Increased IGF-1 in muscle modulates the phenotype of severe SMA mice

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Spinal muscular atrophy (SMA) is an inherited motor neuron disease caused by the mutation of the survival motor neuron 1 (SMN1) gene and deficiency of the SMN protein. Severe SMA mice have abnormal motor function and small, immature myofibers early in development suggesting that SMN protein deficiency results in retarded muscle growth. Insulin-like growth factor 1 (IGF-1) stimulates myoblast proliferation, induces myogenic differentiation and generates myocyte hypertrophy in vitro and in vivo. We hypothesized that increased expression of IGF-1 specifically in skeletal muscle would attenuate disease features of SMA D7 mice. SMA D7 mice overexpressing a local isoform of IGF-1 (mIGF-1) in muscle showed enlarged myofibers and a 40% increase in median survival compared with mIGF-1-negative SMA littermates (median survival 514 versus 10 days, respectively, log-rank P = 0.025). Surprisingly, this was not associated with a significant improvement in motor behavior. Treatment of both mIGF-1NEG and mIGF-1POS SMA mice with the histone deacetylase inhibitor, trichostatin A (TSA), resulted in a further extension of survival and improved motor behavior, but the combination of mIGF-1 and TSA treatment was not synergistic. These results show that increased mIGF-1 expression restricted to muscle can modulate the phenotype of SMA mice indicating that therapeutics targeted to muscle alone should not be discounted as potential disease-modifying therapies in SMA. IGF-1 may warrant further investigation in mild SMA animal models and perhaps SMA patients.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease with a carrier frequency of ~1 in 40 in the population. In severe SMA patients, motor neuron degeneration and muscle atrophy result in severe weakness of proximal more than distal muscles and early death (1,2). SMA is caused by mutation of the survival motor neuron 1 (SMN1) gene and deficiency of the SMN protein (3). Currently, there is no disease-modifying treatment to offer to patients with this devastating disease.

Muscle from severe SMA patients shows widespread small myofibers, which have a developmentally arrested appearance (4) and an immature expression profile of myofibrillar proteins (5). Similarly, in severe SMAΔ7 mice, myofibers are hypotrophic and express an immature pattern of myosins before there is degeneration of innervating motor nerve (6,7). This muscle pathology may be simply secondary to early motor neuron dysfunction. Experiments in SMA mice in which SMN expression has been selectively restored in myofibers (8) or selectively depleted from motor neuron precursors (9) suggest that SMN deficiency in motor neurons is the principal driver of SMA disease pathology. Nonetheless, it is unresolved whether SMN protein deficiency in muscle, particularly in muscle precursor cells, causes intrinsic defects of muscle growth that contribute to the SMA phenotype. It has been suggested, for example, that SMN deficiency in satellite cells or myoblasts impairs myofiber growth and regeneration (10,11). It has also been proposed that SMN-deficient muscle fails to provide the appropriate
Insulin-like growth factor 1 (IGF-1), also known as somatomedin C, is a small polypeptide hormone that belongs to the IGF family. IGF-1 is principally secreted by the liver into the blood in response to growth hormone (GH), but various tissues can also synthesize isoforms of IGF-1 locally, where they may play autocrine or paracrine roles. In muscle, IGF-1 plays an important role during muscle development and induces muscle regeneration after injury and denervation (15). Transgenic mice overexpressing a locally synthesized form of IGF-1 (class I Eα or mIGF-1) in skeletal muscle develop muscle hypertrophy resulting from increased proliferation of satellite cells (16). Local muscle IGF-1 expression attenuates age-related skeletal muscle atrophy (16) and improves muscle function in the mdx mouse model of muscular dystrophy (17). In addition, IGF-1 has been postulated to have retrogradely acting neurotrophic effects on motor neurons (18) and transgenic overexpression of mIGF-1 (19) or viral delivery of IGF-1 (20) to muscle delays the onset and progression of disease in amyotrophic lateral sclerosis (ALS) mice. Overexpression of mIGF-1 in muscle was also recently shown to markedly extend the survival of mice with spinal and bulbar muscular atrophy (SBMA), an X-linked adult form of SMA (21).

