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Review

Molecular mechanisms and animal models of spinal muscular atrophy ☆

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ABSTRACT

Spinal muscular atrophy (SMA), the leading genetic cause of infant mortality, is characterized by the degeneration of spinal motor neurons and muscle atrophy. Although the genetic cause of SMA has been mapped to the *Survival Motor Neuron1* (*SMN1*) gene, mechanisms underlying selective motor neuron degeneration in SMA remain largely unknown. Here we review the latest developments and our current understanding of the molecular mechanisms underlying SMA pathogenesis, focusing on the animal model systems that have been developed, as well as new diagnostic and treatment strategies that have been identified using these model systems. This article is part of a special issue entitled: Neuromuscular Diseases: Pathology and Molecular Pathogenesis.

1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive genetic disorder characterized by the loss of spinal motor neurons, which leads to muscle atrophy, paralysis, and ultimately death [12,54,68]. As the primary genetic cause of infant mortality and the second most common autosomal recessive genetic disorder, SMA affects one in every six thousand live births with a carrier frequency of one in forty people [72,74,84]. More than 98% of patients with SMA have a homozygous disruption of the *Survival Motor Neuron1* (*SMN1*) gene on chromosome 5q13 by deletion, rearrangement, or mutation [35,49]. In addition to the telomeric *SMN1*, humans also contain a centromeric *SMN2* produced by intrachromosomal duplication. *SMN2* differs from *SMN1* by only five nucleotides [13,49]. One silent single nucleotide change within exon 7 of *SMN2* disrupts its splicing [16,17,44]. Thus, *SMN2* produces only a small amount of full-length functional protein and mostly an unstable truncated isoform of SMN lacking exon 7 (SMNΔ7). Therefore, mutations of *SMN1* lead to reduced but not depleted levels of full-length SMN protein in SMA, which are sufficient to sustain the survival of most cell types with the exception of spinal motor neurons.

The expression level of SMN protein is inversely correlated with SMA disease severity. The pathological symptoms are highly variable. Patients can be classified into four categories (Table 1) according to the age of onset and maximum motor function achieved [71]. SMA type I, or Werdning–Hoffman disease, is the most common and severe type. Onset is usually before 6 months of age, and death occurs within the first 2 years of life. These infants have profound flaccid symmetrical weakness and hypotonia, and are unable to sit without support. Bulbar denervation results in tongue weakness and fasciculation with poor suckling and swallowing. SMA type II is of intermediate severity and characterized by onset of disease between 7 and 18 months of age. Patients are capable of sitting independently but do not achieve the ability to walk. Patients with SMA type III (Kugelberg–Welander disease) have onset of symptoms after 18 months and are able to achieve independent walking. Adult onset, or SMA type IV, starts around the second or third decade of life and is characterized by mild weakness without respiratory or nutritional problems [71]. Although the majority of patients (95%) have homozygous deletion of *SMN1* exon 7 or both exon 7 and 8, about 3–4% of patients are compound heterozygotes for the deletion of *SMN1* on one allele and a point mutation on the other. De novo mutations occur at a rate of 2% because of regional instability on chromosome 5 [56]. An updated list of *SMN1* gene mutations is available on Leiden Open Variation Database (http://www.dmd.nl/nmdb2/home.php?select_db=SMN). Mutations include nonsense, frame-shift, missense, deletions, inversions and splicing site changes. The Y272C and 813ins/dup11 mutations are reported to be the most common [96].

The main pathological feature of SMA is neuronal loss in the anterior horn of the spinal cord with chromatolysis, neurophagia and gliosis [3,46]. Neuromuscular junction ultrastructural abnormalities were also noted in humans with SMA [43]. Recently, neuromuscular junction

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Table 1
Classification of spinal muscular atrophy.

	Age of onset	Maximum motor function achieved	Age of death
Type I (severe, Werdnig–Hoffmann disease)	Before 6 months	Unable to sit	Less than 2 years
Type II (Intermediate)	7–18 months	Sit, never walk	More than 2 years
Type III (mild, Kugelberg–Welander disease)	After 18 months	Stand and walk independently	Adult
Type IV (very mild)	Second or third decade	Walking during adulthood	Adult

function was evaluated in SMA type II, III and IV patients using repetitive nerve stimulation. Pathological detrimental response was observed in about half the patients with SMA type II or III, but not in control patients or other motor neuron disease patients, suggesting specific neuromuscular junction dysfunction in SMA [94]. In addition, myotubes were found to be smaller in skeletal muscle tissue from severely affected SMA fetuses, indicating a delay in muscle growth and maturation [59]. Patients with severe SMA also develop congenital heart defect and arrhythmias, vascular abnormalities such as digital necrosis and mild hyperglycemia, suggesting pancreatic dysfunction. The first diagnostic test for a patient suspected to have spinal muscular atrophy should be *SMN1* gene deletion test. If this initial test is negative, further testing, including muscle creatine kinase level and nerve conduction study with repetitive nerve stimulation and electromyography, should be done. If electrophysiological studies suggest a motor neuron disease then sequences of both *SMN1* alleles should be determined.

