Spinal Muscular Atrophy: Journeying From Bench to Bedside

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Abstract Spinal muscular atrophy (SMA) is a frequently fatal neuromuscular disorder and the most common inherited cause of infant mortality. SMA results from reduced levels of the survival of motor neuron (SMN) protein. Although the disease was first described more than a century ago, a precise understanding of its genetics was not obtained until the SMA genes were cloned in 1995. This was followed in rapid succession by experiments that assigned a role to the SMN protein in the proper splicing of genes, novel animal models of the disease, and the eventual use of the models in the pre clinical development of rational therapies for SMA. These successes have led the scientific and clinical communities to the cusp of what are expected to be the first truly promising treatments for the human disorder. Yet, important questions remain, not the least of which is how SMN paucity triggers a predominantly neuromuscular phenotype. Here we review how our understanding of the disease has evolved since the SMA genes were identified. We begin with a brief description of the genetics of SMA and the proposed roles of the SMN protein. We follow with an examination of how the genetics of the disease was exploited to develop genetically faithful animal models, and highlight the insights gained from their

analysis. We end with a discussion of ongoing debates, future challenges, and the most promising treatments to have emerged from our current knowledge of the disease.

Keywords Spinal muscular atrophy · survival motor neuron · animal models · motor neuron · neurodegeneration

Spinal Muscular Atrophy Phenotype and Disease Pathology

The earliest references to spinal muscular atrophy (SMA) were made in the late 1800s by the European physicians, Guido Werdnig and Johan Hoffman. Each described a debilitating, frequently fatal disorder of infants in which the spinal motor neurons were lost and the skeletal muscles atrophied [1–4]. The disorder, which bears their joint names, Werdnig–Hoffman disease (type I SMA) accounts for the largest proportion and the most severe form of the classic SMAs. The spectrum of SMA phenotypes was initially expanded in 1956 by Kugelberg and Welander, who described a relatively mild form of the disease (type III SMA) [5], and again a few years later by Dubowitz [6], who reported a cohort of patients with an intermediate phenotype (type II SMA). The 3 types of SMA, as well as a fourth (adult-onset or type IV SMA) have traditionally been distinguished from one another on the basis of severity and onset (Table 1). While this classification persists, today it is generally recognized that the SMA phenotypes constitute more of a continuum, from the truly severe with disease symptoms detected *in utero* to the very mild with onset during late life. The varying severities notwithstanding, all SMA patients suffer weakness that characteristically affects the proximal muscles more than the distal ones, and the lower limbs more than the upper ones. The diaphragm is spared relative to the intercostal muscles. In severely hypotonic

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Table 1 The four classes of spinal muscular atrophy (SMA) patients

Type	Eponym	Age at onset	Disease characteristics	Age at death	Proportion of total SMA (%)
I	Werdnig–Hoffman disease	< 6 months	Proximal muscle weakness; patients never gain the strength to sit independently	< 2 years	60
II	Dubowitz disease	6–18 months	Proximal muscle weakness; patients gain enough strength to sit unassisted but become wheelchair-dependent; scoliosis of spine	> 2 years	27
III	Kugelberg–Welander disease	> 18 months	Proximal muscle weakness in early life; patients achieve ability to walk unaided	Normal life expectancy	12
IV	–	Adulthood	Proximal muscle weakness following active life during early adulthood; eventually may require walking aid	Normal life expectancy	1

infants, this results in a predominantly diaphragmatic form of breathing and a bell-shaped chest.

