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Review

Spinal muscular atrophy: An update on therapeutic progress

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ABSTRACT

Humans have two nearly identical copies of survival motor neuron gene: *SMN1* and *SMN2*. Deletion or mutation of *SMN1* combined with the inability of *SMN2* to compensate for the loss of *SMN1* results in spinal muscular atrophy (SMA), a leading genetic cause of infant mortality. SMA affects 1 in ~6000 live births, a frequency much higher than in several genetic diseases. The major known defect of *SMN2* is the predominant exon 7 skipping that leads to production of a truncated protein (SMNΔ7), which is unstable. Therefore, SMA has emerged as a model genetic disorder in which almost the entire disease population could be linked to the aberrant splicing of a single exon (i.e. *SMN2* exon 7). Diverse treatment strategies aimed at improving the function of *SMN2* have been envisioned. These strategies include, but are not limited to, manipulation of transcription, correction of aberrant splicing and stabilization of mRNA, *SMN* and *SMNΔ7*. This review summarizes up to date progress and promise of various in vivo studies reported for the treatment of SMA.

1. Introduction

Spinal muscular atrophy (SMA) is a genetic disease caused by homozygous deletion, truncation, mutation or gene conversion of *survival motor neuron 1* (*SMN1*) [1–4]. *SMN2*, a nearly identical copy of *SMN1*, fails to compensate for the loss of *SMN1* owing to a cytosine to thymidine mutation at the 6th position (C6U in the transcript) of exon 7. C6U triggers predominant skipping of *SMN2* exon 7 due to disruption of an exonic splicing enhancer and/or creation of an exonic splicing silencer [5–7]. The resultant decrease in full-length transcript reduces functional SMN, since the translated product (SMNΔ7) of the truncated transcript is unstable and rapidly degraded [8–10]. The copy number of *SMN2* modulates the severity of SMA: the more *SMN2* copies the less severe the disease due to higher levels of the full-length transcript and functional SMN [11–13]. Thus, treatment strategies to halt the disease progression and ameliorate the symptoms have primarily focused on means to increase full-length *SMN2* transcript and functional SMN.

The multifunctional SMN has been implicated in snRNP biogenesis [14–17], transcription [18,19], splicing [20], translation [21], signal transduction [22], stress granule formation [23] and intra-cellular trafficking [24]. With respect to neuron-specific functions, SMN facilitates interaction of mRNA binding proteins and participates in mRNA transport across the axonal processes of motor neurons [25–27]. SMN modulates axon outgrowth and cytoskeletal dynamics through β actin localization [28–30]. Preventing SMN transport across axons causes growth cone collapse [31]. SMN also plays an important role in postnatal muscle nerve terminal maturation and reduction in SMN levels is predicted to negatively affect neurotransmission [32]. Defects in snRNP biogenesis correlate with the severity of SMA, although only a subset of snRNPs is preferentially affected [33]. Supporting these arguments, motor neurons of *Smn* deficient *Drosophila* show decreased expression of a subset of certain genes containing the U12 type introns [34].

Mice, unlike humans, possess only one *Smn* gene, and homozygous deletion of *Smn* is embryonically lethal [35]. Several transgenic mouse models that mimic the SMA pathology have been developed by introducing human *SMN2* into the mouse genome in the context of *Smn* knockout. Two recent excellent reviews describe these models in much detail [36,37]. Preclinical research to identify promising treatments for SMA has relied heavily upon these murine models [22,38–48]. Table 1 lists a few major mouse models utilized in preclinical trials as well as a few other models that may be exploited for these pursuits. Two severe mouse models account for the majority of preclinical studies: the Taiwanese model [38,48] and the Δ7 SMA model [40]. Generally, therapeutic strategies in SMA mice focused on increasing the amount

