Review

Emerging concepts underlying selective neuromuscular dysfunction in infantile-onset spinal muscular atrophy

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Abstract

Infantile-onset spinal muscular atrophy is the quintessential example of a disorder characterized by a predominantly neurodegenerative phenotype that nevertheless stems from perturbations in a housekeeping protein. Resulting from low levels of the Survival of Motor Neuron (SMN) protein, spinal muscular atrophy manifests mainly as a lower motor neuron disease. Why this is so and whether other cell types contribute to the classic spinal muscular atrophy phenotype continue to be the subject of intense investigation and are only now gaining appreciation. Yet, what is emerging is sometimes as puzzling as it is instructive, arguing for a careful re-examination of recent study outcomes, raising questions about established dogma in the field and making the case for a greater focus on milder spinal muscular atrophy models as tools to identify key mechanisms driving selective neuromuscular dysfunction in the disease. This review examines the evidence for novel molecular and cellular mechanisms that have recently been implicated in spinal muscular atrophy, highlights breakthroughs, points out caveats and poses questions that ought to serve as the basis of new investigations to better understand and treat this and other more common neurodegenerative disorders.

Key Words: motor neuron; neurodegeneration; neuromuscular; spinal muscular atrophy; splicing

Introduction

Selective loss of neurons owing to perturbations in housekeeping proteins is a puzzling aspect of many neurodegenerative disorders and the focus of burgeoning research. One reason for the continued interest in studying selective neuronal vulnerability in common diseases is the growing prevalence of neurodegenerative conditions affecting the aging individual. Alzheimer's and Parkinson's diseases are the most prominent examples. Yet, despite progress in identifying genetic factors underlying these two diseases, investigating selective neuronal loss and dysfunction in them is confounded by their largely sporadic incidence and multifactorial nature, and symptoms that often appear only after decades of life. This poses challenges for the creation of appropriate models of the human conditions; rodent models of these diseases often rely on an over-expression of mutant proteins to hasten the appearance of a phenotype (Francardo, 2018; Myers and McGonigle, 2019). One solution to these various challenges is to study common causes of neurodegeneration in simpler disease paradigms. Infantile-onset spinal muscular atrophy (SMA) is the prototypical example. Caused by homozygous mutations in the survival of motor neuron 1 (*SMN1*) gene, invariable presence of a hypomorphic paralogue, *SMN2*, and consequent low levels of their translated product (Lefebvre et al., 1995; Coovert et al., 1997; Lefebvre et al., 1997), the SMN protein, the disease provides a convenient means of investigating why neurons in particular are affected by dysfunction of essential proteins. Advantages of investigating common mechanisms underlying neurodegeneration in SMA instead of in more pervasive, multifactorial neurodegenerative disorders include the monogenic nature of the disease, a phenotype commensurate with the study of selective neuronal vulnerability and, perhaps most importantly, an excellent set of model mice that faithfully recapitulate the signature features of the human disease in a rapid and reproducible manner. Here we review how a careful analysis of the various SMA models has begun to cast light on the decidedly neuromuscular SMA phenotype. We examine the evidence that has been presented in recent studies to challenge existing views about the cellular basis of SMA, highlight research attempting to reveal novel molecular mechanisms underlying spinal motor neuron loss and conclude by identifying new challenges and emphasizing current ones in the quest to understand and effectively treat all patients afflicted with the disease.

Search Strategy and Selection Criteria

Studies cited in this review published from 1995 to 2020 were searched on the PubMed database using the following keywords: survival motor neuron, spinal muscular atrophy, neurodegeneration, splicing, neuromuscular junctions, axons and combinations thereof. The results were further screened by title and abstract to include only studies that are of relevance to our review.