Our current understanding of SMA pathology stems mostly from human material acquired at autopsy. Accordingly, it is limited to observations made in the more severe forms of SMA at the very end of the disease. The pathology of mild SMA has relied primarily on muscle biopsies, while longitudinal studies to trace how SMA pathology evolves over the course of the disease derive mainly from findings in rodent models. Studies of human autopsy material revealed that the pathological hallmarks of SMA included loss of the motor neurons of the spinal cord and brainstem, and consequent atrophy of the skeletal muscles ([7] and references therein). Motor neurons that remained were sometimes mispositioned, often chromatolytic and swollen with infiltrations of phosphorylated neurofilaments [7, 8]. Consistent with motor neuron degeneration, ventral root axons were reduced in numbers [9]. In tissue from severely affected patients, abnormalities also extended to other neurons. Chromatolysis of dorsal root ganglion neurons, Clarke's column neurons and, cells of the various nuclei of the thalamus have all been reported [10–13]. However, considering the source of the tissue (i.e., autopsy material) it is unclear if these latter findings were a direct cause or secondary consequence of the SMA pathology. The pathology of SMA muscle is reflective of the denervation that follows motor neuron death or dysfunction [14]. Muscle from intermediate or mildly affected patients consists of a mix of atrophic and hypertrophic fibers. Moreover, fiber-type grouping, a consequence of repeated waves of denervation and reinnervation, is typical of such muscle. In limited cases, mild SMA muscle may also exhibit myopathic features, such as fiber splitting, central nuclei, basophilia, and an increase in endomysial connective tissue. Serum creatine kinase levels are mildly elevated as a laboratory correlate. In contrast, muscle from severely affected individuals consists of a sea of atrophic fibers within which are sometimes scattered islands of hypertrophic fibers [14]. Owing to the severity of the disease and the inability of motor neurons to recover functionally, muscle from severely affected patients is seldom reinnervated. This is reflected in the persistence of the normal checkerboard pattern of type I and II fibers. In rare instances, muscle fibers from patients with severe SMA may also appear immature and myotube-like, with central nuclei suggestive of a centro-nuclear myopathy [15].

Recent efforts to expand our understanding of SMA pathology have relied on animal models of the disease [16]. These have been remarkably illuminating, revealing, for instance, that motor neuron loss is preceded by distal axonal and neuromuscular synaptic defects [17–19]. Motor axons and nerve terminals were reported to become engorged with neurofilament protein, and the postsynaptic specializations failed to mature (Fig. 1). These observations, initially made in SMA mice, have now been confirmed in human patients and are

suggestive of a disease that begins as a synaptopathy [20]. Although SMA is a predominantly neuromuscular disorder, the most severe form These include the heart, vasculature, pancreas, bone, and liver [21]. Some of these systemic defects, which were initially reported in mouse models, were eventually confirmed in patients [22] and are likely rooted in the global disruption of SMN's housekeeping function of regulating gene splicing.

The Molecular Genetics of SMA

SMA has an incidence of approximately 1 in 10,000 newborns and a carrier frequency of approximately 1 in 50 [23]. It is caused by recessively inherited mutations in the survival of motor neuron 1 (*SMN1*) gene [24]. However, patients invariably retain one or more copies of an almost identical but poorly expressing homolog, *SMN2*, which lies within close proximity of *SMN1*. The chief difference between the 2 genes is a synonymous C to T transition 6 base pairs inside exon 7 of *SMN2* [25]. The nucleotide change creates an ESS that binds the splicing repressor, hnRNPA1, specifically in the copy gene [26]. As a consequence, *SMN2* exon 7 is mostly spliced out resulting in transcripts that commonly lack the exon [27]. *SMN2* does express low levels of the intact transcript, but the full-length (FL) isoform derives primarily from *SMN1*. Although the truncated *SMN2* transcripts lacking exon 7 are translated, the corresponding SMN 7 protein is unstable and degraded [28]. Therefore, *SMN2* produces only low levels of the functional SMN protein, a situation that eventually results in motor neuron death and the SMA phenotype (Fig. 2). Related studies revealed that the 5q locus harboring the SMN genes is uncommonly unstable [29]. Accordingly, humans can have multiple *SMN2* copies. The greater the number of *SMN2* genes in a patient, the higher the levels of functional SMN protein produced and the less severe the SMA phenotype [30–32]. Indeed, an increase in *SMN2* copy number is the most widely recognized explanation for the milder disease phenotypes.