We hypothesized that overexpressing mIGF-1 in skeletal muscle would attenuate the disease features of severe SMA mice. We show that mIGF-1 improves muscle mass and extends the survival of severe SMA mice independent of SMN induction indicating that muscle-directed therapy remains an important consideration for the treatment of SMA patients.

RESULTS

SMAΔΔ7 mice overexpressing mIGF-1 show increased muscle mass

Transgenic mice overexpressing a rat, non-circulating, muscle-specific isoform of IGF-1 (mIGF-1) under control of the rat myosin light chain promoter have been previously described (16). SMAΔΔ7 mice (hSMN2+/+/SMNΔΔ7+/+/ mSmn+/−) were bred to these mIGF-1 transgenic mice in order to obtain SMA mIGF-1POS (mIGF-1+/−/hSMN2+/+/ SMNΔΔ7+/+/mSmn−/−) and SMA mIGF-1NEG (mIGF-1−/−/ hSMN2+/+/SMNΔΔ7+/−/mSmn−/−) mice (See Material and Methods for details of breeding strategy). Heterozygous (Het) mIGF-1POS mice (mIGF-1+/−) were born with expected frequencies, but mIGF-1+/+ mice were never recovered suggesting that homozygosity of the transgenic mIGF-1 allele is embryonically lethal when on the FVB/NJ background.

Quadriceps muscles were isolated from SMA and heterozygous littermate mIGF-1POS and mIGF-1NEG mice at postnatal day 10 (P10), and the expression levels of total IGF-1 transcripts (endogenous mouse + exogenous rat) were determined by quantitative reverse transcriptase-polymerase chain reaction (qRT–PCR). This verified an ~5-fold increase in total IGF-1 transcript levels in mIGF-1POS mice (Fig. 1A). We also measured transcript levels of the IGF-1 receptor, IGFR1 (Fig. 1B), which mediates the biological activity of IGF-1. Both SMA mIGF-1NEG and mIGF-1POS mice showed increased IGFR1 expression compared with corresponding heterozygous littermates (1.3-fold increase in SMA mIGF-1NEG compared with Het mIGF-1NEG and 1.9-fold increase in the SMA mIGF-1POS compared with Het mIGF-1POS, P < 0.01 and P < 0.001, respectively). These data suggest a compensatory upregulation of the IGFR1 in SMA-diseased muscle. Interestingly, heterozygous littermate mIGF-1POS mice showed mildly reduced IGFR1 expression compared with heterozygous littermate mIGF-1NEG mice (1.29 ± 0.09 versus 1.01 ± 0.05, P < 0.05), suggesting a compensatory downregulation of IGFR1 in the presence of excess IGF-1 in healthy mice. This compensation did not occur in SMA mIGF-1POS mice.

In order to examine whether increased mIGF-1 expression resulted in increased muscle size, the muscle masses of the quadriceps, gastrocnemius and triceps muscles were determined in SMA mIGF-1NEG and mIGF-1POS mice at P10 (Fig. 1C and D). The wet weights of quadriceps and gastrocnemius were significantly increased in SMAΔΔ7 mIGF-1POS mice compared with SMAΔΔ7 mIGF-1NEG mice [quadriceps: SMAΔΔ7 mIGF-1POS = 9.98 ± 0.97 (n = 6) and SMAΔΔ7 mIGF-1NEG = 6.67 ± 0.49 mg (n = 7), P = 0.009 and gastrocnemius: SMAΔΔ7 mIGF-1POS = 8.36 ± 0.77 (n = 6) and SMAΔΔ7 mIGF-1NEG = 5.45 ± 0.21 mg (n = 7), P = 0.003]. Histological analysis revealed that myofiber diameter was also significantly increased in SMA mIGF-1POS quadriceps muscle by 1.5-fold (P < 0.05) (Fig. 1C and E). The average total body weights of the cohorts of mice used for muscle analysis were SMA mIGF-1NEG = 3.23 ± 0.20 g and SMA mIGF-1POS = 4.08 ± 0.38 g (P = 0.12).

