



Molecular bases of spinal muscular atrophy: the survival motor neuron gene

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Abstract

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by degeneration and loss of motor neurons of the anterior horn of the spinal cord. The clinical manifestations include proximal symmetric weakness and progressive muscle atrophy. The identification of the SMN1 gene as determinant of SMA has opened alternative ways of studying the disease. Absence of SMN1 (either by deletion or conversion) was detected in the majority of patients and subtle mutations were described in a minority. SMN1 absence was associated with a wide spectrum of manifestations, from congenital disease to asymptomatic cases. Modifier factors, such as the number of copies of SMN2, its homologous copy present in all patients, could influence the phenotype and help to find a treatment for the disease. The SMN gene is expressed in various neuronal populations, although only motor neurons are responsible for the manifestations of the disease. The SMN protein is part of a complex with various proteins involved in the splicing reaction. This apparently essential function of all cells is critical for motor neurons, and warrants further research to elucidate the mechanisms of disease.

Keywords: Spinal muscular atrophy, SMN gene, genotype-phenotype, modifier genes, motor neuron death, RNA metabolism.

Resum

L'atròfia muscular espinal (AME) és una malaltia neuromuscular autosòmica recessiva caracteritzada per la degeneració i la pèrdua de les motoneurons de la banya anterior de la medul·la espinal. Les manifestacions clíniques més característiques són una debilitat proximal simètrica i una atrofia muscular progressiva. La identificació del gen SMN1 com a gen determinant de l'AME obre noves alternatives per a l'estudi de la malaltia. En la majoria dels pacients es detecta l'absència del gen SMN1 (ja sigui per deleció o per conversió), però també se n'han identificat mutacions puntuals en un petit percentatge. L'absència del gen SMN1 s'associa a un ampli espectre de manifestacions clíniques, que van des de formes congènites de la malaltia fins a casos asimptomàtics. Diferents factors modificadors, com el nombre de còpies del gen SMN2 – el gen homòleg present tant en controls com en malalts – poden modificar el fenotip i a la vegada ser útils per investigar un tractament eficaç. Tot i que el gen SMN s'expressa en diferents poblacions neuronals, només les motoneurons són les responsables de les manifestacions de la malaltia. La proteïna SMN forma part d'un complex amb altres proteïnes que participen en la reacció d'empalmament i aquesta funció, essencial per a totes les cèl·lules, sembla ser crítica per a les neurones motores. Cal aprofundir en l'estudi dels mecanismes que condueixen a l'atròfia muscular espinal.

Proximal spinal muscular atrophy (SMA) is a common autosomal recessive disorder –with an incidence of 1/6000 to 1/10000 live births– which results from degeneration and loss of the alpha-motor neurons in spinal cord anterior horn

cells. Clinical manifestations include proximal muscular weakness and atrophy and, as a consequence, scoliosis, respiratory failure and death. SMA patients can be classified into three groups based on age at onset, achieved milestones and life span [1,2]. Type I SMA (Werdnig-Hoffman disease) is the most severe form with clinical onset before 6 months. Type II SMA is the intermediate form with onset before 18 months. Type III SMA (Wohlfast-Kugelberg-Welander disease) is the mild form of the disease with onset af-

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ter 18 months. An adult form of the disease occurs with onset after 30 years, most patients being mildly affected (SMA type IV or adult form). The biological bases for understanding the degeneration and loss of motor neurons remain to be clarified and there is no specific therapy to alleviate or to cure the disease.

Recent advances in our understanding of the genetic bases of childhood SMA will considerably improve the clinical management of the disease and help develop novel strategies for the prevention of motor neuron degeneration.

The SMA locus

The three clinical forms of the disease were mapped to the 5q13 region [3,4]. This region contains an inverted repeat harboring several genes: survival motor neuron (SMN) [5], neuronal apoptosis-inhibitor protein (NAIP) [6], p44 (subunit of basal transcription factor TFIIH) [7,8] and SERF1/ H4F5 [9] (Figure 1). Given the large number of repeated sequences, pseudogenes, and retro-transposable elements, this is a very unstable region, often prone to unequal rearrangements between highly homologous elements, resulting in deletions, duplications or gene conversion events.