The SMN Protein and its Role in SMA

The FL-SMN protein consists of 294 amino acids and migrates at approximately 38 kDa on denaturing gels. It is ubiquitously expressed, but appears to be present at especially high levels in spinal motor neurons [31, 33]. In these cells, as in most others, SMN partitions to both cytoplasm and nucleus, concentrating in the latter into foci that also stain positively for the Cajal body (CB) marker, coilin [34]. While their precise function remains unclear, the SMN foci, termed “gems”, for Gemini of CBs, likely serve as sites of RNA processing and in the recycling and/or biogenesis of splicing factors.

SMN is presumed to be involved in multiple biochemical pathways, a conclusion based on the myriad proteins it is reported to interact with. However, the most widely accepted function of the protein is its role in orchestrating the biogenesis of spliceosomal small nuclear ribonucleoprotein (snRNP) particles [35, 36]. In this regard, and as part of a greater complex, SMN has been shown to direct the assembly of the spliceosomal Sm proteins onto small nuclear RNAs (snRNAs). While Sm core assembly readily occurs, even in the absence of SMN [37, 38], the SMN complex serves as a specificity factor to ensure the nonpromiscuous assembly of only the appropriate Sm core proteins onto their target snRNAs [39]. Following modification of the snRNAs in the cytoplasm, the nascent SMN-bound snRNP combines with factors that facilitate the import of the entire complex into nuclear CBs/gems [40]. Here, SMN dissociates from the snRNP, which proceeds to engage in pre-mRNA splicing. Thus, while SMN does not directly engage in pre-mRNA splicing, low levels alter snRNP concentrations and thereby perturb splicing. Indeed, extracts from cells grown either *in vitro* or from animals expressing low SMN are clearly unable to assemble snRNPs as efficiently as extracts from wild-type cells [41]. Moreover, selective snRNPs—those that constitute the minor spliceosome—are most dramatically affected [41, 42]. However, determining precisely how such defects in snRNP assembly trigger a predominantly neuromuscular disease phenotype remains a central question and has prompted an intense search for disease-relevant genes mis-spliced in SMA. Examples that have emerged from recent work include *Nrxn 2*, a presynaptic organizer; *Uba1*, a protein involved in ubiquitin homeostasis; and *Tmem41b*, a protein whose function remains to be fully defined [43–45]. Each was found when perturbed to cause a motor axon phenotype in a zebrafish model. However, validation of the physiological relevance of these molecules in mammalian SMA models have either yet to be reported (*Tmem41b*, *Nrxn 2*) or were limited to modifying specific aspects of the overall SMA phenotype (*Uba1*). One interpretation of the latter finding is that downstream effectors of SMN alter selective characteristics of the larger SMA disease phenotype. Additional genes whose transcripts are mis-spliced owing to low SMN will no doubt be tested in future studies to determine the link between altered snRNP biogenesis and the SMA phenotype [46].

Whilst the role of SMN in regulating gene splicing may yet fully explain the SMA phenotype, a second school of thought linking the protein to selective motor neuron loss emerged following its localization to the axonal and growth cone compartments of cultured neurons [47]. In these cells, SMN was shown to occur in complexes that shared some of their constituents (Gemins 2 and 3) with the spliceosome but, intriguingly, were devoid of the core spliceosomal Sm proteins [47]. Accordingly, proponents of this school of thought have postulated that SMN, termed the “master assembler”

Fig. 1 Distal motor unit defects in spinal muscular atrophy (SMA). (A) Neuromuscular synapses in the diaphragms of a 10-year-old SMA patient and a 4-year-old control individual. Despite the greater age of the SMA patient, his neuromuscular junctions appear smaller, less complex in structure, and relatively immature as evidenced by persistent expression

of the fetal (γ) isoform of the acetylcholine receptor (AChR) (scale bar=30 μ m). (b) Distal axons of an SMA mouse and its control littermate depicting defects in the form of neurofilament-filled swellings in the former (arrows) (scale bar=5 μ m)

