Peripheral Androgen Receptor Gene Suppression Rescues Disease in Mouse Models of Spinal and Bulbar Muscular Atrophy

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SUMMARY

Spinal and bulbar muscular atrophy (SBMA) is caused by the polyglutamine androgen receptor (polyQ-AR), a protein expressed by both lower motor neurons and skeletal muscle. Although viewed as a motor neuronopathy, data from patients and mouse models suggest that muscle contributes to disease pathogenesis. Here, we tested this hypothesis using AR113Q knockin and human bacterial artificial chromosome/clone (BAC) transgenic mice that express the full-length polyQ-AR and display androgen-dependent weakness, muscle atrophy, and early death. We developed antisense oligonucleotides that suppressed AR gene expression in the periphery but not the CNS after subcutaneous administration. Suppression of polyQ-AR in the periphery rescued deficits in muscle weight, fiber size, and grip strength, reversed changes in muscle gene expression, and extended the lifespan of mutant males. We conclude that polyQ-AR expression in the periphery is an important contributor to pathology in SBMA mice and that peripheral administration of therapeutics should be explored for SBMA patients.

INTRODUCTION

Spinal and bulbar muscular atrophy (SBMA) is one of nine untreatable diseases caused by CAG/glutamine tract expansions. In SBMA, a polyglutamine (polyQ) tract near the amino terminus of the androgen receptor (AR) leads to hormone-dependent protein unfolding and to the loss of lower motor neurons in the brainstem and spinal cord of affected males (Lieberman and Fischbeck, 2000). Clinical onset occurs in adolescence to adulthood and is characterized initially by muscle cramps and elevated serum creatine kinase (Katsuno et al., 2006b; Sperfeld et al., 2002). These myopathic features commonly precede muscle weakness, which inevitably develops as the disease progresses and is most severe in the proximal limb and bulbar muscles. As with all of the polyglutamine disorders, the mechanisms that lead to selective neuronal dysfunction and degeneration are poorly understood, and disease-modifying therapies are currently unavailable.

Several general principles have emerged from the study of SBMA model systems that guide our understanding of disease pathogenesis. Binding of testosterone or dihydrotestosterone to the polyQ-AR promotes ligand-dependent unfolding and nuclear translocation of the mutant protein (Katsuno et al., 2002; Takeyama et al., 2002). These steps are required for pathogenesis and underlie the occurrence of disease only in men. The mutation leads to a partial loss of transactivation function (Chamberlain et al., 1994; Irvine et al., 2000; Kazemi-Esfarjani et al., 1995; Lieberman et al., 2002; Mhatre et al., 1993), and while this may contribute to features of androgen insensitivity, neuromuscular degeneration is mediated by a toxic gain of function conferred by protein unfolding. In SBMA, as in other CAG/polyQ disorders, the mutant protein disrupts multiple downstream pathways, and toxicity likely results from the cumulative effects of altering a diverse array of cellular processes including transcription, RNA splicing, axonal transport, and mitochondrial function (Katsuno et al., 2006a; Kemp et al., 2011; McCampbell et al., 2000; Morfini et al., 2006; Ranganathan et al., 2009; Szebenyi et al., 2003; Yu et al., 2009). The existence of divergent mechanisms of toxicity suggests that potential treatments targeting a single downstream pathway are likely to be incomplete or unsuccessful. In contrast, efforts to target the polyQ-AR as the proximal mediator of toxicity by harnessing cellular machinery to promote its degradation hold promise for therapeutic intervention. Because the Hsp90-based chaperone machinery controls proteostasis of the AR (Morishima et al., 2008; Thomas et al., 2004, 2006; Wang et al., 2010), genetic and pharmacological approaches to promote Hsp70-dependent ubiquitination have been shown to facilitate degradation of the mutant protein (Wang et al., 2013).

Insights into the mechanisms underlying selective neuromuscular degeneration in SBMA have come from the study of mouse

models. Previous analysis of AR113Q knockin mice suggested that pathology arising in skeletal muscle contributes to the disease phenotype (Yu et al., 2006a). In these mice, denervation and myopathy precede spinal cord pathology, consistent with the notion that myopathy is an early disease manifestation (Jordan and Lieberman, 2008). Supporting a role for muscle in pathogenesis are data from transgenic mice that overexpress wild-type (WT) AR only in skeletal muscle and show hormone-dependent myopathy and motor axon loss (Johansen et al., 2009; Monks et al., 2007). That muscle both contributes to the SBMA phenotype and provides a therapeutic target is supported by data showing diminished disease severity in polyQ-AR transgenic mice with genetic overexpression of IGF-1 in skeletal muscle (Palazzolo et al., 2009) or with peripheral IGF-1 administration (Rinaldi et al., 2012).