Abbreviations: ALS, amyotrophic lateral sclerosis; ASO, antisense oligonucleotide; BBB, blood brain barrier; CREB, cyclic AMP response element binding protein; FDA, Food and Drug Administration; GSK-3, glycogen synthase kinase 3; HDAC, histone deacetylase; hnRNP A1, heterogenous ribonucleoprotein A1; ICV, intracerebroventricular; IGF-1, insulin-like growth factor 1; IP, intraperitoneal; ISS-N1, intronic splicing silencer N1; ISS-N2, intronic splicing silencer N2; IV, intravascular; JAK, Janus kinase; NMDA, *N*-methyl D-aspartic acid; NSAID, non-steroidal anti-inflammatory drug; SAHA, suberoylanilide hydroxamic acid; SC, subcutaneous; scAAV, self-complimentary adeno-associated virus; SMA, spinal muscular atrophy; SMN, survival motor neuron; snRNP, small nuclear ribonucleoprotein; STAT5, signal transducer and activator of transcription 5; TIA1, T-cell restricted intracellular antigen 1; TSA, trichostatin A; VPA, valproic acid

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Table 1
Mouse models useful for testing drug efficacy for potential SMA therapy.

Model	Genotype	Survival (days)	Outcome measures	References
Taiwanese	*Smn*−/−; *SMN2(2Hung)*+/± [mice carry 1 or 2 copies of transgene]	Type I: ~10 Type II: ~14 Type III: Normal	Survival; motor function; tail and limb necrosis; motor neuron, NMJ, muscle and heart morphology and/or function	[38,48]
Line89	*Smn*−/−; *SMN2(89Ahmb)*+/+	5	Survival; motor function; motor neuron, NMJ[a] and muscle morphology and/or function	[39]
Δ7 SMA	*Smn*−/−; *SMN2(89Ahmb)*+/+; *SMNΔ7* +/+	~14	Survival; motor function; motor neuron, NMJ, muscle and cardiac morphology and/or function	[40]
3 copy *SMN2*	*Smn*−/−; *SMN2(N11)*; *SMN2*(N46)	14–16	Survival; motor function; motor neuron, NMJ and muscle morphology and/or function	[41]
F7 or exon 7 floxed	*SmnF7/Δ7; NSE-Cre* [exon 7 loss in neurons]	25	Survival; motor function; motor neuron, NMJ and muscle morphology and/or function	[43]
F7 or exon 7 floxed	*SmnF7/Δ7*; *HSA-Cre* [exon 7 loss in skeletal muscle]	33	Survival; motor function; motor neuron, NMJ and muscle morphology and/or function	[42]
2B	*Smn2B*/− [2B is defined by mutation in exon 7 splicing enhancer]	~30	Survival; motor function; neuromuscular junction and muscle morphology and/or function	[22]
SMN^{RT}	*Smn*−/−; *SMN2(89Ahmb)* +/+; $SMN\Delta7^{RT}$ +/+	34	Survival; motor function; motor neuron, NMJ and muscle morphology and/or function	[44]
Olig2-*Cre*	*Smn*F7/−; *SMN2(89Ahmb)*+/+; *Olig2-Cre* [exon 7 loss in motor neuron progenitor cells]	365[b]	Motor function; motor neuron, NMJ and muscle morphology and/or function	[45]
Smn C > T	*Smn*C > T [*SMN2* mutation inserted in mouse *Smn*]	Normal	Motor function; motor neuron, NMJ and muscle morphology and/or function	[46]
Allele C	*Smn*C/C [C is defined by a chimeric gene plus *SMN2*]	Normal	Ear, tail and limb necrosis; cardiac morphology and function	[47]

[a] NMJ, neuromuscular junction.
[b] 70% of these mice survived to 365 days.