The Survival of Motor Neuron (SMN) protein, p53 Nexus in Spinal Muscular Atrophy

Proximal muscles are affected to a greater extent than are distal muscles in patients and model mice with 5q SMA

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(Monani and De Vivo, 2014). Consistent with these findings, spinal motor neurons innervating proximal and axial rather than distal muscles were found to be preferentially lost in severe models of SMA (Murray et al., 2015). In an attempt to explain the selective vulnerability of proximally innervating motor neurons, gene expression profiles of vulnerable and resistant motor neurons were compared (Murray, et al., 2015; Simon et al., 2017). Amongst the most intriguing genes to emerge from this analysis were transcriptional targets of the cell-cycle regulator and death effector, p53. These genes, which include *Cdkn1a* and the TP53 apoptosis effector, *Perp*, were specifically upregulated in vulnerable motor neurons. Not surprisingly, robust p53 activation was also observed early in the course of the disease in these motor neurons, eventually becoming evident in other neurons as well as peripheral tissues (Simon et al., 2017). Blocking p53 activation or preventing phosphorylation of its transactivation domain either pharmacologically or with AAV9 constructs failed to arrest overt disease, deafferentiation of Ia synapses on motor neurons or denervation but was reported to preserve vulnerable motor neurons and significantly improve sensory-motor circuit function as assessed by measuring spinal reflexes; oddly, selectively restoring SMN to motor neurons failed to similarly enhance sensory-motor circuit activity in a parallel study conducted by the authors (Fletcher et al., 2017) raising questions about what was reported for p53. Moreover, inducing p53 activation and phosphorylation of its transactivation domain failed to trigger motor neuron death in wild-type mice suggesting that these events must occur in concert with yet-to-be defined alterations to cause overall neurodegeneration in SMA (Simon et al., 2017). Still, and notwithstanding an independent study that did not find any mitigating effect of reduced p53 on SMA motor neuron loss (Courtney et al., 2019) , the authors concluded that increased p53 signaling is an important driver of the neurodegenerative phenotype.

p53 activation as a prelude to neurodegeneration is by no means unique to SMA, having been observed in myriad chronic neurodegenerative disorders (Culmsee and Mattson, 2005; Ranganathan and Bowser, 2010; Qi et al., 2016). Moreover, an increase in its signaling activity can be triggered in a variety of ways including cell stress and DNA damage (Marcel et al., 2015). Still, in an attempt to link p53 activation and selective neuronal loss in SMA to the canonical function of SMN in splicing, a study examining the effects of low SMN on the p53 repressors Mdm2 and Mdm4 was conducted (Van Alstyne et al., 2018). In an elegant series of experiments, these negative regulators of p53 were shown to be mis-spliced in SMA model mice. Forced mis-splicing of the *Mdm2* and *Mdm4* genes in a synergistic manner was sufficient to activate p53 in the motor neurons of wild-type mice. Conversely, AAV9-mediated restoration of the two genes to SMA mice suppressed p53 activation and, importantly, arrested motor neuron loss. However, consistent with the outcome of experiments in which p53 activation was directly blocked (Simon et al., 2017), restoring Mdm2 and Mdm4 to SMA mice failed to prevent denervation, deafferentiation or mitigate overt disease.

The minor spliceosome is reported to be especially vulnerable to low SMN (Gabanella et al., 2007) but is not thought to be responsible for the splicing of *Mdm2/4* (Van Alstyne et al., 2018). Mis-splicing of genes subject to minor spliceosome post-transcriptional processing has, however, been observed (Doktor et al., 2017) in SMA models. Amongst these, *Stasimon* (*Tmem41b*), which codes for an ER-resident transmembrane protein, has received special attention for its alleged role as a downstream effector of motor circuit dysfunction in SMA. To infer this directly, the correctly spliced form of *Stasimon* was restored to motor neurons of SMA model mice with an AAV9 vector. *Stasimon* repletion was indeed found to normalize

spinal reflexes, improve but not fully rescue sensory-motor connectivity – as assessed by vGlut1-positive puncta on motor neuron soma – and modestly correct impaired motor behavior (Simon et al., 2019).

