Motor neuron degeneration in spinal and Bulbar Muscular Atrophy is a skeletal muscle-driven process: Relevance to therapy development and implications for related motor neuron diseases

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Non-cell autonomous degeneration has arisen as an important mechanism in neurodegenerative disorders. Using a novel line of BAC androgen receptor (AR) transgenic mice with a floxed transgene (BAC fxAR121), we uncovered a key role for skeletal muscle in X-linked Spinal and Bulbar Muscular Atrophy (SBMA), a motor neuronopathy caused by a polyglutamine expansion in exon 1 of the AR gene. By excising the mutant AR transgene from muscle only, we achieved complete rescue of neuromuscular phenotypes in these mice, despite retaining strong CNS expression. Furthermore, we delivered an antisense oligonucleotide (ASO) directed against the human AR transgene by peripheral injection, and documented that peripheral ASO delivery could rescue muscle weakness and premature death in BAC fxAR121 mice. Our results reveal a crucial role for skeletal muscle in SBMA disease pathogenesis, and offer an appealing avenue for therapy development for SBMA and perhaps also for related motor neuron diseases.

Keywords: Amyotrophic lateral sclerosis, androgen receptor, antisense oligonucleotide, motor neuron, neurodegeneration, polyglutamine, skeletal muscle, spinal and bulbar muscular atrophy, transgenic mice

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Non-cell Autonomous Toxicity in Neurodegenerative Disorders

Neurodegenerative diseases are a spectrum of progressive disorders that target specific neuronal populations in different areas of the central nervous system (CNS). Despite widely different etiologies, neuronal degeneration represents a common finding for these diseases, which result in debilitating and typically fatal conditions. Research into the mechanistic basis of neurodegeneration has recently uncovered the importance of non-neuron cell populations for disease pathogenesis. The so-called “non-cell autonomous” nature of neuronal toxicity elicited by populations of supporting cells contributes to—and in some cases, initiates—the pathological cascade that culminates in neuron death.¹,²

In amyotrophic lateral sclerosis (ALS), a motor neuron disease characterized by significant motor neuron loss, a variety of cell types, including astrocytes, oligodendrocytes, and microglia, have been implicated in superoxide dismutase-1 (SOD-1)-mediated motor neuron toxicity.¹,³,⁴ Likewise, non-cell autonomous degeneration events have been described for spinal muscular atrophy (SMA), Huntington’s disease (HD),⁵ spinocerebellar ataxia 7 (SCA7),⁶ and Parkinson’s disease (PD),⁷ suggesting that this is a common feature for most neurodegenerative diseases. Thus, cell-to-cell communication may not only provide trophic support and insure proper network function, but, in the presence of toxic misfolded proteins, may also facilitate neuron dysfunction and destabilize otherwise healthy neurons. This represents a dramatic paradigm shift in our understanding of neurological disease pathogenesis with important implications for therapy development, as targeting non-neural cell types, both within and outside the CNS, could greatly benefit affected patients.

X-Linked Spinal and Bulbar Muscular Atrophy (SBMA): Brain or Brawn?

X-linked spinal and bulbar muscular atrophy (SBMA), also known as
Kennedy’s disease, is a neuromuscular disorder caused by expansion of a CAG triplet (coding for the amino acid glutamine) in the first exon of the Androgen Receptor (AR) gene. Control, unaffected populations carry anywhere from 5 to 34 CAG repeats in the AR gene, whereas SBMA patients have 37 or more CAG repeats. SBMA is characterized by adult-onset proximal muscle weakness due to lower motor neuron (MN) degeneration in the spinal cord and brain stem.

