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Commentary

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Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease that predominantly affects motor neurons, resulting in progressive muscular atrophy and weakness. SMA arises due to insufficient survival motor neuron (SMN) protein levels as a result of homozygous disruption of the *SMN1* gene. SMN upregulation is a promising and potent treatment strategy for this currently incurable condition. In this issue of the *JCI*, two independent research groups report novel observations in mouse models of severe SMA that provide hope that this approach will afford meaningful benefit to individuals with SMA.

Spinal muscular atrophy (SMA) is a frequently fatal autosomal recessive neurodegenerative disorder characterized by motor neuron cell loss in the anterior horns of the spinal cord and brain stem (Figure 1). SMA results in skeletal muscle weakness, atrophy, and premature death in the majority of those affected. With an incidence of 1 in 10,000 births, it is the most commonly inherited motor neuron disease and a leading genetic cause of mortality in infants and children (1). Since its original descriptions by Werdnig and Hoffman independently in the 1890s (2, 3), an increasingly broad spectrum of SMA phenotypes has been identified, ranging from prenatal to adult onset (Table 1). SMA type I, the severe infantile form, accounts for more than 50% of newly diagnosed cases. Infants with this condition manifest hypotonia and weakness before six months of age. An effective therapeutic for SMA has thus far remained elusive. Treatment currently involves managing symptoms and preventing complications. However, the unique genetic and clinical characteristics of SMA make it one of the most promising candidates for the development of an effective and potentially curative therapeutic intervention.

A tale of two SMNs

More than 95% of individuals with SMA possess a homozygous deletion involving exon 7 of their survival motor neuron 1 (*SMN1*) gene (4, 5). Thus, the majority of SMA patients, regardless of phenotype, share a unique genetic signature that is easily identified via a simple and inexpensive DNA test (6, 7). While homozygous *SMN1* deletion is necessary, it is insufficient to cause SMA unless *SMN2* is present. An inverted duplication of chromosome 5q13 unique to a subset of hominids including humans harbors up to several copies of *SMN1* and its nearly identical twin, *SMN2* (8, 9). A critical nucleotide difference in *SMN2* results in the exclusion of exon 7 from the majority of transcripts yet produces a fraction of the identical full-length SMN protein as *SMN1* (ref. 10 and Figure 2). Thus, the presence of *SMN2* rescues the embryonic lethality observed in other species with homozygous *SMN1* deletion, testament to the critical role of SMN in the spliceosomal assembly of small nuclear ribonucleoproteins (snRNPs) (11).

The opportunity to target *SMN2* gene(s) or transcripts in vivo to rescue levels of SMN protein in patients with SMA has been widely viewed as an unprecedented opportunity for therapeutic success (12). Proof of concept for this strategy is based on several compelling observations replicated in numerous laboratories over the past decade. These include the successful targeting of *SMN2* in vitro in cells from human subjects (13–15); the repeated demonstration of successful upregulation, replacement, or repair of *SMN2* in vivo in animal models of SMA and in human subjects (16–20); and the observation that increasing *SMN2* dosage in a severely affected mouse model is sufficient to rescue the phenotype (21). Finally, a modifying impact of *SMN2* dosage on disease pheno-

type in SMA human populations has been repeatedly demonstrated, with infants with SMA type I much more likely to have two or fewer *SMN2* copies as compared to those with milder phenotypes (22, 23). In this issue of the *JCI*, two independent groups of researchers address critical issues that continue to provide uncertainty as we try to translate observations from mouse models to the clinic: in particular, these include the relevance of certain recent observations in severe mouse models, such as cardiomyopathy, and the degree to which already symptomatic patients, particularly those with SMA type I, are likely to benefit from *SNM2* targeting (24, 25).

Prolactin: another therapeutic candidate?