Assuming that Stasimon is a downstream mediator of SMN, one might expect mitigation of disease severity resulting from its repletion to be independent of changes in SMN expression. Intriguingly, however, when the investigators measured SMN levels in the spinal cords of Stasimon-augmented mice, one experiment revealed that fully half of the mutants had a *gain* in SMN expression. Moreover, it was this sub-population of mutants that exhibited improved sensory-motor function – based on an increase in spinal reflexes. Notwithstanding failure to extend lifespan or improve muscle innervation in Stasimon-restored SMA mice, one interpretation of the effect of the protein on motor neuron loss and function is that it is merely a by-product of increased SMN rather than a direct effect of Stasimon. This view also has implications for assertions centering on selective motor neuron loss in SMA. Such loss is reported to involve p53 phosphorylation, a process that was blocked by Stasimon via p38 MAPK (Simon et al., 2019). Considering the observed ability of *Stasimon* to increase SMN, might p53 phosphorylation in vulnerable SMA motor neurons be a product of other yet-to-be identified factors rather than a direct effect of mis-spliced *Stasimon*? Assuming, however, that Stasimon is not just a by-stander and does in fact mediate selective motor neuron loss in SMA through p53 phosphorylation, one is still left wondering why it is especially prone to mis-splice in vulnerable motor neurons. One suggestion is an especially low level of snRNPs and/or SMN in these motor neurons, a view previously proposed and consistent with the heterogeneity of SMN in cultured motor neurons (Rodriguez-Muela et al., 2017). However, establishment of this awaits empirical evidence of SMN levels not just in vulnerable versus resistant motor neurons but also between vulnerable motor neurons; it is clear that even amongst this latter pool of motor neurons, only a proportion perish. Demonstrating that vulnerable motor neurons are prone to expressing low SMN will surely be instructive but will then require an explanation for precisely what makes them intrinsically low-expressors of the protein.

Despite questions raised here, the involvement of p53 activity in the severe form of SMA is clear (Simon et al., 2017; Courtney et al., 2019) and its stabilization and activation via degradation of short-lived, alternatively spliced *Mdm2/4* gene products consistent with outcomes from non-SMA related studies (Perry et al., 2000; Giglio et al., 2010; Bardot et al., 2015). What remains unresolved is if and to what extent this pathway and its activation via SMN's canonical role in splicing has anything to do with motor neuron loss in intermediate and milder forms of SMA. Mild SMA patients and model mice express a two to four-fold increase in SMN over levels observed in the severe form of the disease (Coovert et al., 1997; Monani et al., 2003). Yet, proximal muscle atrophy and motor neuron loss remain signature features of mild SMA. Might neurodegeneration in this form of SMA also involve p53 activation? If so, might the activation also stem from mis-splicing? Such a view is proposed in a study attempting to link the role of SMN in minor spliceosome assembly to the neurodegenerative phenotype by examining and then augmenting reduced levels of the minor snRNAs to purported "mild" SMA model mice (Hammond et al., 2010; Osman et al., 2019). Interestingly, mutant mice treated with AAV9 vectors harboring the minor snRNAs were modestly rescued but, oddly, without any detectable increase in the snRNAs, and absent any mitigation of motor neuron loss. The latter observation is particularly puzzling considering the proposition that Stasimon, whose restoration preserves motor neurons, is dependent on the minor spliceosome for proper splicing (Simon et al., 2019). Repeating these and other experiments in

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bona fide mild models of SMA (Monani et al., 2003; Osborne et al., 2012; Bogdanik, et al., 2015; Deguise et al., 2020) will be instructive in examining the true extent to which p53 activation stemming from mis-spliced SMN targets underlies the selective motor neuron loss common to all forms of SMA.

An equally informative set of studies that could shed light on the contribution of mis-splicing to SMA involves intragenic complementation experiments. Recent work has demonstrated that while single SMN missense mutations are not competent in snRNP assembly or in rescuing the SMA phenotype, combinations of two different mutant alleles with lesions in distinct domains not only restore snRNP assembly but also mitigate disease severity (Blatnik et al., 2020; McGovern et al., 2020). Thus, for instance, while SMN1A111G and SMN1T274I, two mild SMA mutations, are independently unable to prevent cell death or embryonic lethality in the absence of FL-SMN (full-length SMN) from *SMN2*, intragenic complementation involving one each of the two mutant alleles restores competency in snRNP assembly and rescues the SMA phenotype. Such complementation occurs without need for wild-type FL-SMN. One inference from these studies is that SMN's canonical function in RNA splicing is what ultimately drives the SMA phenotype. However, it is worth noting that other combinations of missense mutations such as SMN1A111G and SMN1A2G are just as competent in snRNP assembly assays (Blatnik et al., 2020), yet may not rescue the SMA phenotype. This suggests a dissociation between SMN's canonical role in splicing and the neuromuscular SMA phenotype. Examining the relationship between snRNP assembly and SMA through intragenic complementation studies similar to those conducted by McGovern et al. (2020) could shed greater light on critical SMN functions in SMA.