of full-length *SMN2* transcript and SMN as a means to extend lifespan and correct tissue and motor function abnormalities. We summarize the major avenues of therapeutic interventions explored for SMA with particular emphasis on small molecules and antisense oligonucleotides (ASOs). This review complements a recent report that describes in detail the progress in the field of ASO-mediated therapy of SMA [49]. Due to lack of space and the staggering number of compounds tested for SMA therapy, we are unable to provide details on dose, duration and frequency of delivery for most of the compounds. For the purposes of comparison, we have put major emphasis on the life expectancy as the primary measure of the therapeutic efficacy in severe SMA mice. Until two years ago there was no report of a therapeutic compound that could extend the life span of a severe SMA mouse beyond 30 days. Recently, independent studies have shown an impressive increase in the life expectancy of severe SMA mice treated with ASOs that specifically target an intronic sequence within *SMN2* [refs. in 49]. The noticeable aspect of these studies is the cross validation of ASO efficacy among various mouse models and oligonucleotide chemistries against the same intronic target (described later). However, due to timing of blood brain barrier (BBB) formation and several other features distinct from humans, results in mouse models of SMA should be interpreted with caution. An overwhelming majority of small compounds confer a modest (<1.5-fold) increase in the life expectancy of severe SMA mice (Fig. 1). Consistently, these compounds display poor efficacy in clinical trials. However, possibilities remain that some of these compounds could be further improved to achieve a better therapeutic efficacy.

2. Treatment with small compounds

Small compounds offer several advantages, including an easy transport across biological barriers. Considering SMA is a neurodegenerative disease, compounds that are transported across BBB would be best suited for an effective therapy. A summary of the relative efficacy of small compounds and other treatments is given in Fig. 1 [based on refs. 50–82]. Available reports underscore the diversity of processes that may impact *SMN2* transcription, *SMN2* exon 7 splicing and/or SMN levels within a cell. Given below are the major classes of compounds that have been tested for their efficacy for SMA therapy (Table 2). Q3

2.1. Histone deacetylase (HDAC) inhibitors

HDAC inhibitors prevent deacetylation of histones and increase gene expression through chromatin remodeling [83,84]. Various chemical classes of HDAC inhibitors have been shown to enhance the expression of *SMN2* in SMA patient cells and in mouse models of SMA (Fig. 1) [68,69,80,81,85–88]. Sodium butyrate modestly increased survival in Taiwanese type II mice and reduced tail necrosis [81]. Valproic acid (VPA), a FDA-approved compound with multiple functions including HDAC inhibition, increased motor neuron density in the lumbar spinal cord and ameliorated necrosis of the tail and ears of Taiwanese type III mice [89]. In a follow-up study, type III SMA mice treated with VPA exhibited decreased spinal cord motor neuron degeneration, decreased muscle atrophy and improved neuromuscular junction innervation [90]. However, the effects of VPA in severe SMA mice were less pronounced. Nevertheless, VPA has been extensively examined as a treatment for types I, II and III SMA patients in several clinical trials (Clinicaltrials.gov ID numbers NCT00661453, NCT00227266, NCT00374075, NCT00481013, and NCT01671384). The beneficial effects of VPA in SMA patients, however, have been nominal [91–95]. Another HDAC inhibitor, phenylbutyrate, has been trialed in SMA patients with modest results [96], although more extensive clinical trials have been terminated due to poor treatment compliance or slow enrollment (Clinicaltrials.gov ID numbers NCT00439569 and NCT00439218).

Trichostatin A (TSA), a second-generation HDAC inhibitor, improved motor function and modestly increased the survival of Δ7 SMA mice (Fig. 1) [69]. Addition of a nutritional supplement with TSA treatment augmented the beneficial effects, including a ~2.5-fold increase in lifespan of Δ7 SMA mice (Fig. 1) [68]. Suberoylanilide hydroxamic acid (SAHA), another second-generation HDAC inhibitor, rescued the embryonic lethality and modestly increased survival of Taiwanese type I SMA mice (Fig. 1) [80]. However, higher doses of SAHA resulted in toxicity even in heterozygous mice [80]. A recent study with SAHA showed weight gain and improved motor function in Taiwanese type I SMA

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