Telomeric SMN (SMNt or SMN1) and centromeric SMN (SMNc or SMN2) genes differ by five nucleotide changes (three are exonic, located within exons 6, 7 and 8, and two are intronic) [5, 10, 11] and both copies are composed of nine exons, which encode identical amino acid sequences and span approximately 20 kb.

The NAIP gene contains 17 exons, and is present as a telomeric functional copy, a pseudo NAIP copy, and several truncated copies in the SMA region. NAIP is also duplicated either with exon 5 (NAIPtel) or without exon 5 (NAIP Cen).

The p44 gene is deleted at a frequency similar to NAIP although its role in the disease is unknown [7,8]. Recently, a

new gene located at the 5' end of the SMN gene has been identified and this has been considered as a candidate phenotypic modifier for SMA [9]. This gene (H4F5 or SERF1) contains the polymorphic marker 212 and appears to be deleted in most of the type I chromosomes compared with type II, III or non-SMA chromosomes.

Molecular pathology of the SMN1 gene

Approximately 90% of patients suffering from different forms of SMA are homozygously deleted for both copies of the SMN1 gene exons 7 and 8 [14], whereas homozygous deletions of SMN2 seem to have no clearly demonstrated clinical consequences. A small number of SMA patients show homozygous deletions of SMN Tel exon 7 but not exon 8. In these patients hybrid genes were characterized, most of them being the result of gene conversion events in which exon 7 of SMN2 was fused to exon 8 of SMN1 [13 and references therein]. Further studies have found gene conversions in type II and III patients homozygously deleted for SMN exon 7 and 8, which explains why an apparently equal genotype could manifest different clinical phenotypes [14]. It has been proposed that deletion events are predominant in severe SMA chromosomes while conversions are associated with mild SMA chromosomes [15].

In the cases where the SMN1 gene is present, subtle mutations have been detected, indicating that the SMN1 gene is the determining gene. Lefebvre *et al.* [5] detected two microdeletions in splicing sites of introns 7 and 8. In the Spanish population, a frameshift 4 bp deletion in exon 3 (430del4) has been detected [16]. Subsequently, another microdeletion in exon 3 of the SMN1 gene and an 11 bp duplication in exon 6 (800 ins 11) producing a premature stop codon have been described [17,18]. Moreover, missense mutations have also been reported [12] (Figure 2).

In a recent study [19], we analyzed 364 families (168 type I, 118 type II and 78 type III) looking for mutations in the