In order to evaluate whether IGF-1 expression altered the expression of SMN, we measured full-length (SMN678) and truncated forms of SMN (SMN68) transcripts in the quadriceps muscle of SMA mice at P10. There was no change in the expression levels of SMN in response to mIGF-1 overexpression (Fig. 2A). We also evaluated transcripts encoding myosin heavy chain (MyHC) isoforms and acetylcholine receptor (AChR) subunits. We have previously shown increased expression of perinatal and reduced expression of adult IIb MyHC as well as increased neonatal α and reduced adult ε AChR subunit expression in SMA muscle consistent with a pattern of immaturity (7). SMA IGF-1POS muscle showed no statistical differences with SMA mIGF-1NEG muscle, except for the ε subunit of the AChR, which was 4-fold increased in mIGF-1POS muscle (Fig. 2B and C). Interestingly, Het mIGF-1POS mice also showed a 4-fold increase in the expression of the AChR ε subunit compared with Het mIGF-1NEG mice (data not shown) indicating that mIGF-1 overexpression in muscle activates expression of the mature subunit of AChR independent of disease state.

Overexpression of mIGF-1 in muscle increases survival of SMA mice

We next evaluated the survival of mIGF-1NEG and mIGF-1POS SMA mice. SMA mIGF-1NEG mice showed a median survival...
of 10 days (Fig. 3A). This is less than the approximate 14-day survival generally reported for SMA\(D\)7 mice (22) and may relate to mating the SMA\(D\)7 mice, which are on a mixed FVB background, to the mIGF-1 transgenic mice, which are on a pure FVB background. Supporting this idea is the observation that SMA\(D\)7 mice made congenic on a FVB background have a median survival of 10 days (23). Compared with SMA mIGF-1\(^{-}\)NEG mice, SMA mIGF-1\(^{+}\)POS mice had an increased median survival of 14 days indicating that mIGF-1 overexpression in muscle modestly extends survival by 40% (log-rank \(P = 0.025\)). Maximal survival of SMA mIGF-1\(^{+}\)POS mice was not increased, however. A survival benefit was more evident in SMA mIGF-1\(^{+}\)POS mice, when the mother was also mIGF-1\(^{+}\)POS (Supplementary Material, Fig. S1). Gender was not found to significantly influence survival (data not shown).

Like survival, weight gain and maximal weight achieved were reduced in SMA mIGF-1\(^{-}\)NEG mice compared with standard SMA\(D\)7 mice (Fig. 3B). However, weight gain was modestly improved in SMA mIGF-1\(^{+}\)POS mice compared with mIGF-1\(^{-}\)NEG mice, particularly toward the end of the first postnatal week suggesting that IGF-1 may have accelerated