[48], is involved in the assembly of diverse RNP complexes, some of which may be important in the transport and localization of RNAs critical to the health of motor neurons. Studies in support of this theory have shown SMN to interact with, among others, HuD, a protein that binds the acetylcholinesterase transcript, hnRNP-R, which binds the actin mRNA and KH-type splicing regulatory protein (KSRP), which binds the p21 and GAP43 transcripts [49–52]. It is thought that by complexing with such RNPs, SMN regulates the transport of

the various transcripts into the axons and terminals of motor neurons in response to local or developmental cues. This motor neuron-centric view of the role of SMN in SMA has gained additional traction, albeit indirectly, from studies claiming not only to have uncoupled motor neuron dysfunction from snRNP biogenesis [53], but also to have provided a plausible explanation for the alleged disruption of the minor spliceosome in fly models of the disease [54]. The contrasting views of the critical role for the SMN protein in explaining the

Fig. 2 Genetic basis of spinal muscular atrophy (SMA). The cartoon depicts the two survival of motoneuron (SMN) genes and the proportions of the full-length (FL)-SMN and SMN Δ 7 transcripts/protein expressed

by them. *SMN1* but not *SMN2* is lost in SMA patients. Ex = exon; ESS = exonic splicing silencer

SMA phenotype clearly argue for additional careful work, and it is not unlikely that multiple pathways, including one in which low protein disrupts histone mRNA processing [55], all contribute, in varying measures, to the overall disease. Teasing out these multifarious pathways and their relative contributions to SMA may require novel model systems in which the timing of the investigation during the course of the disease will likely be critical to the success of the experiments.

SMA: Novel Insights From Model Mice

Although the human genome is not the only one to harbor multiple SMN genes [56], it is the only one to contain an *SMN2* gene. Moreover, commonly used model organisms possess just 1 copy of an SMN gene—an *SMN1* equivalent [57, 58]. Early work demonstrated that ablation of this gene results in an embryonic lethal phenotype [59]. However, this can be circumvented by introducing 1 or more genomic copies of the human *SMN2* gene onto the null *Smn*^{−/−} background [60, 61]. In mice, 2 copies of *SMN2* are sufficient to rescue the embryonic lethality associated with homozygous loss of murine *Smn*, but the resulting animals express very low levels of the protein and succumb to an SMA-like phenotype in the first week of life [60]. In contrast, increasing the number of *SMN2* copies to 8, not only rescues *Smn*^{−/−} embryonic lethality but also the SMA phenotype [60]. Expectedly, SMN levels in 8-copy *SMN2* *Smn*^{−/−} mice were restored to near normal. In the years since this early work was reported, the strategy of exploiting the human *SMN2* gene has been modified in subtle ways to generate a number of additional SMA model mice [62–66]. Their use, as described below, has not only led to novel insights into the cellular and molecular basis of the human disease, but has also served as a springboard for the design and development of promising therapies for the patient population.

Defining Where SMN is Required

An important determinant of successfully treating diseases caused by protein insufficiency lies in precisely defining where the protein is required in order to arrest or reverse the disease phenotype. Animal models of human disease lend themselves well to addressing such questions. Accordingly, attempts to define the cellular sites of action of the SMN protein followed shortly after the first SMA model mice were reported, and focused, not surprisingly, on motor neurons and muscle. Initial work in which SMN was purported to be selectively restored either neuronally or in muscle suggested that the latter was unlikely to be a significant contributor to the disease [67]. Caveats of the study included the specificity and timing of SMN repletion. Indeed, the initial conclusions made have since been refined by other investigations, suggesting not

only that restoring SMN in muscle early enough in its development, in committed progenitors, is beneficial, but also that motor neurons or, indeed, neurons in general are unlikely to be sole contributors to the SMA phenotype [68–71]. These findings have been bolstered, albeit indirectly, by reports of profound defects of the neuromuscular junctions (NMJs) in SMA mice that affect the pre- as well as postsynapse [16–18]. The precise contribution of muscle to the NMJ and, indeed, overall SMA phenotype remains unclear and may require a somewhat different strategy from those employed so far—one that involves selective SMN depletion in the tissue.

