SMA, spinal muscular atrophy; SMN, survival of motor neuron; snRNP, small nuclear ribonucleoprotein; SR, serine/arginine-rich; TDP-43, transactive response DNA-binding protein 43; TLS, translocated in liposarcoma.

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presence of multiple *SMN2* alleles raises the levels of SMN protein.

In a clinically significant way, SMA affects only the lower motor neurons in the ventral horn of the spinal cord, but other regions of the CNS are involved as well. Thus SMA patients exhibit alterations in the lateral thalamus and in Clark's column of sensory neurons [5]. In the *Smn*^{-/-} mouse model transgenic for human SMN2, which faithfully mimics both the genotype and phenotype of the human patients, local neuron circuits in the spinal cord have been implicated in pathogenesis ([6], but see also [7]). Importantly, ablation of *Smn* in motor neurons only causes a mild non-lethal MND [8], and rescue of *Smn* expression specifically in motor neurons of the SMA mouse has only a limited benefit [7,9], indicating that other cell types also contribute and that SMA, like ALS, is not a cell-autonomous disease of the lower motor neurons [10].

Molecular causes of SMA

SMN is ubiquitously expressed and has a crucial role in a critical step of mRNA metabolism, namely pre-mRNA splicing. SMN catalyses the assembly of the seven Sm proteins on to the snRNPs (small nuclear ribonucleoproteins) U1, U2, U4/U6 and U5 that form the functional core of the splicing machinery [11]. Without SMN, functional snRNPs do not form and pre-mRNA splicing does not take place: SMN is required for cell survival, independently of cell type. In the disease, a reduced level of SMN protein is expressed from the *SMN2* gene, which obviously suffices to support cell survival. Two hypotheses have been put forward to explain the cell-specific effect of SMN depletion.

Instead of shutting down pre-mRNA splicing, a limiting supply of snRNPs might modulate the choice of exons to be included in the mature mRNA. In fact, in mammals, nearly 95% of the genes produce more than one mRNA from a single primary transcript in a process termed AS (alternative splicing) [12]. AS greatly contributes to enlarging the proteome since, in most cases, these mRNAs encode different protein isoforms. AS regulation is mediated by the interaction of the basal splicing machinery with proteins that either stimulate or inhibit the assembly of the processing complex on alternative exons. Three classes of factors play a major role in splice-site selection: the SR (serine/arginine-rich) proteins, the hnRNPs (heterogeneous nuclear ribonucleoproteins) and the snRNPs U1 and U2 [12]. The SR and related proteins bind to the pre-mRNAs and normally activate nearby splice sites, so that the bound region is included in the spliced mRNA as an exon. hnRNP binding usually silences nearby splice sites and makes the respective region an intron. The U1 snRNP, finally, recognizes potential splice donors, and U2 snRNP interacts with possible splice acceptors. SR proteins and snRNPs mutually enforce their interaction with the pre-mRNA. The competition between these positive splice regulators and the hnRNPs determines the choice of the splice sites [12].

Some snRNPs are down-regulated and AS is altered in the SMA mouse model [13], but AS changes are not confined to motor neurons or to the CNS (central nervous system) [13], and are mostly observed late in disease progression [14], implying that disturbed AS is not a cause of the disease. Still, this finding leaves the interesting possibility that one or more mRNA(s) with a crucial role for motor neuron function is spliced to yield predominantly the wrong isoform(s). An mRNA encoding an unknown tetraspanin has been proposed, as it contains a U12 snRNP-dependent intron that is poorly spliced in animal models of the disease [15].

The second hypothesis calls for an additional function of SMN. SMN seems to be involved in the assembly and/or function of additional RNPs, although the requirement for SMN has not actually been demonstrated [16]. One process of RNA metabolism is particularly important for the functioning of neurons with very long neurites, such as motor neurons: mRNP (messenger ribonucleoprotein) transport. mRNAs can be packed in the cell body into translationally silent mRNPs that are then transported to distant compartments of the cell, where the encoded proteins are then produced on demand. In SMA motor neurons, localization of actin mRNA in the axonal growth cone is impaired and axonal growth is reduced [17]. How SMN favours mRNA localization is not yet clear. hnRNP R has been implicated, as it is part of the transported mRNPs and interacts with SMN. Another intriguing possibility are the LSm (like-Sm) proteins that are structurally related to the Sm proteins and share the same post-translational modification that renders the Sm proteins high-affinity-binding targets for SMN. LSm proteins are part of localized mRNPs in all neuronal dendrites and in some axons, most notably of the spinal cord motor neurons [18].

Molecular causes of ALS: factors involved in mRNA metabolism

More and more genetic factors are being identified in ALS which carry obvious links to mRNA metabolism, such as FUS (fused in sarcoma)/TLS (translocated in liposarcoma), TDP-43 (transactive response DNA-binding protein 43) or *c9orf72*.

A significant number of mutations in familial cases of ALS map to a gene on chromosome 16 that encodes a DNA/RNA-binding protein called FUS/TLS [19,20]. FUS/TLS has the domain structure of an hnRNP, binds to mRNAs [21–23] and to SR proteins [24], and influences splice site selection of reporter gene constructs [24,25]. Most, but not all, of the 60 mutations identified so far are clustered in the extreme C-terminus of the protein [26] and hit the nuclear localization signal that resides in the region [27,28]. Consequently, the protein is retained in the cytoplasm of FUS-ALS patients [20] where it can form inclusions [19].