AR is a member of the steroid/thyroid hormone receptor family, and is expressed in both motor neurons and skeletal muscle. AR binds to androgens, in particular testosterone and dihydrotestosterone, and thereafter translocates into the nucleus, where it activates a network of androgen-responsive genes. Ligand-dependent nuclear localization of polyglutamine-AR is necessary for toxicity, and mutant AR accumulates in nuclear inclusions in motor neurons as well as in non-neuronal cells. Although such proteinaceous accumulations are a hallmark of polyglutamine disorders, evidence now strongly suggests that they may represent a protective cellular response to the mutant protein, sequestering toxic species into non-reactive inclusions. Ultimately, the sequestration of the mutant protein fails and visible aggregates are observed in the context of an ongoing process of neuronal demise. Polyglutamine-AR neurotoxicity is due to a loss-of-function of the mutant protein, resulting in varied downstream effects, including transcription dysregulation, impaired axonal transport, and mitochondrial dysfunction. SBMA patients also display signs of androgen insensitivity, including gynecomastia, reduced fertility, and testicular atrophy, indicating that a loss-of-function of normal AR action also contributes to disease pathogenesis.

Traditionally, SBMA has been regarded as a motor neuron disease, with most studies focusing on the mechanisms of motor neuron dysfunction and degeneration. However, several lines of evidence suggest that the mechanism underlying the neuromuscular phenotypes in SBMA is more complex than previously anticipated. First, muscle biopsies of affected SBMA patients consistently show mixed pathology, with both neurogenic and myopathic features. Second, overexpression of wild-type, non-expanded AR in skeletal muscle is sufficient to generate neuromuscular phenotypes reminiscent of SBMA (including axonopathy and gender bias) in mice. Third, muscle pathology is an early finding in a knock-in mouse model of SBMA, detectable long before any abnormalities appear in motor neurons. This suggests that the myopathic features observed in SBMA patients may not be due entirely to simple denervation, and that skeletal muscle could be playing a key role in the disease process.

We sought to directly test the contribution of skeletal muscle to SBMA disease pathogenesis by dissecting the role of skeletal muscle using an in vivo platform. To accomplish this, we generated a novel bacterial artificial chromosome (BAC) transgenic mouse model, featuring a floxed first Androgen Receptor exon to permit cell-type specific excision of the human AR transgene. We engineered the human AR transgene to carry 121 CAG repeats (BAC fxAR121), and the presence of large endogenous sequences, both upstream and downstream of the gene, allowed for proper expression of the transgene. Indeed, expression levels of transgenic human AR were very similar to endogenous mouse AR, at both the mRNA and protein levels. Furthermore, BAC fxAR121 mice develop a gender-restricted progressive neuromuscular phenotype, characterized by weight loss, motor deficits, muscle atrophy, myopathy, and a shortened lifespan.

By crossing the BAC fxAR121 mice with a human-skeletal actin Cre recombinase driver line (HSA-Cre), we obtained virtual elimination of AR-polyQ protein expression in skeletal muscle, without any appreciable reductions in polyglutamine-AR expression in the spinal cord. Behavioral testing and histopathology analysis revealed a complete rescue of SBMA neuromuscular and pathological phenotypes in BAC fxAR121—HSA-Cre bigenic mice. Our results indicate that AR-polyQ in skeletal muscle plays a primary role in SBMA pathogenesis, superseding motor neurons as the key site of AR-polyQ toxicity.

Skeletal Muscle: A ‘Meaty’ Target for SBMA Therapeutics

Our results predict that muscle-directed therapies hold great promise as definitive treatments for SBMA motor neuron disease. This is in agreement with previous reports suggesting that improving muscle function by insulin-like growth factor 1 (IGF-1) over-expression or supplementation can rescue SBMA phenotypes. To take this a step further, we targeted skeletal muscle polyQ-AR, using antisense-oligonucleotide (ASO) technology designed to target the CAG repeats expanded AR mRNA for destruction. Using the BAC fxAR121 mouse model described above, we found that peripheral ASO treatment reduces polyglutamine-AR protein expression levels in muscle tissue, but not in the spinal cord. This translated into significant rescue of weight loss, muscle weakness, and lethality in BAC fxAR121 mice. More importantly, this effect could be achieved even if peripheral ASO treatment began after disease onset, suggesting that this approach might be particularly relevant for patients, who are generally diagnosed after clinical findings appear. Similar results of phenotype rescue by ASO treatment after disease onset have been reported for Huntington’s disease mice, suggesting that polyglutamine repeat disorders are indeed highly amenable to this avenue of therapy.