Whereas most of the early work to define the tissue-specific requirements of SMN centered on motor neurons and muscle, more recent studies have demonstrated that loss of protein, at least in severe SMA, also affects other organ systems ([72] and references therein). Cardiac defects were amongst the first non-neuromuscular abnormalities reported in severe SMA model mice. These have been extended to include multiple additional organ systems. However, as the observations were predominantly made in mice expressing ubiquitously low SMN, it is still not clear if they reflect primary or more incidental consequences of the initial pathology. Nevertheless, if preclinical studies of mice are predictive of future results in humans, the treatment of severe SMA will likely require augmenting SMN or its function(s) systemically [73].

Defining When SMN is Required

Equally important in successfully treating diseases caused by protein deficiency is determining how early or late during disease progression an intervention is required. This, in essence, involves defining the temporal requirements for the protein. In a mouse model of another pediatric neurological disease, Rett syndrome, repletion of MeCP2, the protein in question, was shown to confer significant benefit even after disease onset [74]. Conversely, depletion of protein in healthy adult mice eventually precipitated disease [75], suggesting that optimal levels of the protein are constitutively required to maintain neuronal health. In a mouse model of a much better known neurodegenerative disease, Huntington's chorea, suppression of mutant protein in fully symptomatic mice was also shown to reverse disease symptoms [76, 77]. Similar transgenic strategies have been remarkably informative in defining the temporal requirements for the SMN protein. Studies to determine how late SMN might be restored in severe SMA demonstrated that an incremental benefit accrues the earlier the protein is augmented [78, 79]. The extent of rescue appears to correlate tightly with the extent of damage at the NMJ. The greater the damage, the less likely the SMA mice recover from disease. Yet, these studies determined that arrest, indeed reversal, of disease is possible if SMN is restored early enough in symptomatic animals. A similar

outcome was obtained using virus-based strategies to restore SMN and in a conditional zebrafish model [80, 81].

SMA onset is observed early in life, suggesting that there is a greater requirement for SMN during early postnatal development. To investigate this question, SMN was depleted at various time points postnatally. Expectedly, depletion during the first 12 days of life rapidly led to an SMA-like phenotype and death [82]. However, depletion at PND15 or later had a relatively muted effect, with the preponderance of mutants surviving 6 months or more. Tellingly, this abrupt transition closely coincided not only with a steep decline in snRNP assembly activity that was noted in spinal cord tissue by two weeks of age [83] but also with the full maturation of the NMJ. Given the profound and early effect of low SMN on the NMJ, one conclusion from these results is that it is especially important for wild-type levels of the protein to be present during maturation of the neuromuscular synapse. Moreover, once the synapse matures, the tolerance for low SMN greatly increases. This line of thought is consistent with observations suggesting an enhanced requirement for motor neuronal SMN as NMJs remodel and mature following nerve injury [82]. From a clinical standpoint, the results suggest that, in contrast to Rett syndrome, constitutive high levels of the SMN protein may not be required in treating the disease unless there is widespread damage to the peripheral synapses during adult life. From a mechanistic standpoint, the observations point to events in the maturation of the pre- and/or postsynapse as key to the SMA phenotype. Interrogating changes in motor neurons and/or muscle during NMJ maturation in the presence or absence of SMN could be one strategic way of investigating this possibility.

Treating SMA

Current Options

Current treatment options for SMA remain palliative at best, and despite ongoing clinical trials of promising therapeutic agents (see below), clinicians mostly focus on treating secondary complications arising from the disease. In severely affected SMA patients, respiratory failure has historically been the most common cause of mortality. While this is still the case, aggressive intervention using assisted ventilation protocols have dramatically enhanced the survival of patients with severe SMA. Indeed, patients born between 1995 and 2006 were reported to exhibit a 70 % reduced risk of death relative to those born between 1980 and 1994 when followed over an approximately 50-month period [84]. Additional complications in infants at the more severe end of the disease spectrum include the propensity to tire when feeding. In many instances, this eventually leads to the placement of gastrostomy tubes within the infant to ensure proper sustenance. Clinicians also frequently treat gastrointestinal issues such as dysphagia

and constipation. In patients with intermediate SMA, complications that are orthopedic in nature are common. Scoliosis is almost inevitable, requiring surgical intervention to support the spine. In patients with mild SMA, falls and fractures associated with fatigue and gait abnormalities are commonly reported and require the incorporation of preventive strategies into patient management plans [85]. Efforts to more directly address the cause of SMA—low SMN—mostly focused on histone deacetylase (HDAC) inhibitors, a class of compounds known to have a general effect on the expression of subset of genes [86]. Examples of such agents that were entered into clinical trials for SMA include hydroxyurea, phenylbutyrate, and valproic acid [87–90]. However, the outcomes were disappointing, as neither SMN levels nor functional outcome were enhanced in the type II and III patients studied.