Figure 1. Overexpression of mIGF-1 results in increased muscle mass in SMA mice. (A) IGF-1 and (B) IGFR1 mRNA expression levels were determined by qRT–PCR in quadriceps muscles from P10 mIGF-1\(^{-}\)NEG and mIGF-1\(^{+}\)POS SMA and heterozygous (Het) littermate mice \([^*P < 0.05, ^{**}P < 0.01 and ^{***}P < 0.001, n = 6\) mice per group except for SMA mIGF-1\(^{-}\)NEG \((n = 7)\). (C) H&E stained cross-sections of quadriceps muscle from P10 SMA mIGF-1\(^{-}\)NEG (left) and a P10 mIGF-1\(^{+}\)POS SMA mice (right). Scale bar = 50 \(\mu\)m. (D) Weights of gastrocnemius (gastroc), quadriceps (quad) and triceps muscles in P10 mIGF-1\(^{-}\)NEG and mIGF-1\(^{+}\)POS SMA mice \([^*P < 0.05, n = 7 \text{ mIGF-1}^{-}\text{NEG} \text{ and } n = 8 \text{ mIGF-1}^{+}\text{NEG} + \text{SMA} \text{ mice}]. \) (E) Myofiber diameter in the P10 quadriceps muscle in SMA mIGF-1\(^{-}\)NEG and SMA mIGF-1\(^{+}\)POS mice \([^*P < 0.05, n = 3 \text{ mIGF-1}^{+}\text{NEG} \text{ and } n = 4 \text{ mIGF-1}^{+}\text{POS} \text{ SMA mice}]. \) Values represent average ± SEM.
neonatal muscle growth in SMA mice. The maximal weight
achieved was equivalent in the two groups, however, and
occurred at P9. For SMA mIGF-1NEG mice, this was 3.24
+ 0.34 g (n = 22) and for SMA mIGF-1POS mice this was
3.29 + 0.29 g (n = 26). Of note, an increase in body weight
was not evident in mIGF-1POS compared with mIGF-1NEG
control littermate mice until the fifth postnatal week (Sup-
plementary Material, Fig. S2) indicating that mIGF-1 may
not have maximal effects on muscle growth until this stage
of postnatal development. These observations are in agree-
ment with prior work indicating maximal muscle hypertrophy
at 6 weeks in mIGF-1 transgenic mice (16).

We also examined motor function in SMA mIGF-1NEG and
mIGF-1POS mice. Latency of time to right showed no
statistically significant difference between groups (Fig. 3C).
At P10, SMA mIGF-1POS mice had a righting time latency
of 24.2 ± 3.5 s (n = 23) and in SMA mIGF-1NEG mice, it
was 21.9 ± 3.7 s (n = 22), P = 0.48. The hind-limb suspen-
sion test (24) was also performed and a composite score of
this test (25) showed no statistically significant differences
between groups (Fig. 3D).

Trichostatin A increases survival of SMA mice
We next evaluated whether the therapeutic effects of IGF-1 tar-
geted to muscle might be synergistic with a drug that induces
SMN expression. We have previously shown that the histone
decayelase (HDAC) inhibitor, trichostatin A (TSA), activates
SMN expression in spinal cord and muscle tissues (26,27) and
increases the survival of SMAΔ7 mice by 40% when delivered
starting at P2 (27). We have also shown that nutritional repletion
can further enhance this therapeutic effect (27). Given this, we
provided nutritional support to all animals starting at P3 (see
Material and Methods for details) and culled all litters to 6
pups. SMA mIGF-1NEG and mIGF-1POS mice were treated
with daily intraperitoneal injections of vehicle or TSA at a
dose of 8 mg/kg. This dose was chosen rather than the 10 mg/
kg dose used in prior studies (26,27) because toxicity was wit-
tnessed with TSA 10 mg/kg in these relatively more fragile
mice. Culling and nutrition alone extended the median survival
of SMA mIGF-1NEG mice from 10 to 14 days with these inter-
ventions alone (data not shown). Vehicle-treated SMA
mIGF-1NEG mice showed a median survival of 14 days and
TSA increased this survival to 22 days (median extension of
survival = 57%, P = 0.059) (Fig. 4A). Vehicle-treated SMA
mIGF-1POS mice had a median survival of 16 days and TSA
increased this to 25 days (median extension of survival = 56%,
P = 0.005) (Fig. 4A). Examination of body weights and
latency to righting showed improvements in TSA-treated com-
pared with vehicle-treated SMA mice; however, no further
benefit was seen in SMA IGF-1POS mice treated with TSA
(Fig. 4B and C).

