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Muscle Expression of Mutant Androgen Receptor Accounts for Systemic and Motor Neuron Disease Phenotypes in Spinal and Bulbar Muscular Atrophy

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SUMMARY

X-linked spinal and bulbar muscular atrophy (SBMA) is characterized by adult-onset muscle weakness and lower motor neuron degeneration. SBMA is caused by CAG-polyglutamine (polyQ) repeat expansions in the androgen receptor (AR) gene. Pathological findings include motor neuron loss, with polyQ-AR accumulation in intranuclear inclusions. SBMA patients exhibit myopathic features, suggesting a role for muscle in disease pathogenesis. To determine the contribution of muscle, we developed a BAC mouse model featuring a floxed first exon to permit cell-type-specific excision of human AR121Q. BAC fxAR121 mice develop systemic and neuromuscular phenotypes, including shortened survival. After validating termination of AR121 expression and full rescue with ubiquitous Cre, we crossed BAC fxAR121 mice with Human Skeletal Actin-Cre mice. Muscle-specific excision prevented weight loss, motor phenotypes, muscle pathology, and motor neuronopathy and dramatically extended survival. Our results reveal a crucial role for muscle expression of polyQ-AR in SBMA and suggest muscle-directed therapies as effective treatments.

INTRODUCTION

X-linked spinal and bulbar muscular atrophy (SBMA, Kennedy's disease) is an inherited neuromuscular disorder characterized by adult onset proximal muscle weakness due to lower motor neuron degeneration. SBMA patients also display signs of androgen insensitivity, including gynecomastia, reduced fertility, and testicular atrophy (Katsuno et al., 2012). This finding, together with the X-linked inheritance, led to analysis of the androgen receptor (*AR*) gene as the potential cause of SBMA. While a CAG repeat in the first exon of the *AR* gene varies in length from 5–34 triplets in normal individuals, SBMA patients were found to harbor repeats ranging from 37–66 CAG repeats (La Spada et al., 1991). As CAG encodes the amino acid glutamine (Q), SBMA was the first disorder identified to result from expansion of a CAG-polyQ repeat tract. Eight other inherited neurodegenerative disorders were subsequently found to be caused by expanded CAG repeats; hence, in addition to SBMA, the CAG-polyQ repeat disease category includes Huntington's disease (HD), dentatorubral pallidoluysian atrophy (DRPLA), and six forms of spinocerebellar ataxia: SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 (La Spada and Taylor, 2010).

For decades, research into the basis of neurological disease focused upon the contribution of neuronal dysfunction to disease pathogenesis. However, over the last 15 years, there has been a growing appreciation of the importance of nonneuronal cells in maintaining neuron function and contributing to

neurological disease pathogenesis (Garden and La Spada, 2012). In amyotrophic lateral sclerosis (ALS), the inability to recapitulate SOD1 neurotoxicity in transgenic mice upon expression of mutant SOD1 in motor neurons argued against cell autonomous degeneration (Lino et al., 2002; Pramatarova et al., 2001). Chimeric expression of mutant and normal SOD1 in the spinal cords of mice demonstrated a role for nonneuronal cells in ALS motor neuron degeneration and revealed that astrocytes and microglia are key determinants of disease onset and disease progression (Clement et al., 2003). Subsequently, conditional gene silencing of the ubiquitously expressed SOD1 mutant within astrocytes and microglia indicated that mutant SOD1 within either glial cell type is a key determinant of disease progression (Boillée et al., 2006; Yamanaka et al., 2008), while similar mutant gene silencing in $NG2^+$ precursor cells of oligodendrocytes is a key contributor to disease onset (Kang et al., 2013). In the CAG-polyQ repeat disease field, careful study of a line of SCA7 transgenic mice revealed that Purkinje cell neurodegeneration occurred even when the polyQ-ataxin-7 transgene was not expressed in Purkinje cells, leading to a hypothesis of noncell autonomous SCA7 neurodegeneration (Garden et al., 2002). As Purkinje cell neurons are intimately associated with a specialized astroglial cell type known as the Bergmann glia, SCA7 transgenic mice were engineered to express mutant ataxin-7 in only Bergmann glial cells in the cerebellum. These animals developed cerebellar ataxia and Purkinje cell degeneration, demonstrating the noncell autonomous nature of polyQ neurodegeneration (Custer et al., 2006). Studies in HD and in Parkinson's disease mouse models have similarly shown that expression of mutant disease protein in one cell type is capable of producing dysfunction and demise of a different neuronal cell type, especially for cells in direct communication with one another (reviewed in Ilieva et al., 2009). All of this preceding work suggests an overarching theme in neurodegenerative disease pathogenesis: preferential degeneration of select neuron populations does not necessarily stem from intrinsic molecular pathology restricted to the neuron subtype of interest (i.e., cell autonomous toxicity), but rather often results from pathological processes occurring in one or more neighboring cell types that perform crucial functions upon which the exquisitely vulnerable neuron subtype stalwartly depends (i.e., noncell autonomous toxicity).