Interestingly, members of the FUS/TLS family bind to spliceosomal snRNAs [29,30], raising the intriguing possibility that it could have a role in snRNP biogenesis or localization, i.e. it could be on the same pathway as

SMN. Indeed, FUS/TLS and SMN interact, and pathogenic FUS/TLS mutations change the subcellular distribution of SMN [31–33]. We have shown that FUS/TLS binds, in addition to SMN, to the spliceosomal snRNAs U1 and U2. Pathogenic mutations in FUS/TLS do not disturb snRNA binding; instead, the subsequent mislocalization of FUS/TLS to the cytoplasm causes the retention of a portion of the snRNAs in the same compartment. Since the total amount of snRNA in the cell does not change, this means that the effective concentration of the snRNA in the nucleus decreases [34]. This strongly suggests that mutated FUS/TLS and SMN depletion interfere with the same molecular pathway, causing a decrease in snRNPs available for the splicing machinery, and thus potentially modulating alternative splicing, which therefore could well be the underlying common cause of MND pathogenesis.

The gene encoding TDP-43 on chromosome 1 is found to be mutated in ~3% of familial ALS cases. The vast majority of the mutations are found in the aggregation-prone C-terminus of the protein [26]. Although aggregation of the mutated protein is not necessary for the development of motor neuron symptoms [35], it is of note that TDP-43 inclusions are found in many patients suffering from ALS or the clinically related FTLD (frontotemporal lobar degeneration), even when TDP-43 is not mutated. This suggests that misfunction of TDP-43 could have a general role in ALS pathogenesis [36].

Like FUS, TDP-43 has the domain structure of an hnRNP, binds to RNA, interacts with other hnRNPs as well as SR proteins, and thus modulates alternative splicing [36]. Consistently, mice expressing wild-type [37] or mutated [35] TDP-43 show alterations in AS. Importantly, TDP-43 is also linked to SMN and U snRNPs biogenesis. Thus the dysregulation of TDP-43 has an effect on SMN levels [38], changes its subcellular distribution and also has an effect on snRNA abundance in neuroblastoma [37,38]. FUS and TDP-43 interact [33] and co-localize [37], and it therefore seems likely that they collaborate in the U snRNP formation and/or localization. As a consequence, U snRNAs are depleted in the CNS of ALS patients [38].

Expansions of a non-coding GGGGCC repeat in the first intron in the gene *c9orf72* are the most common form of familial ALS, and seemingly non-genetic ALS cases also carry expanded *c9orf72* repeats [39]. Neither the role of the protein encoded by *c9orf72* nor the effect of mRNAs carrying the expanded exanucleotide, which have a propensity for forming highly stable quadruplex structures in RNA [40,41], are known. Expanded RNA repeats might represent the toxic species that compromises the function of the affected neuron. A similar case has been elucidated in the DM (myotonic dystrophies), a multi-systemic disorder caused by a CUG trinucleotide expansion in the 3' untranslated region of the *DMPK* (dystrophia myotonica-protein kinase) gene or a CCUG expansion in intron one of the *ZNF9* (zinc-finger protein 9) gene. The expansion of CUG or CCUG repeats is thought to lead to sequestration and up-regulation of the RNA-binding proteins muscleblind and CUGBP (CUG-binding protein) respectively, and hence

cause defects in RNA splicing of their natural targets [42,43]. A similar pathogenic scheme is predicted in *c9orf72* ALS by the presence, in samples from *c9orf72* patients, of RNA foci containing the repeat sequence [44] and by the observation that the expression of the repeat RNA is sufficient to cause neurodegeneration both in *Drosophila* and in mammalian cells [45]. The ALS-linked *c9orf72* repeats bind proteins of the hnRNP A family [45,46] and hnRNP A3 forms neuronal cytoplasmic and intranuclear inclusions in the hippocampus of patients with *c9orf72* repeat extensions [46]. Interestingly, a pathogenic mutation in hnRNP A1 was recently described in an ALS family where all other known ALS gene mutations could be excluded [47]. It is thus conceivable that accumulation/aggregation of the *c9orf72* repeats likewise alter AS.

Conclusions and future perspectives

In the present review, we have shown how SMN, the protein depleted in SMA, interacts with FUS and TDP-43, two ALS-causing factors. As a consequence, snRNA expression is misregulated and snRNPs mislocalize and are not available for the splicing machinery, which could well change splice-site selection in the patient. Another ALS mutation, the hexanucleotide expansion in *c9orf72*, causes mislocalization of hnRNPs that are also involved in splice-site selection. One could thus argue that AS is disturbed in SMA and at least some ALS subtypes. A particular vulnerability of AS could well be the common cause of SMA and many forms of ALS, and indicate novel targets in the design of a therapeutical approach.

However, there are also other steps in mRNA metabolism that could be the culprit. SMN has been implicated in mRNA transport (see above). Likewise, both TDP-43 and FUS/TLS localize to hippocampal dendrites [48,49] and were identified in dendritic RNA transport granules [50,51]. Consistent with a defect in local protein synthesis, FUS/TLS-knockout neurons show an aberrant spine morphology [49]. It is thus clear that these proteins have a role in mRNA transport. For example, we would like to speculate that FUS/TLS could bind, in addition to the spliceosomal Sm–snRNP complexes, to the structurally related LSm–mRNP complexes: LSm proteins accompany localized mRNAs from the nucleus to their final destination near the dendritic spines [18]. Finally, the C9ORF72 repeats can be aberrantly translated, even though they lack a proper ORF [52,53]. The resulting dipeptide polymers might exert a similar toxicity to that of polyglutamine repeats, but could also be pathogenic by deviating some translation factors that are required for the translation of the repeat RNA. Note that SMN has also recently been implicated in translation regulation [54], and that the control of translation is tightly coupled to mRNA transport: only silent mRNAs travel. Thus there are at least two more mRNA pathways where SMA and ALS touch, and more work is needed to elucidate the precise cause of motor neuron vulnerability.

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