Targeting skeletal muscle for SBMA therapies has many practical advantages over targeting the CNS. First, delivery of any compound to muscle is much more tractable than attempting to do the same for the spinal cord. Second, muscle is readily available for tissue sampling during clinical trials, allowing for directed analysis of pharmacological hallmarks. Third (and perhaps most important for patients), preferentially targeting AR in muscle could bypass the secondary side-effects associated with CNS reduction of AR function, which include loss of libido, lack of focus, and general malaise. This is particularly significant in light of our finding that intraventricular delivery of ASOs, which targets spinal cord but not skeletal muscle expression of polyglutamine expanded AR, had no effect on disease onset or progression in SBMA mouse models. We are currently undertaking similar motor neuron specific-genetic excision experiments.
in our lab, which will directly measure the contribution of motor neuron ‘cell autonomous’ toxicity in SBMA mice. However, our latest data indicating a primary role for skeletal muscle in SBMA pathogenesis is highly provocative, highlighting skeletal muscle as an appealing target for SBMA therapy development.

The mechanistic basis of muscle-mediated polyQ-AR toxicity to motor neurons remains unclear. Skeletal muscle provides trophic support and electrical stimuli for innervating motor neurons. But how does excision of polyQ-AR from muscle result in disease rescue in BAC fxAR121 mice? SBMA patients and transgenic mice have reduced expression of the key neurotrophic factors vascular endothelial growth factor (VEGF) and type II transforming growth factor β receptor (TGFbeta). Similarly, AR113Q-transgenic muscle shows decreased glial cell line-derived neurotrophic factor (GDNF) and neurotrophin-4 expression, along with aberrant myotonic electrical activity. Thus, polyQ-AR mediated transcriptional dysregulation of growth factors could be directly diminishing the trophic ability of SBMA muscle. In support of this, genes implicated in muscle function, myogenesis, and energy balance are dysregulated in skeletal muscle of models of SBMA. Removal of polyQ-AR from BAC fxAR121 muscle could, thus, restore its ability to sustain and nourish motor neurons, resulting in disease rescue. Consistent with this hypothesis, muscle-restricted overexpression of IGF-1, a known muscle anabolic factor, improved survival and neuromuscular phenotypes in SBMA mice. Furthermore, similar IGF-1 therapeutic benefits have been reported for models of ALS and SMA, further underscoring the responsiveness of neuromuscular diseases/MNDs to muscle-targeting therapeutics.

Current treatments for SBMA and other neurodegenerative proteinopathies remain unsatisfactory and focus mainly on managing symptoms to improve patients’ quality of life. Our work, however, suggests that reducing AR toxicity in muscle might be sufficient to generate important, quantitative effects in SBMA disease progression. Particularly, whereas IGF-1 clinical trials have failed to show benefits for ALS and SMA patients, the ability of IGF-1 to reduce polyQ-AR toxicity via activation of the PI3K/Akt pathway as well as directly induce muscle growth and regeneration merit a revisit of this molecule for SBMA preclinical trials.

**Muscle in Other Motor Neuron Diseases**

Muscle integrity appears to have a substantial effect on motor neuron survival in the face of polyQ-AR toxicity. Could this be applicable to other MNDs? Motor neurons and skeletal muscle are both spatially and functionally linked. Skeletal muscle is a major source of trophic and pro-survival factors for motor neurons, and this symbiotic relationship supports motor neuron axonal growth and regulates muscle innervation by incoming axons. Thus, it is not surprising that muscle homeostasis directly contributes to neuronal survival and, indeed, that muscle dysfunction may also play a key role in related motor neuron disorders, such as ALS and SMA.