Emerging Treatments

Careful laboratory research during the last decade has led to a much greater appreciation of the mechanisms governing SMN gene expression and, consequently, the cause of low SMN protein in SMA. This understanding and proof-of-concept experiments attesting to the feasibility of modulating *SMN2* gene expression have, not surprisingly, focused considerable attention on the gene as a therapeutic target. Accordingly, much effort has been expended on enhancing *SMN2* gene expression either transcriptionally or by modulating its splicing. In combination with cell and gene replacement approaches, the pharmacologic strategies are beginning to yield what may be the first truly promising treatments for SMA. We expand further on these approaches below.

Pharmacologic Agents

The pharmacologic approaches to treating SMA have largely focused on the *SMN2* gene. Given the fact that that it is invariably present in SMA patients, that its full-length protein product is identical to that of *SMN1*, and that this protein, if expressed from the gene at sufficient levels, is capable of fully rescuing the disease phenotype in model mice, *SMN2* is an intuitive choice of therapeutic targets for SMA. Two primary classes of pharmacologic agents have emerged from cell culture and animal studies as modulators of *SMN2* expression. The first, histone deacetylase inhibitors are reported to transcriptionally activate the gene, resulting in increased levels of each of the SMN2 RNA isoforms. However, only a handful of the inhibitors effectively increased SMN *in vivo*. Noteworthy examples include trichostatin A and the hydroxamic acid, LBH589 [91, 92].

In combination with the proteasome inhibitor, bortezomib, trichostatin A also significantly enhanced survival of severely affected SMA mice [93]. A second group of compounds that has received much attention include those shown to alter *SMN2* splicing. Amongst the most promising of these is a

proprietary molecule identified at PTC Therapeutics, Inc., which alters *SMN2* splicing to increase levels of the FL-SMN transcript. Accordingly, it enhances SMN protein and rescues the SMA phenotype of severely affected model mice [94]. This compound is currently being optimized, and is expected to enter phase 1 trials shortly. An additional noteworthy, quinazoline compound that targets the DcpS enzyme but whose mode of action in increasing SMN is not clear has also been deemed promising enough to enter clinical trials [95]. One or more of these small molecule-type drugs will likely constitute the next generation of therapeutic agents for the treatment of SMA. While small molecule-type drugs often have the distinct advantage of freely traversing the blood–brain barrier (BBB), they also present the potential for off-target effects. This concern combined with new information about the mechanisms regulating *SMN2* splicing has spurred the development of a new group of pharmacologic agents—antisense oligonucleotides (ASOs)—in the treatment of SMA. Blocking one particular intronic element, ISS-N1, in *SMN2* with an ASO was found to be as effective in mitigating the SMA phenotype as the most potent small molecules [96, 97]. Moreover, in mice, the ASO was found to be long-lasting, with persistent effects on *SMN2* splicing 6 months after initial administration [98]. This agent, which is being developed by Isis Inc., is currently in phase 2 clinical trials and, based on early promising results, is expected to advance still further. One caveat of ASOs as therapeutic agents is their inability to penetrate the BBB. Their utility in treating neurodegenerative diseases such as SMA therefore requires intrathecal administration, often repeatedly.

Pharmacologic agents that exploit a third strategy, the stabilization of the SMN 7 protein by forcing transcriptional read-through of the truncated transcript, include aminoglycosides. One such compound, TC007, was found to increase SMN gems in cells in culture and also to enhance total spinal cord SMN protein in mice [99]. However, its effect on the SMA phenotype of model mice was modest at best, and it is unlikely that such compounds hold sufficient promise to advance to clinical trials for SMA. Pharmacologic agents whose therapeutic action is independent of SMN expression have also received attention [100, 101]. Olesoxime, a neuroprotective agent reported to target the mitochondrial permeability pore is emerging in European clinical trials as one promising candidate in the treatment of SMA [100].