Spinal Muscular Atrophy and the mRNP Connection

Despite the varying degrees to which motor neuron loss and motor behavior are attenuated by restoring SMN-dependent splicing abnormalities and preventing p53 activation, denervation in the SMA mice proceeds unimpeded and animals rapidly succumb to disease. Clearly then, blocking p53 activation and/or restoring levels of the minor spliceosome are insufficient to confer more than a modest degree of protection from low SMN. As acknowledged in the literature, this implies other, disease-relevant SMN functions. One appealing possibility stemming from a number of studies initiated as early as 2003 (McWhorter et al., 2003; Rossoll et al., 2003; Tisdale et al., 2013) is a role for SMN in assembling other RNP complexes including those consisting of mRNAs whose transport, localization and expression in axons and growth cones is vital to overall neuronal health. Indeed, SMN has been shown over the years to interact with a number of mRBPs including HuD and ZBP1, proteins critically important in properly localizing GAP43 and β-actin transcripts respectively to axonal growth cones (Akten et al., 2011; Fallini et al., 2011, 2014, 2016; Hubers et al., 2011). Low SMN via reduced interaction with HuD and ZBP1 not only impairs the axonal localization of these specific transcripts but also, more generally, affects the compartmentalization of the larger population of poly (A) mRNAs that interact with it via the PABC1 protein. Overall, this results in the assembly of fewer and smaller mRNP granules under SMA conditions and, importantly, reduced association of the granules with molecular motors involved in axonal transport of locally regulated transcripts and their protein products (Donlin-Asp et al., 2017). Thus, SMN plays an important role as a chaperone of mRNPs, a function that may have an equal if not even more profound effect on the neuromuscular SMA phenotype than its canonical function in orchestrating the splicing cascade.

The Survival of Motor Neuron (SMN) Protein at the Neuromuscular Junction

Although one school of thought argues for a pre-eminent role for SMN in maintaining sensory-motor connectivity within the spinal cord, suggesting that deafferentiation of motor neurons must be the initiating event driving neurodegeneration in SMA, early disturbances at the distal motor unit – the neuromuscular junction (NMJ)– cannot be ignored. Indeed, NMJ defects in SMA (**Figure 1**) have been widely reported across the disease spectrum and observed as early as E18.5 in model mice (Kariya et al., 2008, 2009; Murray et al., 2008; Kong et al., 2009; Ling et al., 2010; Ruiz et al., 2014); deafferentiation has not been reported in mild SMA. Moreover, a careful analysis of the timing of the appearance of motor unit defects in three different mouse models of severe SMA concluded that defects at the NMJ precede both deafferentiation as well as motor neuron cell body loss (Buttner, 2020 unpublished results). Finally, controlled depletion of SMN during adulthood promptly triggers NMJ defects, but without any accompanying evidence of motor neuron defects or loss in the spinal cord (Kariya et al., 2014). These and other investigations have long-argued for a role for the SMN protein in both the early postnatal development and later maintenance of the NMJ. One set of results that supports this proposition stems from a revealing series of investigations into the causes of discordant phenotypes in sibs with identical 5q haplotypes (Oprea et al., 2007; Hosseinibarkooie et al., 2016; Reissland et al., 2017; Janzen et al., 2018). The outcomes of these studies make a compelling case for a role for the SMN protein in ensuring proper neurotransmission at the NMJ by regulating the process of endocytosis. Indeed, in each case, modifying factors that were identified were shown to rescue defective endocytosis in SMA cells or model organisms. Notably, restoring normal expression or augmenting the modifiers in SMA model mice as a means of restoring endocytosis not only reversed distal defects of the motor unit but also prevented deafferentiation and rescued the overt SMA phenotype. Importantly, such phenotypic rescue reportedly was not accompanied by any gain in SMN expression. Although these and similar studies (Dimitriadi et al., 2016) establish an exciting disease-relevant basis to the neuromuscular SMA phenotype, it remains unclear precisely how low SMN disrupts endocytosis or compromises the fidelity of neurotransmission at NMJs. One common thread may derive from the modulation of the various modifiers by Ca^{2+} entering the nerve terminal during neurotransmission. Ca^{2+} homeostasis is reportedly disrupted in SMA (Ruiz et al., 2010), perhaps as a consequence of mis-localized $CaV_{2.2}$ channels (Jablonka et al., 2007). This in turn would be expected to disrupt neurotransmission (Sudhof, 2012), an outcome possibly rectified in SMA by altering the expression of the modifiers, their Ca^{2+}-dependent interactions with key endocytic regulators and, consequently, endocytosis itself. Whether any of these pathways rely on SMN's canonical function in splicing is not certain. However, precedence for NMJ defects in SMA deriving from splicing defects has been established (Zhang et al., 2013; Kim et al., 2017). These studies linked aberrant splicing of the NMJ organizer, Agrin, to the characteristic developmental arrest of the post-synaptic acetylcholine receptor (AChR) clusters in SMA model mice. Significantly, genetic or pharmacologic repletion of the reduced Z^+ Agrin isoform to model mice mitigated disease (Kim et al., 2017; Boido et al., 2018), but without preventing neurodegeneration. Still, the incomplete rescue in this and other studies involving the modulation of individual disease mediators is consistent, at least in the limited context of severe SMA, with a diverse set of genes and pathways being affected by very low SMN. Identifying which of these is specific to selective motor neuron loss and neuromuscular dysfunction in SMA will require a greater focus on models of mild SMA in which disruptions originating from SMN's housekeeping function(s) prove less confounding to the overall analysis.