SMA is a recessive neurodegenerative motor neuron disorder caused by mutations leading to reduced expression of survival motor neuron (SMN) protein. Muscle abnormalities are present in SMA models, including early muscle pathology that precedes motor neuron death, deficits in myoblast fusion, and an inability to sustain innervations, even from healthy nerves. Furthermore, motor neuron specific excision of SMN (Olig2-Cre SMA mice) yields only a mild version of SMA disease, with no appreciable muscle pathology, and peripheral delivery of ASOs that promote proper splicing of a duplicate SMN gene, known as SMN2, is a highly effective therapy in SMA mice. This suggests that non-neuronal cells are also significantly contributing to SMA disease severity. Indeed, SMN2 restoration by peripheral ASO delivery also rescued lower circulating levels of IGF-1 in SMA mice, an effect that correlated with enhanced hepatic expression of IGF-1. While muscle insulin/IGF-1 signaling (IIS) was not analyzed in this context, skeletal muscle is both a principal source and target of IIS, suggesting that some of the observed SMN2 ASO effects could be originating from muscle. In agreement with this, muscle-specific over-expression of IGF-1 does extend lifespan and rescues muscle wasting in SMA mice. Thus, improving muscle homeostasis (by enhancing IIS signaling or other survival and growth factor pathways) does result in SMA phenotype improvement. While these results contrast with the mild improvements observed in IGF-1 human clinical trials of SMA patients, timing and delivery of treatment may confound these results.

ALS is another motor neuron disease that results in major motor neuron loss and muscle atrophy. Elegant genetic studies have shown that glia and astrocyte toxicity mediate motor neuron death in SOD-1-linked ALS. However, while muscle expression of SOD-1 is indeed, intrinsically toxic to skeletal muscle, there was little to no motor neuron degeneration observed. Further studies revealed no appreciable rescue of disease phenotypes after muscle-specific excision of the mutant SOD-1 transgene, suggesting that muscle plays a secondary role in SOD-1-mediated neuron cell death. However, muscle expression of SOD-1 G93A protein did induce an inflammatory response in the spinal cord (particularly microglial cells) reminiscent of pre-symptomatic ALS1 mice. Since microglia play a key role in SOD-1-linked ALS disease progression, the possibility of a skeletal muscle/microglia response axis for disease phase modulation remains. Furthermore, SOD-1 related mutations are estimated to represent only about 10% of familial ALS. A recently discovered, highly penetrant, hexanucleotide expansion in C9orf72 is now believed to account for about 40% of familial ALS cases. Similarly, TDP-43 (TARDBP) and FUS/TLS mutations are also a common cause of familial ALS cases, with TDP-43 histopathology emerging as the hallmark finding in almost all sporadic ALS patients and most non-SOD1 familial ALS cases. As no studies have yet addressed the role of skeletal muscle in the context of these novel ALS-related mutations, skeletal muscle pathology cannot be excluded as a possible pathogenic factor for most ALS patients. Determining the contribution of skeletal muscle in ALS will have major implications for the development of therapies for this currently untreatable disorder.
Conclusions

In recent years, the use of conditional mouse models and cell co-culture systems has revealed the complex nature of neuronal dysfunction in many neurodegenerative disorders. Non-cell autonomous degeneration appears to be an overarching theme for these disorders. In particular, the symbiotic relationship between skeletal muscle and motor neurons has complicated the unraveling of cell-type intrinsic deficits that lead to neuromuscular disease. Our recent work indicates that skeletal muscle plays a primary role in SBMA disease pathogenesis, and provides strong proof of concept that therapies targeting muscle can have significant impact on patients suffering from this disease. More importantly, this realization underscores the need to carefully examine the role of skeletal muscle in related motor neuron disorders, as such work could suggest novel paths to effective treatments for SMA and ALS.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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