DISCUSSION
Here, we have evaluated the effects of muscle-restricted over-
expression of mIGF-1 on the phenotype of severe SMAΔ7 mice.
SMA mice overexpressing mIGF-1 showed increased muscle mass and a 40% increase in median survival, but this
was not accompanied by a measurable improvement in
motor function. Combining this intervention with daily injec-
tions of the HDAC inhibitor TSA further extended survival,
but this combination did not show significantly synergistic
benefits.

SMA is caused by insufficient expression levels of the SMN
protein. SMN plays a key role in the assembly of a specific
class of RNA–protein complexes, the uridine-rich small ribo-
nuclear proteins (U snRNPs) (28,29), which are central com-
ponents of the spliceosome. Deficiency of SMN impairs
assembly of Sm proteins onto snRNA (30,31) and thus is pre-
dicted to affect splicing. Splicing changes have been reported in
various tissues of SMA mice with increased numbers of
alterations present in older mice (31,32). The consequence

Figure 2. Expression of SMN is not changed by mIGF-1 overexpression.
(A) SMN678 and SMN68 mRNA levels were determined in quadriceps isolated
from P10 SMA mIGF-1NEG (n = 7) and SMA mIGF-1POS mice (n = 6).
(B) Embryonic, perinatal and adult MyHC and (C) AChR γ and AChR ε
mRNA levels were measured in the same muscles (*P < 0.05). Values represent
average ± SEM.
Figure 3. Overexpression of mIGF-1 modestly improves survival of SMA mice. (A) Kaplan–Meier survival curves comparing mIGF-1\textsuperscript{NEG} and mIGF-1\textsuperscript{POS} SMA mice. Median survival in mIGF-1\textsuperscript{NEG} SMA mice was 10 days and median survival in mIGF-1\textsuperscript{POS} mice was 14 days (log-rank $P = 0.025$, $n = 52$ mIGF-1\textsuperscript{NEG} and $n = 33$ mIGF-1\textsuperscript{POS} SMA mice). (B) Body weight was increased in SMA mIGF-1\textsuperscript{POS} mice on P7, P8 and P9 compared with SMA mIGF-1\textsuperscript{NEG} mice (\*\(P < 0.05\) and \**\(P < 0.01\), as determined by multiple imputation test). (C) Average righting time showed no difference between SMA mIGF-1\textsuperscript{NEG} and mIGF-1\textsuperscript{POS} mice ($n = 52$ and $n = 33$, respectively). (D) The composite tube test score also did not show statistically significant differences between mIGF-1\textsuperscript{NEG} and mIGF-1\textsuperscript{POS} SMA mice ($n = 8$ mice per group). Values represent average $\pm$ SEM.
of these splicing changes and their role in SMA has yet to be defined; however, their presence in multiple tissue types raises the possibility that although motor neurons are most susceptible to the molecular deficits caused by SMN deficiency, other tissues may also be affected. Indeed, recent studies have increasingly pointed to previously unrecognized abnormalities of the heart and vasculature in both SMA mice and humans (33–39). It has long been debated whether intrinsic...
abnormalities of skeletal muscle contribute to SMA disease manifestations. Determining whether treatments exclusively targeted to muscle, when given alone or in combination with other treatments, could be beneficial for SMA patients is a priority for SMA therapeutics development.