Cellular Basis of Motor Unit Dysfunction in Spinal Muscular Atrophy

Sensory and motor neurons

SMA is traditionally described as a motor neuron disease. However, recent findings have prompted some in the field to challenge the received wisdom and define it more as a disorder of the motor circuit (Imlach et al., 2012; Tisdale and Pellizzoni, 2015; Fletcher et al., 2017). Yet the distinction is superfluous, even specious, considering the fact that the motor neuron does not exist in isolation but rather constitutes part of the larger motor unit. Other cells comprising the unit, especially those directly synapsing with diseased motor neurons, would not therefore be expected to remain morphologically normal. Nevertheless, attributing findings of diminished proprioceptive neuron synapses on motor neurons and motor neuron hyper-excitability to a cell-autonomous effect of SMN deficiency in sensory neurons is, in our minds, an over interpretation and disregards the results of numerous other studies (Park et al., 2011; Gogliotti et al., 2012; Martinez et al., 2012; Thirumalai et al., 2013; Arumugam and Tabares, 2017). The earliest of these investigations assessed the relative contribution of motor neurons to the overall SMA phenotype by generating model mice selectively depleted of SMN in these cells (Park et al., 2010). Although the mutants were shown to exhibit a relatively mild phenotype, they were nevertheless also found to suffer significant motor neuron loss and severe deafferentiation. Importantly, proprioceptive neurons in the mutants continued to express normal levels of the SMN protein, suggesting that deafferentiation of motor neurons and indeed their eventual loss in SMA is triggered from within motor neurons. Two subsequent studies employing distinct models corroborated this claim. Each selectively restored SMN to motor neurons of SMA mice to assess effect. Consistent with the report of Park et al (2010), motor neuron cell bodies were preserved and, importantly, Ia afferents on motor neurons fully restored (Gogliotti, et al., 2012; Martinez et al., 2012) – notwithstanding modest overall phenotypic rescue. Interestingly, inadvertent use in one of the studies (Martinez et al., 2012) of a leaky Myf5-Cre driver which robustly and ectopically restored SMN to DRG neurons of SMA mice did nothing to prevent deafferentiation, once again suggesting that sensory-motor dysfunction and