Gene and Cell Replacement Protocols

Although pharmacologic approaches to treating SMA have gained the most attention, gene therapy for the disease also appears promising. In part, this is a consequence of the identification of new serotypes, for example adenoassociated virus (AAV)9, of the adenoassociated virus which are reported to efficiently penetrate BBB of neonatal mice and infect multiple

cell types [102]. Such a vector harboring *SMN1* infected not only neurons, but also peripheral tissue, dramatically rescuing the SMA phenotype in severely affected model mice [80, 103, 104]. The promise of this strategy in preclinical murine models has prompted the development of protocols that can be applied to larger models and eventually SMA patients. Caveats of this strategy include questions about the ability of AAV9 vectors to traverse the BBB of human infants, and the design of the construct, which is intended to ensure that the therapeutic gene remains episomal. The inability of the vector genome to integrate into the chromosomes of the cell means that the replacement gene will eventually be lost in dividing cells.

The ability to differentiate embryonic stem cells into neuronal lineages has spurred interest in cell replacement-type strategies for the treatment of neurodegenerative disorders [105]. Such a strategy has been employed in murine models of SMA [106]. However, it is unlikely that the modest benefit the animals derived was a result of reinnervation of muscle. Indeed, fewer than 2 % of the transplanted cells eventually established themselves within the parenchyma of the spinal cord and expressed the motor neuronal marker Hb9::green fluorescent protein. Fewer still extended axons into the periphery.

These results suggest that the benefit to the SMA animals accrued through trophic support supplied by the transplanted cells rather than from improved motor connectivity. Nevertheless, cell replacement continues to be pursued as a potential therapeutic avenue for the treatment of neurodegenerative diseases and may complement future pharmacologic therapies for SMA.

Perspectives on the Future of SMA Research

Progress within the SMA field since the SMN genes were identified has been rapid. This is particularly true from a clinical perspective and is, in no small measure, a consequence of the unique and fascinating genetics of SMA. Mainly, it has involved the autosomal recessive nature of the disease, the near-complete identity between the two SMN genes, the invariable presence of *SMN2* in patients, and the proven potential of this gene as a viable therapeutic target. Therapeutic targets have therefore focused predominantly on restoring the SMN protein. This approach does not require a thorough understanding of the molecular function(s) of the protein. The ability to diagnose rapidly the disease, and a basic appreciation of the cellular and temporal requirements of the protein are sufficient to effect SMN-based therapies. Yet, most would agree that a precise understanding of how low SMN causes the various types of SMA would surely enable clinicians to refine current and emerging treatments. Accordingly, we advocate that basic investigations into the molecular basis of SMA continue unabated, and indeed be expanded. We would not be surprised if this effort eventually begins to impact motor neuron diseases more broadly. Indeed, the

intersection of SMA and another common motor neuron disease, amyotrophic lateral sclerosis has already been reported [107–109]. Accordingly, we pose the following questions which we believe could define future SMA research. Does impaired snRNP assembly truly lie at the root of the SMA phenotype? If so, are there specific mediators of the motor neuron pathology of the disease? Might these be more efficiently uncovered by narrowing our analyses to specific tissues at specific points during the course of the disease? Alternatively, could one take advantage of “discordant” sibs, patients with identical SMA haplotypes but distinct disease phenotypes to address this question? If a disruption in the canonical function of SMN truly lies at the heart of the SMA phenotype, it might be naïve to expect that just 1 or 2 distinct molecules are responsible for the overall pathology of the disease. By extension, the search for mediators of the motor neuron-specific phenotype of SMA might best be focused on less severe forms of the disease wherein perturbations of additional, more general SMN targets do not confound the analyses. We do not expect the present paucity of answers to the above questions to hinder the progress of promising emergent SMN-restoring therapies. However, we firmly believe that the information obtained in the process of answering them will serve as the basis of the next generation of SMA treatments.

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