IGF-1 is essential for normal growth and development (reviewed in 15). Several transcript isoforms of IGF-1, which originate from alternative splicing and promoter usage, are divided into two classes (reviewed in 40). IGF-1 class II isoforms predominate in the liver, are circulating and are highly GH responsive. Class I isoforms are widely expressed, but are preferentially confined to the tissue of origin and act in autocrine/paracrine way. Class I α IGFR-1 (mIGF-1) is synthesized locally in muscle. It is transiently increased in response to muscle damage and results in increased satellite cell activation and muscle growth. This isoform of IGF-1 is not thought to enter the circulation in significant quantities (16,41). Interestingly, we observed that the survival benefit in SMA IGF-1^POS mice was more evident when the mother was also IGF-1^POS (Supplementary Material, Fig. S1). Although this might relate to nonspecific effects of a healthier intra-uterine environment or less traumatic birth from Fig. S1), although this might relate to nonspecific effects of a circulating mIGF-1 from IGF-1^POS mothers crossed the placenta and had beneficial effects on SMA pups in utero. Although severe SMA mice overexpressing mIGF-1 showed a modest survival benefit, there was no motor behavioral improvement despite an increase in total weight and muscle mass. Muscle strength is difficult to evaluate in neonatal mice and our motor behavioral measures may not be sufficiently sensitive to detect small differences in muscle strength. Nonetheless, these results raise the possibility that increased survival is not a direct result of improved muscle power, but may result from other effects such as improved metabolic status. Type I SMA patients have severe muscle wasting and are at high risk for entering a fasting state with resulting hypoglycemia and muscle proteolysis (42,43). SMA mice have also been shown to be hypoglycemic (44) and show evidence of muscle breakdown as indicated by increased expression of muscle-specific ubiquitin ligases late in disease (personal unpublished data). Although mIGF-1 overexpression did not improve low glucose levels in SMA mice (Supplementary Material, Fig. S3), increased muscle bulk may have protected mIGF-1^POS mice against muscle breakdown by as yet undefined mechanisms. Optimizing muscle bulk in SMA patients might have similar beneficial effects. Indeed, increased survival of type I SMA patients in recent years can be ascribed in part to aggressive nutritional support with gastrostomy feedings despite little change in muscle strength (45).

Although increasing muscle bulk may benefit SMA patients, the lack of improvement in motor behavior suggests that increasing muscle mass alone does not substantially improve the function of motor neurons in severe SMA mice. The results from this study are consistent with a previous study in which we reported that inhibition of myostatin increased muscle mass and myofiber diameter, but did not improve survival or motor function of severe SMA mice (46). This failure to increase muscle strength in the face of larger muscles may relate to impaired activity of motor neurons in SMA mice either due to intrinsic abnormalities of motor neuron excitability (47) or to impaired synaptic activation of motor neurons (48,49). Without restored excitation of the SMN-deficient motor neurons themselves, muscle may not be stimulated to contract regardless of muscle size and power capacity. This leaves open the possibility, however, that activation of SMN-deficient motor neurons (either by restoration of SMN in motor neurons or by other means) together with strategies to increase muscle bulk could give additive or even synergistic effects. Surprisingly, in this study, TSA treatment of SMA IGF-1^POS mice did not result in significant synergistic effects.

In addition to promoting muscle growth, IGF-1 has been shown to have neurotrophic effects on motor neurons and therefore has been considered a promising therapy for motor neuron diseases (50). The mechanism for these neurotrophic effects is not known, but it is intriguing that we observed a 4-fold increase in the expression of the mature e subunit of the AChR in IGF-1 overexpressing mice. The adult e subunit-containing AChRs have distinct gating properties with a reduced channel open time and increased ion conductance compared with embryonic δ subunit-containing AChRs (51,52), and one might speculate that these properties play important roles in NMJ synapse stability particularly during disease. In two other motor neuron disease animal models, mIGF-1 overexpression was shown to have substantial effects on survival and motor function. When SBMA mice were bred to the same line of mIGF-1 overexpressing mice used in this study, median survival was increased by ~200% and there was a marked improvement in grip strength and rotarod scores (21). SOD1G93A ALS mice overexpressing mIGF-1 showed a 24% increase in median survival and enhanced walking distance (19). Why are such effects not seen in severe SMA mice? Part of the answer may lie in the ability of diseased motor neuron axons to respond to IGF-1’s ability to promote distal axonal sprouting. IGF-1 has been shown to induce sprouting of motor neurons in vitro (18) as well as in vivo when expressed in target muscle (18,53). Partial denervation with reinnervation of myofibers by sprouting of remaining intact intramuscular motor axons is a feature of both ALS and SBMA, but unlike in these adult-onset motor neuron diseases, there is little evidence of reinnervation in severe SMA human patients or mice. In contrast, milder SMA patients have robust reinnervation resulting in very large motor units evident on neurophysiological testing (2). Recently, it was demonstrated that mSmn^−/− mice, which have a 40% reduction in anterior horn cell number, appear to compensate for this loss with collateral sprouting that prevents muscle weakness (54). This process is dependent, at least in part, on another neurotrophic factor, ciliary neurotrophic factor (54). Distal sprouting has also been reported in mild A2G SMA mice (55). These observations suggest that milder forms of SMA disease may show enhanced benefit from IGF-1.