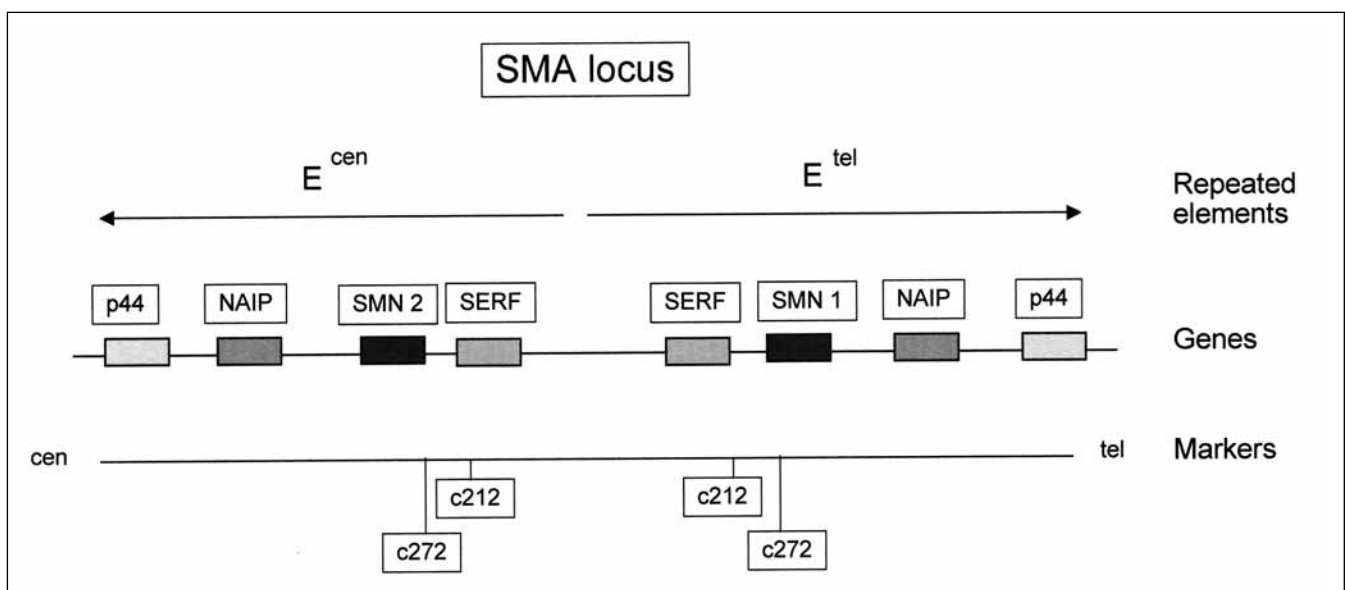


Figure 1. Scheme of the SMA locus in 5q13. The SMA locus consists of two inverted repeated elements containing four genes. Markers C212 and C272 are located at the 5' end of both SMN genes.