SMN has been implicated in several functional processes, including pre-mRNA splicing, mRNA transport, and axon growth [1,63,64,76,80]. Although SMN is ubiquitously expressed in all tissues, spinal motor neurons are particularly vulnerable to diminished levels [68]. The underlying mechanisms of spinal motor neuron susceptibility remain largely unknown. Currently there is no effective therapy for SMA. There are, however, a number of prevailing and not mutually exclusive hypotheses based on the data yielded from disease models that may begin to explain. That the wild-type SMN protein has an established function in small nuclear ribonucleoprotein (snRNP) assembly suggests a role for pre-mRNA splicing in SMA disease progression [76]. Alternatively, because motor neurons have highly specialized, far-extending axons, it has been postulated that the localization of mRNAs to these distal processes is affected in SMA, which may be a driver of the selectivity for motor neuron degeneration [80]. At present, no direct target of mRNA splicing defect has been identified that can explain SMA pathogenesis, and there is equally sparse evidence to support the hypothesis that defects of distal mRNA transport and localization lead to SMA. Despite these unanswered questions concerning disease development and progression, much has been gleaned by modeling SMA with laboratory model systems. Here, we review the animal model systems that have been developed, as well as new mechanistic insights, diagnostic and treatment strategies that have been identified using these models.

1.1. Caenorhabditis elegans

The nematode *Caenorhabditis elegans* has been an efficient model for studying various disease-related gene functions. Genetic mutations in *C. elegans* can be induced by exposing worms to mutagens, including ethyl methanesulfonate (EMS) and gamma irradiation. RNA interference (RNAi)-mediated knockdown of gene expression can be easily achieved by feeding worms with small interfering RNA (siRNA) libraries. The *C. elegans* genome contains a single *SMN* ortholog, *smn-1*, that encodes an SMN protein 36% identical to the human ortholog (Fig. 1) [6]. Reducing the expression of *smn-1* by RNAi causes larval lethality, suggesting that *smn-1* is essential for survival of *C. elegans* animals [67]. A null mutation of *smn-1*, *smn-1*(*ok355*), that deletes most of the *smn-1* coding region, causes developmental arrest, reduced lifespan and progressive loss of motor functions [10]. Neuronal expression of an *smn-1* transgene partially rescues the developmental arrest and motor defects, while muscle-specific expression of the transgene does not, suggesting that the *C. elegans smn-1* primarily functions in neurons [10].

Extensive genetic screens have been performed to identify genes capable of modifying the deleterious phenotypes of *smn-1*-deficient *C. elegans* mutants [26]. Among these genes, the small conductance Ca^{2+}-activated K^+ channel (SK channel) was identified and manipulated pharmacologically to seek new modifiers of SMN functions for potential SMA therapy. Activating the SK channel by the neuroprotective drug Riluzole improved the motor functions of the *C. elegans smn-1*(*ok355*) null mutant and restored axon outgrowth in *Smn*-deficient rat hippocampal neurons [25], suggesting that genes identified in these screens could be potential targets for treating *SMN*-related defects. A caveat of using the *smn-1*(*ok355*) null mutant for screens is that severe defects of the mutant make identification of modifiers of the phenotype very demanding. To overcome this drawback, a point mutation in *smn-1* that mimics a human SMA disease mutation was isolated, which causes weak motor defects and a slightly reduced lifespan [88]. This mutant was used to screen a library of chemicals for potential drugs that could ameliorate the mild defects. Six chemicals were identified for further analysis [88]. The most effective ones include two FDA-approved drugs, 4-AP (a potassium channel blocker) and gaboxadol hydrochloride (a $GABA_A$ receptor agonist), and one novel compound Neu5Ac (a monosaccharide) [88]. With this, the *C. elegans smn-1* mutants represent an efficient discovery tool for performing large-scale screen for modifiers of SMN function.

1.2. Drosophila

The *Drosophila* genome contains a single copy of *SMN* ortholog, *Smn*, with 41% sequence homology to human *SMN1* (Fig. 1) [66]. Ectopic expression of SMA disease-related human SMN1 or truncated forms of *Drosophila* SMN causes pupal lethality and developmental arrest in a dominant-negative manner [66], suggesting that *Smn* is essential for *Drosophila* survival. Consistent with this finding, the *Drosophila* ortholog of Gemin 3, an SMN-interacting protein, is required for larva motor functions and animal survival [18,87]. *Drosophila* models carrying different *Smn* mutations have been developed and extensively studied. Mutant animals carrying an *Smn* point mutation similar to that in human SMA patients exhibit reduced excitatory post-synaptic currents, disorganized motor neuron boutons, loss of glutamate receptors at the neuromuscular junctions and compromised motor abilities [19]. Hypomorphic *Drosophila Smn* mutants isolated by Rajendra et al display defective axonal arborization in motor neurons and a failure to form thin filaments in muscles [78]. Altogether, these studies establish an essential role for *Smn* in regulating motor neuron and neuromuscular functions in *Drosophila*.

The analysis of mouse *Smn* mutants has identified defective expression of both major intron snRNAs and minor intron snRNAs, consistent with the role of *Smn* in snRNP assembly and pre-mRNA splicing [8,30,98]. Reduced snRNA expression was also observed in *smn*-deficient *S. pombe* [14]. Conversely, major intron snRNA expression in *Smn*-deficient *Drosophila* was not obviously affected [78], and reduced expression of minor intron snRNAs does not apparently affect the splicing of minor introns [77], suggesting that the effects of *Smn* on snRNA expression is species-dependent.

That the splicing of both major and minor introns was altered in *Smn* mutants raises the question as to which intron type accounts for the

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