In this study, we have shown for the first time that a therapeutic directed specifically to muscle can improve the survival and weight of severe SMA mice. These effects are modest and not accompanied by improved motor behavioral outcomes, suggesting that targeting muscle alone does not directly improve the function of severely SMN-deficient motor
neurons. Further studies are needed to address whether IGF-1 or similar muscle-directed therapeutic strategies will have better effects in milder models of SMA in which SMN-deficient motor neuron retain the capacity for distal reinnervation as seen in mild SMA patients. Furthermore, the potential benefits of IGF-1 delivered to the nervous system should also be explored in SMA disease models in the future.

MATERIALS AND METHODS

Animals

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Johns Hopkins University Animal Use Committee. Breeder pairs for SMAΔ7 mice (22) on a FVB background (Stock number 005025; FVB.Cg-Tg(SMN2 x delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1tm3Msd/l) were purchased from Jackson Laboratories. These mice were genotyped by PCR of tail DNA as previously reported (26). SMA mice were null at the mSmn allele (mSmn−/−) and control littermates were heterozygous (mSmn+/−) or wild-type (mSmn+/+) at the mSmn allele. Transgenic mice on a FVB background expressing IGF-1 in muscle under control of the myosin light chain promoter have been described previously (16). The myosin light chain promoter is first active at embryonic day 9.5. Mice were genotyped by PCR of tail DNA using the primers IGF-1-F: 5′-TTCCTG TCTACAGTGCTGTAAT-3′ and IGF-1-R: 5′-GAGCTGACTT TGTAGGCTCTCA-3′ as previously described (16). To determine the copy number of mIGF-1 transgene, mice were genotyped by qPCR of genomic DNA. Quantitative primers to detect mIGF-1 (Mm00439560_m1) were purchased from Applied Biosystems. A primer recognizing the promoter sequence of the mSmn gene, MSP0, previously reported in Kernochan et al. (56), was used as an endogenous control as its copy number is stable in all mice. qPCR reactions were run in triplicate using the ABI Prism 7900 Sequence Detector System (Applied Biosystems). All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drug treatment, nutrition and behavior

Drug treated, nutrition and behavioral effects in milder models of SMA in which SMN-deficient motor neuron retain the capacity for distal reinnervation as seen in mild SMA patients. Furthermore, the potential benefits of IGF-1 delivered to the nervous system should also be explored in SMA disease models in the future.

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cycle (Ct) method using 18s as an endogenous control. Values were normalized to the mean of the IGF-1−/− mice for each gene, which was assigned as 1.

**Statistical analysis**

Survival data were analyzed using GraphPad Prism 5 software package. Statistical differences were compared with the log-rank test of Kaplan–Meier curves for two experimental groups, whereas comparisons of survival curves between three or more groups were done using the Bonferroni post hoc test. Behavioral, pathological and biochemical data were analyzed using Excel and SAS software packages, and statistical significance was determined using either Student’s t-test or two-way ANOVA followed by Bonferroni multiple comparisons, as appropriate. Missing data from body weight and righting times curves were analyzed using multiple imputation assuming a simple multi-level model with random intercepts and later analyzed with two-way ANOVA followed by the Bonferroni test. Significance was set at a P-value of less than 0.05.

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

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**Conflict of Interest statement.** None declared.

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