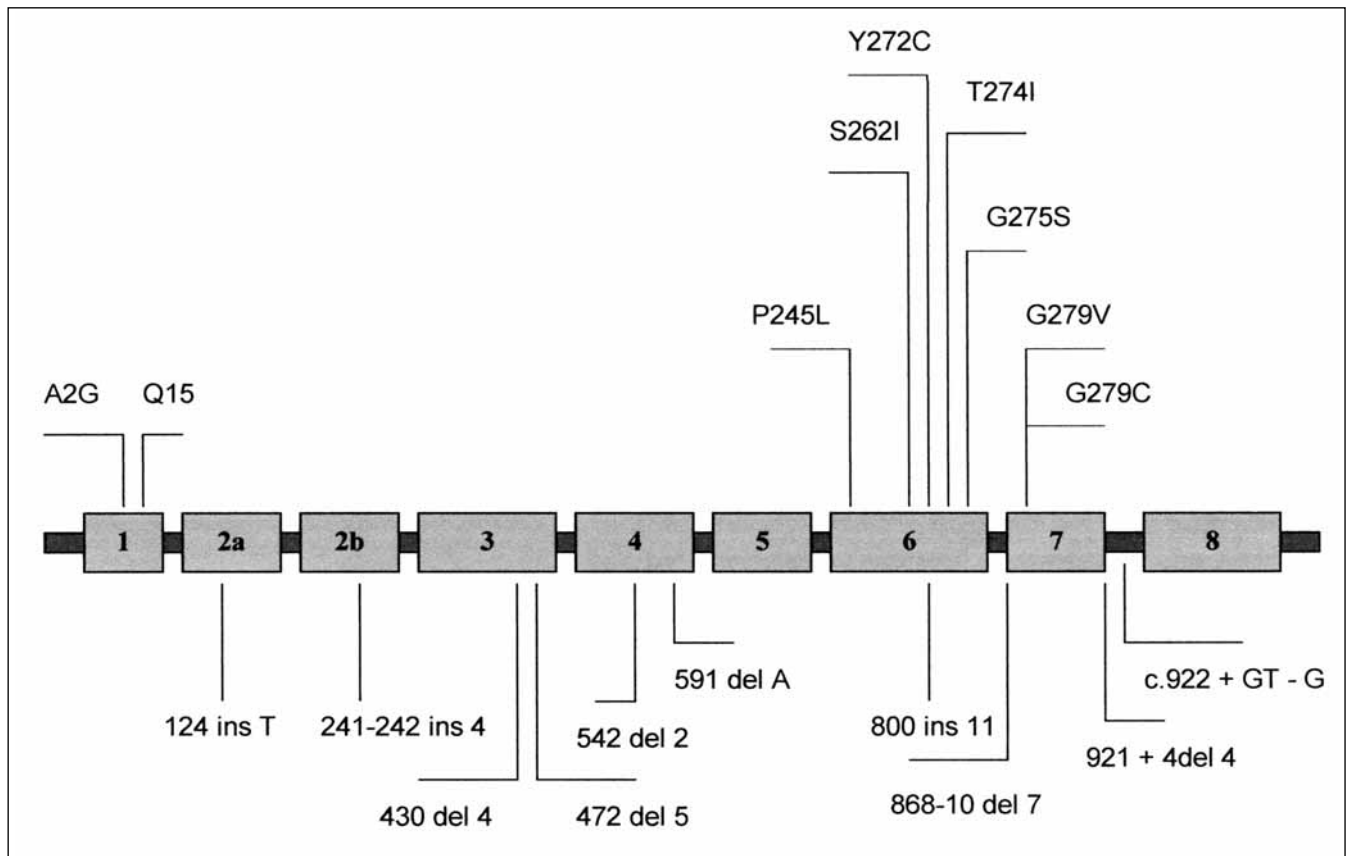


Figure 2. Location of mutations described in the SMN1 gene.

SMN1 gene. Of these patients, 308 (85%) had deletions of the exon 7 and 8 of the SMN1 gene; centromeric-telomeric hybrid genes were detected in 18 patients (5%); 8 cases (2%) showed the 4 bp deletion in exon 3 (430del4) and finally, one type II case, showed an 11 bp duplication in exon 6. No mutation has been identified to date in the remaining 28 patients (7%). All the types of mutations described were associated with the three different phenotypes. The 430del4 was detected in two type I cases, three type II and three type III cases. Haplotype analysis of chromosomes bearing the 4 bp deletion revealed a single origin of this mutation in the eight Spanish families.

Genotype-phenotype correlation. Modifier genes

Although mutations of the SMN1 gene are associated with SMA, no obvious genotype-phenotype correlation has been observed since the absence of SMN1 exon 7 and/or 8 occurs in patients with either the severe form (type I) or the milder forms (type II and type III) (Figure 3). The fact that severe type I SMA is associated with a higher frequency of homozygous deletions (approximately 45%) compared with the milder forms (12-18% of type II and III patients) suggests a partial involvement of NAIPtel in the SMA phenotype. Moreover, onset and the mean age of death occur earlier in patients where SMN1 and NAIPtel are absent [20]. However, the contribution of NAIP to the final phenotype of SMA is still unknown. The comparison of clinical data with the patient genotype (SMN, NAIP and p44) showed that large-scale deletions involving all these loci are observed in the majority

of type I patients, although smaller rearrangements involving the SMN1 gene alone can still result in a severe phenotype. Moreover, homozygous deletions of NAIP or p44 genes have been observed in healthy controls unrelated to the SMA families [6,8]. The H4F5 or SERF1 gene maps 6.5 kb upstream of exon 1 for both SMN1 and SMN2 and contains the C212 marker in its last intron. Almost 94% of SMA chromosomes are deleted for one or both copies of C212 and therefore for the SERF1 gene. Recent studies indicate that SERF is a nuclear protein in HeLa and COS-1 cells (particularly in nucleoli) although it is not associated with the SMN complex [21]. The nucleolar localization coupled with deletion data suggests that SERF1 could be a candidate phenotypic modifier of SMA patients.

The SMN2 gene is always present in SMA patients and allows them to express a certain amount of SMN protein [22]. Interestingly, no patient has been diagnosed with a homozygous absence of both SMN1 and SMN2 genes, implying that the total absence of SMN would be lethal *in utero*. Most SMN2 transcripts lack exon 7, and are thus functionally defective, reinforcing the view that the disease is the result of an insufficient amount of intact SMN protein. Analysis of parents and patients with type II and III forms has shown that these individuals have on average a larger number of SMN2 copies than type I patients [22, 23]. These findings indicate that the homozygous absence of SMN1 is the result of a true deletion in type I cases and a gene conversion in type II or III cases. However, the correlation between the number of SMN2 genes and the clinical phenotype is not absolute. It