In this issue, Farooq and colleagues demonstrate that the delivery of a novel therapy, prolactin (PRL), increases SMN expression in both mouse models of SMA and human cell lines via a transcriptional mechanism (24). In aggregate, their in vitro data indicate that the effect of PRL on SMN is almost certainly via the STAT5 pathway. STAT5 is a member of the STAT family of transcription factors that are direct targets of JAKs, which are activated, in turn, by ligand binding via specific cytokine receptors. Prolactin is a small polypeptide protein that binds to PRL receptors triggering the JAK2/STAT5 signaling pathway. In the animal model of severe SMA, the ability of subcutaneously delivered PRL to upregulate SMN in brain and spinal cord resulted in a marked improvement in motor function, but only a modest improvement in survival (24). What makes this story potentially more compelling than that of other candidates with similar capacity for SMN upregulation is that this effect may prove more robust in humans than in mice because of the presence of a higher number of STAT5-binding sites in the human *SMN2* promoter. In addition, PRL crosses the blood-brain barrier, and since recombinant PRL has been previously tested and proven safe in mothers of preterm infants
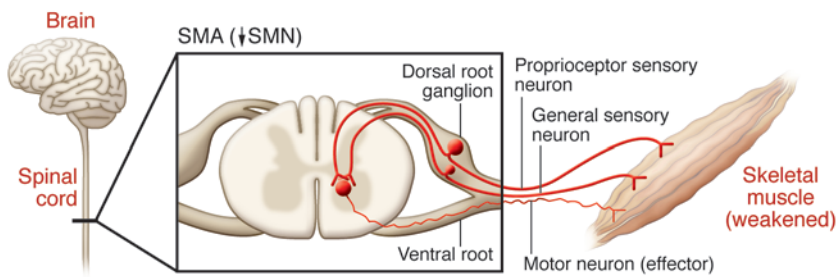
Figure 1
SMA is a neurodegenerative disease that predominantly affects motor neurons. SMA is caused by insufficient levels of SMN protein as a result of homozygous disruption of the *SMN1* gene. The primary target of SMN deficiency appears to be a relatively selective degeneration of motor neurons in the anterior horn cells of the brainstem and spinal cord, resulting in skeletal muscle atrophy.

to augment lactation, the immediate potential therapeutic implications are evident as we await other SMN-targeted therapies to navigate the hurdles of the clinical trials pipeline (26). As such, PRL joins a short list of candidates potentially worthy of further testing in SMA patients in the near term.

Inducing SMN in SMA mice: a postsymptomatic rescue

Since patients present at various stages of disease progression and SMN restoration therapy in individuals with SMA would occur after symptom onset, a key question is the following: When is SMN upregulation or replacement too late, and when is it "just in time"? To try to answer this question, Lutz and colleagues generated SMA model mice harboring an inducible *Smn* rescue allele (25). Inducing expression of SMN to an estimated 40% of wild-type levels following onset of symptoms at P4 in an animal model of severe SMA resulted in a profound improvement in motor function and survival. While induction at P4 clearly and dramatically improved survival, that at P6 and later time points resulted in a much more modest impact on survival and motor function that was entirely absent by P10. In mice normally expected to die by P17, this is a compelling achievement, but what does it really mean for patients with SMA? At P4, motor neuron cell counts in spinal cord in affected mice are equivalent to those in wild-type mice. This observation, along with functional studies in mice demonstrating impaired synaptic vesicle release at presynaptic neuromuscular junctions (NMJs), have led investigators to postulate that motor neuron cell loss is a rather late event in SMA disease pathogenesis (27). However, in contrast to patients, these symptomatic mice, untreated until P4, have much more subtle evidence of denervation and certainly more preserved motor function as compared with symptomatic SMA type I infants. Thus, exactly how well the observations of Lutz and colleagues (25) will translate to patients with SMA, at least to those with the severe infantile form, is not entirely clear.