deafferentiation originates in and is primarily dependent on events within motor rather than proprioceptive sensory neurons. In this vein, it is instructive to emphasize two additional studies which, respectively, concluded that motor neuron hyper-excitability and deafferentiation are most likely consequences of motor neuronal rather than proprioceptive sensory neuron abnormalities (Thirumalai et al., 2013; Arumugam and Tabares, 2017). The first (Arumugam and Tabares, 2017) showed that cultured motor neuron-like cells expressing low SMN were hyper-excitable even in the absence of sensory neuron synaptic input, a result consistent with that of an *in vivo* study (Gogliotti et al., 2012) demonstrating that selectively restoring SMN to motor neurons of SMA mice rescued the hyper-excitable phenotype. The second by Thirumalai et al. (2013) hypothesized that if low SMN truly affected proprioceptive sensory neurons in a cell-autonomous fashion, reduced Ia boutons would not merely be a characteristic of motor neurons but extend to other connecting neurons as well. Accordingly, calbindin-positive Renshaw cells which receive direct monosynaptic inputs from muscle spindles were examined. vGlut1-positive boutons on these cells were reported to be no less abundant in SMA mice than in control littermates – even at the end stage of disease. In light of these collective findings, the results of Fletcher et al. (2017), which are based on selective repletion of SMN to proprioceptive neurons, is curious and hard to reconcile with the consensus. One way of potentially reconciling the discrepant data would be to selectively deplete SMN in proprioceptive sensory neurons of wild-type mice and assess effect. If these cells contribute in any significant manner to the overall SMA phenotype, one would not only predict deafferentiation of motor neurons but also an overt phenotype. Even if, as suggested by most studies, sensory-motor connectivity defects originate in motor neurons, additional work will be required to identify mediators of the deafferentiation phenotype. Clearly, a number of factors including *Stasimon*, *Uba1*, *Gars*, *Pls3* and *Ncald* have been implicated in this phenotype (Hossenibarkooie et al., 2016; Reissland et al., 2017; Shorrock et al., 2018; Simon et al., 2019). Which, if any, of these is principally involved is yet to be resolved.
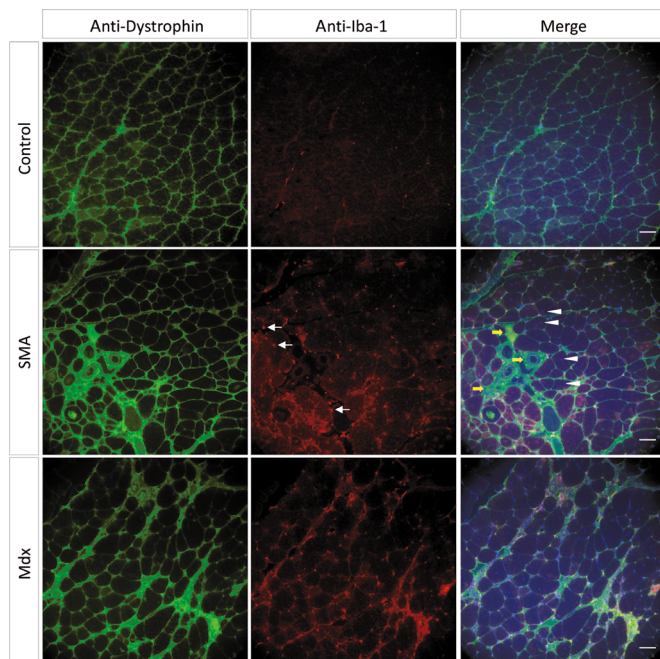
Muscle