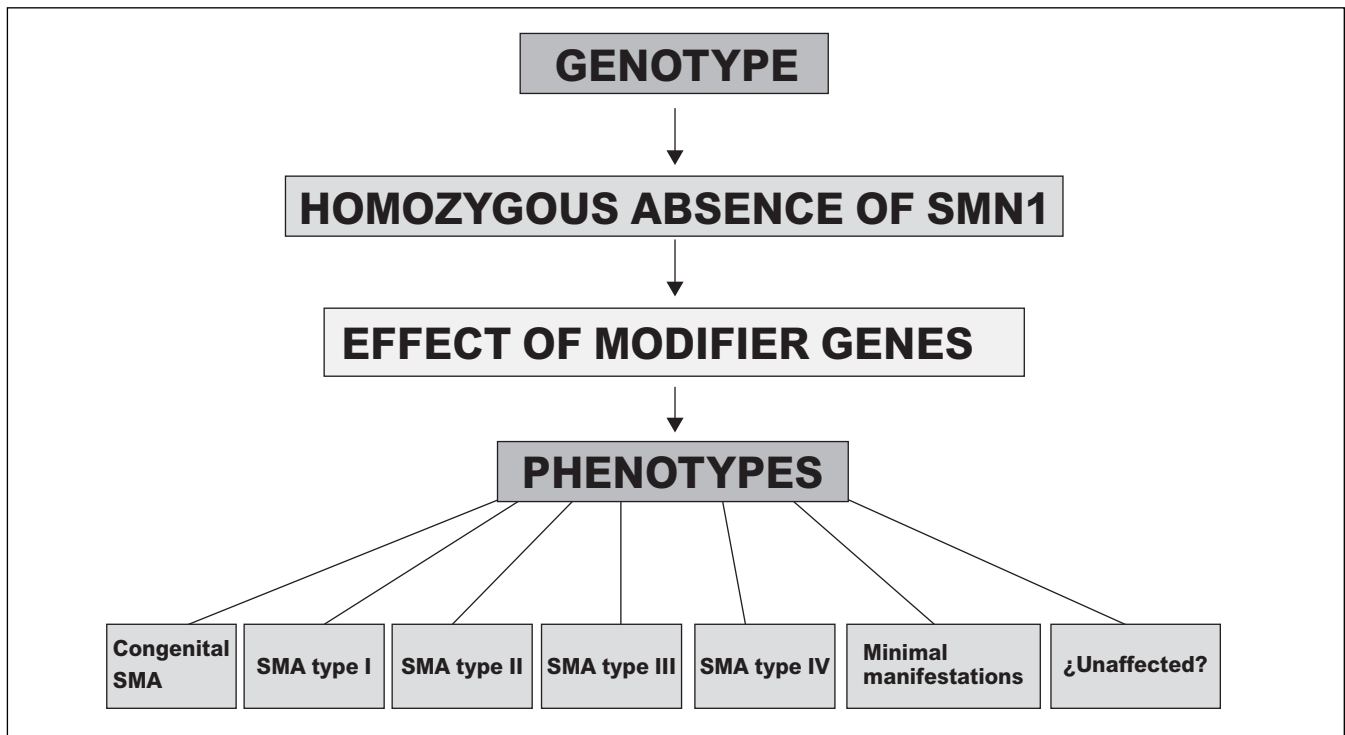


Figure 3. Different phenotypes reported in association with homozygous absence of the SMN1 gene under the influence of modifier genes.

has been reported in a few SMA families whose index cases had the chronic form of the disease (type II and III) that «unaffected» siblings and parents are also carriers of a homozygous absence of SMN1 [24,25]. However, minimal manifestations and EMG abnormalities have been detected in some of these cases, indicating the wide range of manifestations of the disease [26] (Figure 3). Different phenotypes have also been found in the families with the 430del4 mutation: one homozygous (430del4/430del4) daughter was minimally affected in one consanguineous family while her three homozygous sisters were wheelchair-bound since infancy [19]. They had the same number of SMN2 copies, indicating that modifier genes outside the SMA locus were also responsible for the phenotype.