Table 1
Spectrum of phenotypic manifestations in proximal SMA

SMA type	Typical age of onset	Typical life span	Also called	Clinical characteristics / Maximum milestones achieved
0	Prenatal	< 6 months	SMA — arthrogryposis multiplex congenita type	Congenital hypotonia, weakness, respiratory failure, proximal joint contractures Unable to breathe unsupported
I	Birth–6 months	~32% survival probability > 2 years[A]	Werdnig-Hoffman disease	Infantile onset of generalized hypotonia, weakness, impaired bulbar function, respiratory insufficiency Unable to sit unsupported
II	6–12 months	~70% survival to adulthood[A]	SMA, Dubowitz type	Able to sit independently Onset of limb weakness as infants or toddlers Progressive weakness, respiratory insufficiency, scoliosis, joint contractures in childhood
IIIa	After 12 months	Normal	Kugelberg-Welander disease	Onset of proximal muscle weakness in childhood
IIIb	After 3 years		Kugelberg-Welander disease	Able to ambulate independently, although 50% with type IIIa lose independent ambulation by 12 years of age
IV	Adulthood	Normal		Onset of proximal leg weakness in adulthood, able to ambulate independently

[A]Data from ref. 29.
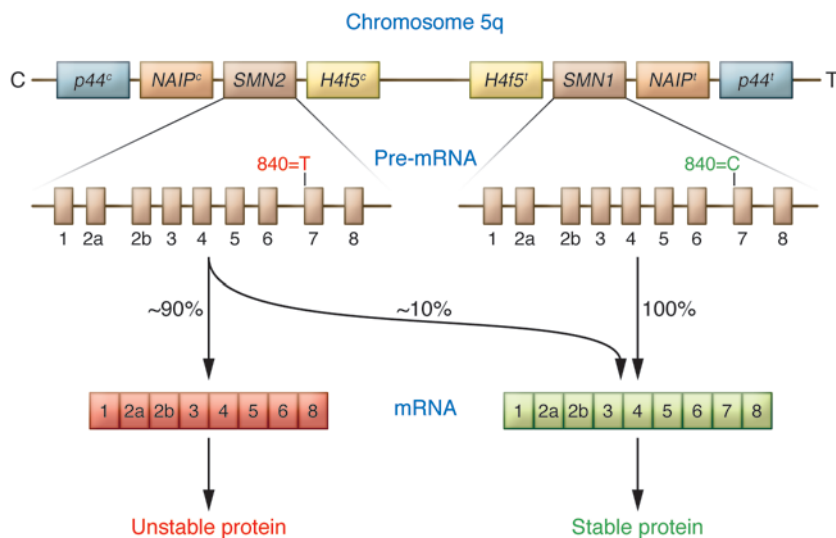
Figure 2
Gene organization and expression in the region of human chromosome 5q containing the *SMN1* and *SMN2* genes. *SMN* is one of four duplicated genes within a duplicated inverted 500-bp region on chromosome 5q. The two copies of *SMN* differ by a few base pairs with only two of them being in the exonic sequence. Functionally critical is the single nucleotide difference in exon 7 at position 840. Although translationally silent, the C→T transition in *SMN2* affects splicing. *SMN* is one of four genes in this inverted duplicated region; the others have not been clearly demonstrated to contribute to the pathogenesis of SMA. H4F5, protein 4F5; NAIP, NLR family, apoptosis inhibitory protein.

Parallel studies of NMJ pathology, muscle denervation, spinal cord pathology, and motor function aren't feasible in human subjects, although such studies have been beautifully performed in mice by Lutz and colleagues (25), and others. However, observations in prospectively evaluated infants with a positive family history and documented homozygous *SMN1* exon 7 deletion suggest that distal denervation is indeed a sentinel event in infants at a time when other symptoms of the disease are relatively modest, in contrast with the mouse model data (28). The early and rapid progression of distal denervation in the first few weeks to months of life in infants predicted to develop type I SMA represents perhaps the single greatest challenge to successfully targeting SMN in humans. Infants destined to develop SMA type I unfortunately account for the largest proportion of those born at risk for SMA, highlighting the urgency for prospective identification of such cases via newborn screening to better assess the potential benefit of earlier diagnosis and intervention on improving outcomes for a majority of those who will develop SMA.