Here, we test an alternative strategy to ameliorate toxicity in mouse models of SBMA by suppressing polyQ-AR expression using antisense oligonucleotides (ASOs). We use these compounds to specifically target polyQ-AR expression in the periphery. We demonstrate using two mouse models that peripheral gene suppression of the polyQ-AR rescues deficits in muscle weight, fiber size, and grip strength; reverses changes in muscle gene expression; and extends lifespan of mutant males. We conclude that polyQ-AR expression in the periphery is an important contributor to pathology in SBMA mice and that peripheral administration of therapeutics should be explored for SBMA patients.

RESULTS

Subcutaneous ASO Suppresses PolyQ-AR Expression in the Periphery but Not the Spinal Cord

We sought to define the contribution of peripherally expressed polyQ-AR to the phenotype of SBMA mice and to determine whether peripheral tissue is a therapeutic target. To accomplish this, we suppressed AR expression by subcutaneous administration of ASOs. Because these compounds do not cross the blood-brain barrier (Geary, 2009; Yu et al., 2007), this strategy selectively targeted AR in peripheral tissues such as skeletal muscle. We developed 16-mer chemically modified ASO complementary to human and mouse or human AR transcripts (ASO1 and ASO2 respectively; Table S1). These 2′,4′-constrained ethyl (cEt) gapmer ASOs show increased stability, tolerability, and potency upon in vivo administration (Seth et al., 2009). Initial characterization demonstrated dose-dependent suppression of human and mouse AR mRNAs in cell culture by targeted, but not control, ASOs (Figure 1A). Similarly, subcutaneous administration of targeted, but not control, ASOs led to dose-dependent suppression of AR mRNA and protein expression in skeletal muscle of WT male mice (Figures 1B and 1C). Serum testosterone levels of these males exhibited modest variability, and treatment with targeted ASOs did not result in a significant alteration (Figure S1).

We used these compounds to determine the extent to which suppressing peripheral expression of the polyQ-AR rescued the phenotype of SBMA mice. This was accomplished using both AR113Q knockin (Yu et al., 2006a; Yu et al., 2006b) and human bacterial artificial chromosome/clone (BAC) fxAR121 transgenic

mice (Cortes et al., 2014). Both of these models express the full-length polyQ-AR under the regulation of its endogenous promoter. These mice display a similar androgen-dependent phenotype characterized by weakness, muscle atrophy, and early death. In both models, subcutaneous administration of ASOs decreased AR expression in skeletal muscle, but not spinal cord. To determine the targeting efficacy and specificity in skeletal muscle, BAC fxAR121 transgenic males were treated with the human AR-targeted ASO2. Subcutaneous administration of human AR-targeted ASO2, but not control ASO, led to dose-dependent suppression of transgene expression in skeletal muscle without affecting expression of the endogenous mouse allele (Figure 1D). While treatment with ASO2 (50 mg/kg, twice weekly, starting at 11 weeks) specifically suppressed transgene expression in skeletal muscle of BAC transgenic mice, the human and mouse cross-reactive ASO1 suppressed both transgenic human AR and endogenous mouse AR mRNA (Figure 2A), demonstrating target selectivity. Quantitative real-time RT-PCR demonstrated >95% reduction of human AR mRNA levels in skeletal muscle of treated males. No significant change in mouse or human AR mRNA levels was detected in brain or spinal cord of treated mice (Figure 2A), indicating that subcutaneous administration selectively targeted peripheral AR expression. The decrease of AR mRNA in muscle was associated with comparable reduction in AR protein immunoreactivity in skeletal muscle nuclei following treatment (Figure 2B).

AR113Q knockin males express a hybrid humanized AR in which most of mouse AR exon 1 has been replaced by human sequence (Yu et al., 2006b). Therefore, we used ASO1, a human and mouse AR cross-reactive ASO, to treat these mice. Subcutaneous administration of ASO1 (50 mg/kg, twice per week for 4 weeks and then once per week) or saline was initiated at 8 weeks and continued until 26 weeks. Treatment resulted in a significant decrease in AR mRNA levels in quadriceps muscle, but not spinal cord (Figure 2C). This decrease in AR expression was long-lived, as partial mRNA reduction was detected in skeletal muscle harvested from mice at 36 weeks of age, 10 weeks after the termination of treatment; by 46 weeks of age (20 weeks posttreatment washout), AR mRNA levels in muscle approached those of saline-treated controls. Similarly, sustained suppression of transcripts by ASOs has been reported in skeletal muscle of myotonic dystrophy mice (Wheeler et al., 2012). Knockdown of AR mRNA levels in muscle was associated with a ∼90% decrease in AR protein levels (Figure 2D). We observed a slow recovery in AR protein levels in muscle after the termination of treatment, with expression remaining ∼80% lower than controls at 10 weeks posttreatment and ∼65% lower than controls at 20 weeks posttreatment (age 46 weeks). In contrast, AR mRNA and protein expression in spinal cord were not significantly altered by peripheral ASO delivery.

Peripheral PolyQ-AR Suppression Rescues Disease in SBMA Mice

Significant amelioration of disease phenotype following AR gene suppression in the periphery was observed in both SBMA mouse models. BAC fxAR121 mice exhibited an age-dependent loss of grip strength and body mass (Figures 3A