Skeletal muscle is a major source of trophic support for innervating motor neurons and has been shown to contribute not only to neuron survival during development, but also to synaptic activity and axonal function (Funakoshi et al., 1995). SBMA patients often exhibit features of myopathy, as progressive muscle weakness occurs in the context of elevated serum creatine kinase levels (Katsuno et al., 2012). Muscle biopsies of SBMA patients reveal mixed pathological findings, with both myopathy and neurogenic atrophy features (Sorarù et al., 2008). Knockin mice expressing AR with 113 glutamines (AR113Q) develop early myopathy findings with little or no significant motor neuron loss until late in their disease course (Yu et al., 2006), consistent with muscle as a key site for SBMA disease pathogenesis. Moreover, while widespread transgenic expression of human AR20Q at levels comparable to endogenous AR does not produce a neuromuscular phenotype (Sopher et al., 2004), skeletal muscle-specific overexpression of wild-type AR (AR22Q) in mice is sufficient to produce SBMA-like neuromuscular disease, accompanied by denervation of target muscle and motor neuron axon degeneration, complete with androgen dependence, gender bias, axonopathy, and muscle wasting (Monks et al., 2007). Additionally, testosterone treatment of asymptomatic female transgenic mice overexpressing the AR22Q transgene in muscle yielded pronounced neuromuscular deficits, but without detectable motor neuron pathology (Johansen et al., 2009). Although development of SBMA-like disease phenotypes upon skeletal-muscle-specific expression of AR22Q may simply stem from the very high level of AR transgene overexpression in this model (Monks et al., 2007), the SBMA-like phenotype in males can be reversed upon cessation of testosterone treatment. These findings indicate that muscle-restricted AR toxicity may underlie SBMA disease pathogenesis, and therapies targeting skeletal muscle may prove beneficial for patients. In support of this thesis, transgenic expression of anabolic insulin growth factor-1 (IGF-1) directed to muscle can rescue nerve pathology in SBMA transgenic mice, producing a significant extension in lifespan (Palazzolo et al., 2009).

Although the preceding studies suggest a role for muscle dysfunction as a component of SBMA motor neuronopathy, the necessity of polyQ-AR expression in muscle for SBMA disease pathogenesis is yet to be investigated. To directly examine the role of muscle expression of AR in SBMA pathogenesis, we developed a BAC transgenic mouse model featuring a floxed first exon to permit cell-type-specific excision of the human AR gene. We engineered the human AR transgene to carry 121 CAG repeats (BAC fxAR121) and found that BAC fxAR121 mice develop a gender-restricted, progressive neuromuscular phenotype, characterized by weight loss, motor deficits, muscle atrophy, myopathy, and shortened lifespan. By conditionally terminating expression of mutant polyQ-AR in the skeletal muscles of BAC fxAR121 male mice, we document a crucial role for muscle expression of mutant polyQ-AR in SBMA disease pathogenesis and predict that muscle-directed therapies hold great promise as definitive treatments for SBMA motor neuron degeneration.

RESULTS

Generation and Expression Analysis of BAC fxAR121 Transgenic Mice

The human *AR* gene is composed of eight exons that span ~180 kb of DNA. Using the Human Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway), we identified two overlapping BACs that span the entire length of the *AR* gene. Through a recombineering strategy, we fused these two BACs to create an *AR* BAC construct that, in addition to all eight *AR* exons, includes ~50 kb of DNA 5′ to the first *AR* exon and ~30 kb of DNA 3′ to the last *AR* exon (Sopher and La Spada, 2006). With this recombineering approach, we also introduced a 121 CAG repeat tract and engineered two loxP sites flanking *AR* exon 1 to create a floxed AR CAG121 BAC (BAC fxAR121) transgenic construct (Figure 1A). We then derived BAC fxAR121 transgenic mice, and when we performed RT-PCR analysis of human AR (hAR) transgene expression, we determined that hAR RNA