The SMN protein

The SMN encodes a 38 kD protein, which bears no resemblance to any other proteins in the database. A tight correlation between the level of SMN protein and the severity of the disease has been observed in tissues and cells derived from SMA patients [22,27]. A number of observations indicate that SMN could be involved in RNA metabolism. First, a clustering of missense mutations in the SMN gene has been described in SMA patients in a region of amino acids 262-279 containing a tyrosine/glycine-rich motif which is present in various RNA binding proteins [28]. Secondly, the SMN protein has been located by immunohistochemistry in a novel structure termed «gems», which is more commonly associated with coiled bodies. Ramon y Cajal described the coiled bodies in 1903, and there is considerable evidence that both structures are involved in RNA metabolism [29]. The role of SMN appears to be related to two essential cellular processes

such as the biogenesis of spliceosomal U snRNPs and the pre-mRNA splicing [30-32]. The SMN binds to itself, to Gemin2, (former SIP1 or SMN interacting protein 1) and to small nuclear RNPs U1 and U5 of the spliceosome, the catalytic core of the splicing reaction. Recently, Gemin3 and Gemin4 were reported as components of the SMN complex [33,34]. Mutant forms of the SMN such as the deletion of exon 7 and the Y272C are incapable of joining and regenerating snRNPs [35]. The interaction of SMN with these proteins possibly plays an important role in the assembly of snRNPs in the cytoplasm and in the nuclear regeneration of snRNPs and spliceosomes. In the light of these observations, SMN must have a critical function for all cells. If SMN is only necessary in large amounts in motor neurons, it could either play an alternative role in these cells or just require a high quantity for splicing factors.

Expression of SMN and the mechanism of neuron degeneration and death in SMA

The expression of the SMN gene has been detected in the motor neurons of the spinal cord as well as in different neuronal populations such as sensory neurons of the dorsal root ganglia, neurons of the medulla oblongata, Purkinje cells of the cerebellum and pyramidal cells of the cortex [36]. This expression is present in the spinal cord at 8 weeks of fetal life, and throughout postnatal and adult life. The sharp expression of SMN in the motor neurons of the human spinal cord, the target cells in SMA, indicate that this gene is implicated in neuronal development and in the pathogenesis of the disease (Figure 4). SMA is caused by a reduction in SMN levels, which leads to the loss or degeneration of only part of a specific neuronal group. It can be hypothesized that the

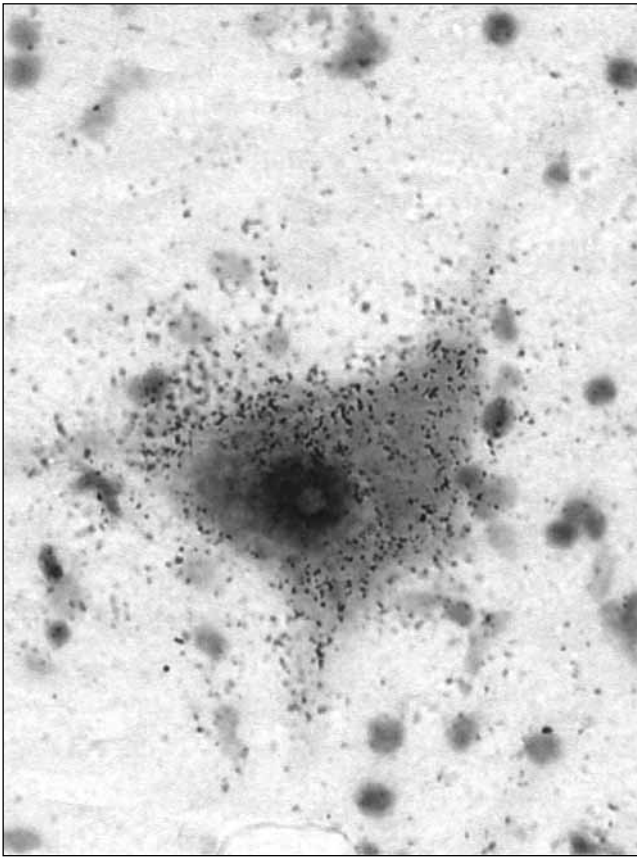


Figure 4. In situ hybridization of the anterior horn of human adult spinal cord showing a motor neuron with positive signal of SMN messenger RNA detected with an antisense SMN S³⁵ riboprobe (hybrid RNA-RNA). Toluidine blue, 600x.