Considering the neuromuscular SMA phenotype, the role of muscle in contributing to the SMA had always been suspected. However, studies to directly assess if and how low SMN in muscle triggers disease arrived at disparate conclusions (Cifuentes-Diaz et al., 2001; Guettier-Sigrist et al, 2002; Gavrilina et al., 2008; Boyer et al., 2013; Bricceno et al., 2014; Iyer et al., 2015; Ripolone et al., 2015). Still, ascertaining the effect of low SMN in muscle or indeed any cell-type is of paramount importance not only from a mechanistic standpoint but also from a clinical perspective; SMN repletion is now an approved means of treating SMA and continues, in the main, to be effected largely to the CNS by means of a splice-switching oligonucleotide (Kim and Monani, 2018). To directly assess the *in vivo* effect of low SMN in muscle, selective paucity of the protein was engineered in muscle progenitors of model mice expressing one or two human *SMN2* copies (Kim et al., 2020). Mutants thus generated and expressing one copy of the *SMN2* gene rapidly developed a disease phenotype characterized by reduced body size, poor movement and a lifespan that rarely exceeded two weeks. In contrast, mutants harboring two *SMN2* copies initially appeared asymptomatic. However, by 6 months of age overt disease as well as cellular pathology was readily apparent. Behaviorally, the animals exhibited muscle weakness and reduced survival. This was accompanied by profound pathology of the myofibers (**Figure 2**), morphological and functional abnormalities of the NMJs, and impaired muscle contractility in the form of a significant reduction in peak specific force generated during *ex vivo* experiments (Kim et al., 2020). A notable outcome of the overall study was the ability to at least partly mitigate disease by restoring SMN to symptomatic animals. Although detailed molecular pathways linking low SMN in muscle to pathology and overall disease are yet to be defined, individual factors dysregulated in SMA muscle have been identified (Mutsaers et al., 2013; Rehorst et al., 2019). Determining how these factors are perturbed and if it involves disruptions in splicing remains an area ripe for investigation. Still, the basic observation of a cell-autonomous role for skeletal muscle in triggering a disease phenotype is especially relevant in ensuring the most effective outcomes in current SMN repletion therapies.
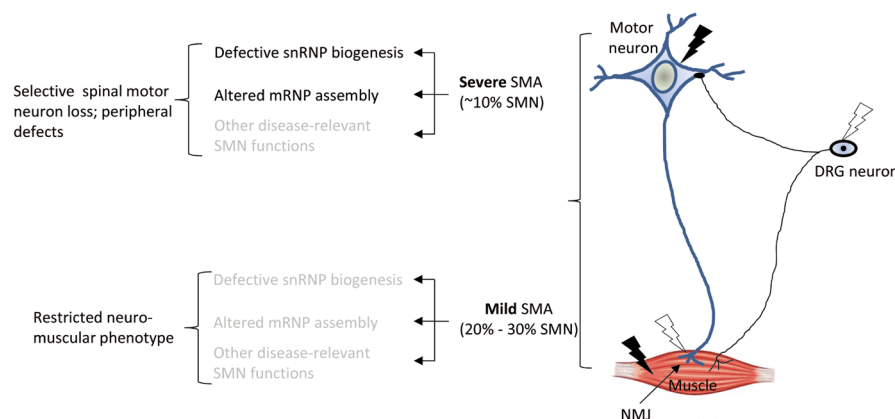
Peripheral tissue pathology in SMA

Despite the recognition that SMA predominantly affects the motor neurons, it is increasingly evident that low SMN also has systemic effects (Hamilton and Gillingwater, 2013; Nash et al., 2016). Peripheral organs and cells reportedly affected in the disease include the heart, pancreas, liver, kidney, bone and immune cells (Heier et al., 2010; Bowerman et al., 2012; Deguise et al., 2017; Maxwell et al., 2018; Deguise et al., 2019; Nery et al., 2019; Allardyce et al., 2020; Hensel et al., 2020). However, these defects have almost exclusively been identified in studies of the most severe (type 1) form of SMA in which SMN levels are profoundly (80–90%) depleted. Given its indispensable role in splicing, the adverse systemic effects of very low SMN are not surprising. Yet, while the systemic pathology has clinical implications, it also obscures and confounds attempts to investigate mechanisms underlying the neuromuscular aspects of the SMA phenotype. One solution

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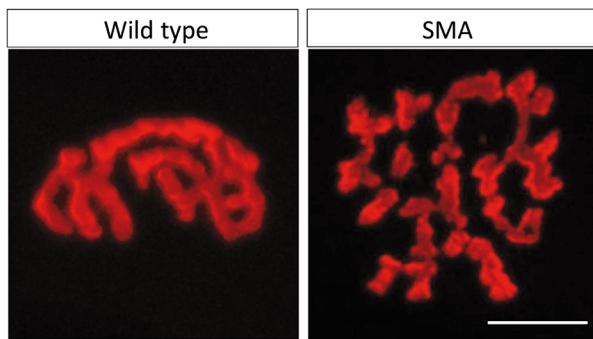
Figure 1 | Representative NMJs in wild-type and SMA mutant mice.
Acetylcholine receptor clusters from adult mice stained with labeled alpha-bungarotoxin depicting the characteristic fragmentation of the clusters in SMA mutant. Scale bar: 50 μm. NMJs: Neuromuscular junctions; SMA: spinal muscular atrophy. Sourced from the authors' laboratory.

