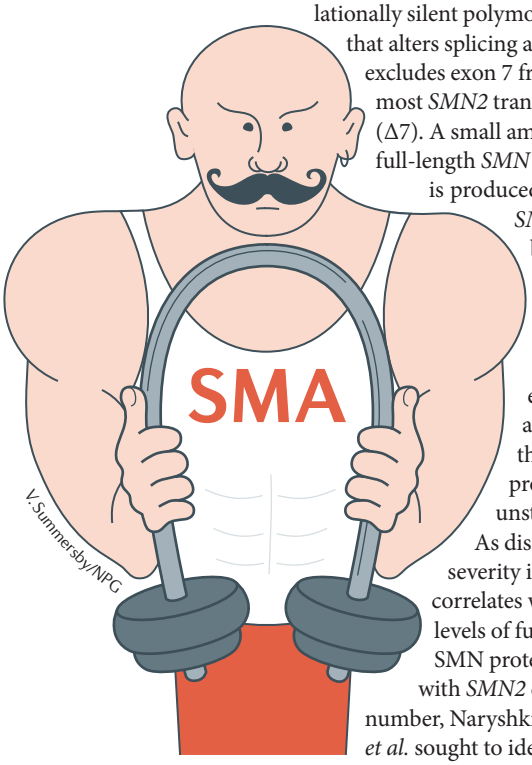
NEUROMUSCULAR DISORDERS

Beefing up the right splice variant to treat spinal muscular atrophy

Spinal muscular atrophy (SMA), the leading genetic cause of infant mortality, is characterized by progressive degeneration of motor neurons in the anterior horn of the spinal cord and atrophy of skeletal muscles. SMA results from mutations in survival of motor neuron 1 (*SMN1*). Naryshkin *et al.* have now identified orally available small-molecule compounds that alter the splicing of the nearly identical *SMN2*, which can improve motor function and extend lifespan in mouse models of SMA.

SMN2 is thought to have arisen from an *SMN1* gene duplication event. The coding regions of *SMN1* and *SMN2* differ by only one translationally silent polymorphism that alters splicing and excludes exon 7 from most *SMN2* transcripts (Δ7). A small amount of full-length *SMN* mRNA is produced from *SMN2*, but most of the transcripts lack exon 7, and the Δ7 protein is unstable. As disease severity inversely correlates with levels of functional SMN protein and with *SMN2* copy number, Naryshkin *et al.* sought to identify compounds that alter *SMN2* splicing, thereby increasing the production of full-length *SMN2* transcripts. By screening a library of ~200,000 compounds and performing further chemical optimization for molecules that increase the ratio of full-length to Δ7 *SMN2* mRNA, they found three compound series — exemplified by compounds named SMN-C1, SMN-C2 and SMN-C3 — that increased the fraction of *SMN2* mRNAs that contain exon 7.



These compounds were then tested in two cell types from patients with SMA: motor neurons generated from induced pluripotent stem cells and fibroblasts. Treatment with any one of the three compounds altered *SMN2* splicing, thereby increasing the levels of both full-length *SMN* transcripts and SMN protein in both cell types. Of note, these compounds did not seem to affect overall splicing, transcription or translation, as the expression of only twelve genes was altered by a factor of >two in SMA fibroblasts that were treated with SMN-C3. Furthermore, an analysis of annotated splice junctions within the transcripts identified only a handful of splicing alterations in response to SMN-C3 treatment.

The investigators then used a mouse model of mild SMA ($Smn^{C/C}$), in which the animals have a normal lifespan but have muscle weakness, peripheral necrosis and reduced weight. Daily oral treatment of these mice with SMN-C2 or SMN-C3 shifted *Smn* gene expression to favour the production of full-length *Smn2* mRNA (from 40% full-length, 60% Δ7 to 90% full-length, 10% Δ7). After 10 days of treatment with SMN-C3, protein levels were partially restored in the brain and spinal cord, and fully restored in muscle to those levels observed in unaffected heterozygous $Smn1^{+/-}$ mice.

In a mouse model of severe SMA (Δ7 mice), administration of SMN-C2 or SMN-C3 (by intraperitoneal injection until postnatal day 23 and oral administration thereafter) increased SMN protein levels and prolonged survival; approximately 90% of animals survived beyond postnatal day 65, when the study was terminated. By contrast, untreated Δ7 mice died within 3 weeks of birth (median survival 18 days). Treating mice with SMN-C3 also restored weight gain and motor function to normal or near-normal levels.

The observed systemic effects in the Δ7 mice treated with SMN-C3 were associated with reduced SMN-related neuromuscular pathology. Loss of spinal cord neurons, denervation of the neuromuscular junction in the splenius capitis and atrophy of the extensor digitorum longus were all prevented by treatment with SMN-C3 in a dose-dependent manner.

These newly identified small molecules have nanomolar potency, penetrate into muscle and the central nervous system and seem to be highly selective for *SMN* splicing. Analogues of these compounds are currently in clinical testing, and could become a promising therapy for patients with SMA.

Megan Cully

ORIGINAL RESEARCH PAPER Naryshkin, N. A. *et al. SMN2* splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science* **345**, 688–693 (2014)