Translating lessons from mice to humans

Whether the mouse models of severe SMA currently in use are robust surrogates for the severe infantile form of the human disease across the spectrum of manifestations observed in the mouse model remains a point of controversy; nevertheless, the increasing number of successful therapeutic interventions targeting SMN in such severe mouse models promise great hope

for the development of an effective therapy for SMA. The dramatic nature of the rescue after symptom onset achieved by Lutz et al. (25) provides encouragement that reversing disease pathology may be feasible in patients with SMA. As such, it represents another significant milestone in our quest for demonstrating proof of concept for a meaningful therapeutic benefit with targeted upregulation of SMN. For clinicians who have closely followed developments at the bench to glean insights to their translational relevance, the already demonstrated, although less dramatic, survival benefit observed with interventions targeting increased SMN expression after symptom onset are also compelling. Avila et al. previously demonstrated that treatment with trichostatin A at P5 in the mouse model of severe SMA resulted in a modest impact on survival and motor function (18); likewise, the administration of scAAV9-SMN by Foust and colleagues at P5 demonstrated a modest effect (19). What sets the publication by Lutz and colleagues (25) apart is the dramatic nature of the rescue, heretofore previously matched only by SMN replacement at P1 or P2 (22, 23). Thus, the weight of the evidence to date, fortified by the work of Lutz and colleagues (25), suggests that all is not lost after symptom onset in SMA.

Translating the work of Farooq and colleagues (24) from mouse to human is perhaps even more challenging, due to the substantial differences identified between the PRL promoter in mice and humans. However, if the authors prove correct in their interpretation that PRL may prove substantially more potent in upregulat-

ing *SMN2* in vivo in humans than it did in mice, then it may indeed prove a promising intervention in patients with SMA, even for those already exhibiting symptoms.

Predicting value for a given therapy is profoundly more difficult in patients than in animal models and analogous in some respects to peering into a crystal ball. As we look to the future, we should avoid the temptation to squander the opportunity to achieve modest yet meaningful benefit in already symptomatic patients. The substantial progress of the past decade, particularly the dramatic benefits achieved with a variety of therapeutic strategies targeting SMN in animal models, provides us with an increasing likelihood that at least one of the current therapies under development will prove successful in the next few years. The wealth of data continues to support a much greater benefit for mice treated as early as possible in the course of the disease. This exposes a painful truth that is hard for those of us who are both clinicians and clinical trialists to swallow. In order to have the best chance for a "cure," or even definitive proof of a more modest benefit, we may have to target our early efforts to those with less severe disease, early in the clinical course. For that reason, a broader adoption of newborn screening for SMA may prove the most rapid and effective means of identifying a cohort of infants and children who represent the best candidates for promising therapeutic treatments in the near term. Ultimately this will benefit the greatest number of patients by allowing early qualification of promising therapies.

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Quantity, not quality, of antibody response decreased in the elderly

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The burden of disease during seasonal influenza epidemics is felt most keenly among the very young and the elderly. Although vaccination effectively protects children and young adults against infection, it has limited efficacy in elderly individuals. This has been linked to a reduced ability to induce a robust serum antibody response. In this issue of the *JCI*, Sasaki et al. identify some of the cellular and molecular deficits that underlie the reduced serum antibody response induced by influenza vaccination in elderly individuals. Importantly, they show that it is the quantity of the response, and not its quality, that needs to be improved if we are to enhance the success of influenza vaccination in this vulnerable population.

Influenza epidemics are associated with an estimated 200,000 hospitalizations and 35,000 deaths each year in the United States alone (1). Most of these adversely affected individuals are young children or elderly individuals. Vaccination against influenza has been described to be effective in preventing infection in healthy children and young adults; however, many of the elderly individuals who receive the vaccine still contract the infection and have secondary complications that lead to hospitalization and/or disability (2, 3). Despite this, influenza vaccination is still universally recommended for elderly individuals, because it does reduce disease severity, but understanding the mechanism(s) that underlies the limited response of the elderly to influenza vaccines could help direct the development of more effective approaches.