Figure 2 | Muscle-autonomous defects in SMA.
Photomicrographs of muscle from adult SMA and control mice sectioned in the transverse plane. Selective depletion of SMN in skeletal muscle causes widespread pathology of the myofibers including centralized nuclei (arrowheads), dystrophic fibers (yellow arrows) and infiltration by inflammatory microglia evidenced by Iba1-positive puncta (white arrows). Such pathology is absent in wild-type controls but evident in muscle from mdx mutants, a model of muscular dystrophy. Scale bars: 50 μm. Adapted from Kim et al. (2020). SMA: Spinal muscular atrophy; SMN: survival of motor neuron.

that will likely be instructive even beyond SMA is a greater focus on models of milder forms of the disease. We suspect that these models are not handicapped by widespread dysfunction of the periphery, enabling perturbations that are detected to be attributed primarily to abnormalities of the motor unit. Whether these abnormalities are nevertheless triggered by defects in the canonical function of SMN remains largely unresolved. Accordingly, we advocate for a greater emphasis on mild SMA as investigators continue to define key mechanisms underlying selective neurodegeneration in this and other more common diseases. Such a rationale and a summary of what is currently known about SMN in SMA are depicted in **Figure 3**.

Conclusions and Future Directions

Much has been learned about the molecular and cellular biology of SMN since the discovery of the *SMN1* and *SMN2* genes a quarter century ago. Still, our understanding of the mechanisms driving the SMA phenotype remains far from complete. One view is that defining the intricacies of these mechanisms is less important considering the advent of SMN repletion to treat SMA (Finkel et al., 2017; Mendell et al., 2017). We could not disagree more. While SMN repletion as a therapy for SMA is certainly reason to cheer, it is not, by any means, a cure-all. Moreover, its very success derives from work on pre-clinical models that were used to inform when and where SMN in required and, accordingly, to guide the timing of intervening therapies. Continued investment in the sort of basic research that led to these insights ought therefore to remain a fundamental priority. No greater argument need be made for this than the greatly varied and frequently imperfect therapeutic outcomes observed thus far in the many thousands of patients who have undergone treatment. Clearly, early treatment is best (De Vivo et al., 2019), but may be less durable than desired, turning what was a frequently fatal disease into a chronic one. Outcomes of treating symptomatic patients are modest at best, suggesting a ceiling effect of the SMN protein and/or limited benefit from the way repletion is currently effected. One potential solution is to identify not only the most critical developmental and maintenance functions that SMN performs within the neuromuscular system, but also mediators of these functions. Parallel studies to define mechanisms that potentially downregulate these functions – under normal circumstances –could inform ways of re-activating them in symptomatic individuals as a means of regaining lost function. Considering the confounding systemic effects of extremely low SMN, we advocate for these studies to be conducted in models of mild SMA wherein disease is primarily driven by dysfunction in the motor unit. It is not unreasonable to suggest that this sort of work could rapidly assume broad relevance, informing not just the biology and treatment of SMA but also other neurodegenerative conditions.

Figure 3 | Cellular sites of action of low SMN within the motor unit and defects associated with low protein.
Indicated are cells and cellular compartments affected in SMA. Solid lightning bolts denote cells empirically shown to contribute cell-autonomously to the SMA phenotype; open bolts denote cells/compartments suspected but not unequivocally shown to be primary drivers of SMA. Also indicated are the most widely accepted SMN functions affected by low protein and their contribution(s) to SMA. Black text indicates functions that have been experimentally established; gray text symbolizes functions assumed to be affected. SMA: Spinal muscular atrophy; SMN: survival of motor neuron.

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