other neuronal groups and tissues are protected by unknown genetic or cellular factors when SMN is interrupted. Recent studies in human fetal spinal cord suggest that the programmed neuron death is enhanced in SMA during development, which results in a loss of approximately half of the neurons at birth. However, morphologic changes in motor neurons such as peripheral displacement of the nuclei or central reduction in the number of Nissl corpuscles consistent with early central chromatolysis [37], were observed only postnatally. Given that most of the SMA motor neurons in the neonatal period appear with pathologic findings that had been undetected at earlier stages, it can be hypothesized that type I SMA results in differential age-dependent responses, leading to cell death and motor neuron degeneration during development [38]. Further investigation is necessary to attribute the function of SMN in RNA biogenesis to a possible mechanism involving cell death increase in fetal SMA spinal cord or motor neuron degeneration. The development of animal models is necessary to test these hypotheses.

Animal models

In mice, where the SMN gene exists as a single copy, the total loss of the SMN gene due to homologous recombination results in massive cell death in the early blastocyst stage, a period which corresponds to the initiation of embryonic RNA

transcription [39]. The massive cell death detected in these SMN-deficient mouse embryos reinforces the previous hypothesis that the complete absence of SMN1 and SMN2 is lethal in humans [5], thereby lending support to the crucial role of SMN. Recently, four studies have described the development of SMN-deficient mice models that mimic human SMA. Two different research groups [40,41] have generated mice with a different number of transgenic copies of SMN2, developing a phenotype resembling severe SMA type I (one or two copies of SMN2) or milder forms (up to 8 copies) of the disease. Monani *et al.* [40] observed that neuron death appeared after birth in the final stages of the disease. There are neither degenerative changes in motor neurons nor signs of skeletal muscle denervation in mice dying at 6 days of life, suggesting that an acute degenerative process could occur. However, given that some mice died shortly after birth, degenerative changes in the motor neurons could begin in the fetal stages. Jablonka *et al.* [42] reported a SMN heterozygous mouse with reduced levels of SMN protein without signs of denervation or motor defect, showing postnatal loss of spinal and facial motor neurons until month 6, decreasing thereafter. Finally, Frugier *et al.* [43] developed a conditional deletion of the murine exon 7, generating a mouse with a reduced life expectancy consistent with chronic SMA (type II and III). These authors reported severe skeletal muscle denervation with morphological changes in motor neurons such as indentation in the motor neuron nuclear membranes and nuclear fragmentation despite the absence of motor neuron loss. The latter finding suggests that there was an apoptotic process and that motor neuron loss was a late manifestation of the disease in these animals. These changes had not been reported in human SMA, and the authors attributed them to early events of neuronal degeneration that subsequently led to motor neuron loss.

Perspectives

The identification of the gene defect in SMA has opened up new possibilities of studying and diagnosing the disease. Aside from the advances in the characterization of molecular defects in SMA patients, the possibility of preventing, stopping or reversing motor neuron degeneration based on the knowledge of the SMN function is eagerly awaited. An interesting therapeutic alternative is upregulation or overexpression of the SMN2 gene, which is present in all SMA patients [44]. SMN2 produces a partially functional protein that could alleviate the severity of the disease. Moreover, preliminary results indicate that a full-length SMN expression can be restored from SMN2 after stimulation *in vitro* with Htra2-b1, a serine/arginine-rich splicing factor [45]. High throughput drug screenings are in progress to identify compounds that activate or replace SMN protein. Furthermore, studies are currently being undertaken to target and deliver SMN protein to motor neurons *in vivo*, develop efficient gene therapy vectors to motor neurons, and to confirm the hypothesis that stem cell transplantation can replace lost motor neurons.

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