Molecular Pathophysiology and Disease-Modifying Therapies for Spinal and Bulbar Muscular Atrophy

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Spinal and bulbar muscular atrophy (SBMA), or Kennedy disease, is an adult-onset lower motor neuron disease characterized by slowly progressive muscle weakness and atrophy. The disease is caused by the expansion of a trinucleotide CAG repeat encoding a polyglutamine tract within the first exon of the androgen receptor (AR) gene. During the 2 decades since the discovery of the AR gene mutation in SBMA, basic and clinical research have deepened our understanding of the disease phenotype and pathophysiology. Spinal and bulbar muscular atrophy exclusively affects men, whereas women homozygous for the AR mutation do not fully develop the disease. The ligand-dependent nuclear accumulation of pathogenic AR protein is central to the pathogenesis, although additional steps, eg, DNA binding and interdomain interactions of AR, are required for toxicity. Downstream molecular events, eg, transcriptional dysregulation, axonal transport disruption, and mitochondrial dysfunction, are implicated in the neurodegeneration in SBMA. Pathogenic AR-induced myopathy also contributes to the degeneration of motor neurons. Several potential therapies, including hormonal manipulation, have emerged from animal studies, some of which have been tested in clinical trials.


Spinal and bulbar muscular atrophy (SBMA), or Kennedy disease, is a late-onset, hereditary motor neuron disease characterized by slowly progressive muscle weakness and atrophy of bulbar, facial, and limb muscles. It is estimated to be 1 to 2 per 100,000 with no known racial preferences. Spinal and bulbar muscular atrophy fully manifests only in men, whereas female patients may express subclinical abnormalities in electrophysiological or laboratory test findings. The onset of weakness is typically between 30 and 60 years of age but is often preceded by nonspecific symptoms, eg, postural tremor, fatigue, and muscle cramps. Patients often demonstrate signs of androgen insensitivity, including gynecomastia and testicular atrophy. Creatine kinase serum levels are elevated in the majority of patients, while hypertension, hyperlipidemia, liver dysfunction, and glucose intolerance may also be observed. The progression of SBMA is usually slow, but a considerable number of patients need assistance to walk in their 50s or 60s. Respiratory tract infections due to bulbar palsy are the leading cause of death.

Spinal and bulbar muscular atrophy is caused by the expansion of a trinucleotide CAG repeat encoding a polyglutamine tract within the first exon of the androgen receptor (AR) gene. This CAG repeat ranges from 9 to 36 in normal subjects to 38 to 62 in patients with SBMA. There is an inverse correlation between the CAG repeat size and the age at onset of SBMA as reported in other polyglutamine-mediated neurodegenerative diseases, including Huntington disease, dentatorubral-pallidoluysian atrophy, and several forms of spinocerebellar ataxia. The cardinal histopathological finding is the loss of lower motor neurons.
motor neurons in the anterior horns of the spinal cord and in the brainstem motor nuclei except for the third, fourth, and sixth cranial nerves.\textsuperscript{5}

Basic studies using animal models led to the elucidation of the hormone-dependent pathogenesis of SBMA and the development of molecular-targeted therapies, the efficacy of which have yet to be clarified in clinical trials. Herein, we outline the molecular pathophysiology, summarize the therapeutic strategies currently being developed, and discuss the future direction of translational research of SBMA.

**MOLECULAR PATHOPHYSIOLOGY OF SBMA**

The AR is a 110-kDa nuclear receptor that belongs to the steroid/thyroid hormone receptor family and contains the following functional domains: amino-terminal transactivating domain, in which the CAG trinucleotide repeat is located, DNA-binding domain, and ligand-binding domain (Figure 1). The expansion of the polyglutamine tract attenuates the transcriptional activity of AR, suggesting that the loss of its normal function possibly contributes to androgen insensitivity in patients with SBMA.\textsuperscript{3} The pivotal cause of neurodegeneration in SBMA is, however, believed to be a gain of toxic function of pathogenic AR due to the expansion of the polyglutamine tract, since the complete loss of functional AR does not lead to motor impairment in humans or mice.\textsuperscript{7} While the elongated polyglutamine tract itself is capable of causing neuronal damage, the domains outside the polyglutamine tract also modify the toxicity of pathogenic AR.\textsuperscript{8}

**Nuclear Accumulation of the Mutant AR**

The expansion of the polyglutamine stretch alters the conformation of AR, resulting in the diffuse nuclear accumulation of the proteins, the extent of which is dependent on the CAG repeat size.\textsuperscript{9} This nuclear accumulation of pathogenic AR is observed in the affected neurons and in visceral organs, including scrotal skin.\textsuperscript{2} The toxicity of the polyglutamine-expanded AR is abolished by a mutation in the receptor’s nuclear localization signal or the addition of a nuclear export signal to the protein, strongly supporting the idea that nuclear localization is a prerequisite for toxicity.\textsuperscript{10,11} Polyglutamine-expanded AR also forms microscopically visible intraneuronal inclusion bodies, a classic histopathological hallmark of polyglutamine diseases, although their role in pathogenesis is likely to be neuroprotective.\textsuperscript{8}

**Ligand-Dependent Toxicity of Pathogenic AR**

Spinal and bulbar muscular atrophy is unique among the polyglutamine diseases in that the manifestation of the neuromuscular phenotypes is sex dependent. Although this disease exclusively affects men with a high penetration, homozygous women do not show detectable motor dysfunction, suggesting that SBMA is not a canonical X-linked recessive disorder.\textsuperscript{12} The AR forms a multimeric in-active complex with interacting proteins, including heat shock proteins, in the cytoplasm and translocates into the nucleus when it binds to ligands. This ligand-dependent intracellular trafficking of AR appears to play a causative role in the pathogenesis of SBMA. In transgenic mouse models of SBMA, testosterone deprivation by castration reverses the motor dysfunction of male mice, suggesting that the ligand amplifies the neurotoxicity of pathogenic AR by accelerating its nuclear translocation.\textsuperscript{13,14} Conversely, administration of testosterone aggravates the symptoms and histopathological features in mice and humans.\textsuperscript{2} The ligand-dependent neurotoxicity of pathogenic AR is also demonstrated in a fly model of SBMA, in which androgen does not only facilitate the nuclear translocation of pathogenic AR but alters its conformation.\textsuperscript{10} Furthermore, Akt-mediated phosphorylation of AR reduces ligand binding and thus attenuates the toxicity of polyglutamine-expanded AR.\textsuperscript{15}

Although it is clear that ligand-dependent nuclear localization is necessary for the induction of neurodegeneration by pathogenic AR, recent studies indicate that nuclear translocation is not sufficient for its toxicity. For instance, a mutation that inhibits the binding of pathogenic AR to DNA without affecting ligand binding abolishes neurodegeneration, indicating that DNA binding is necessary for pathogenic AR-mediated neurodegeneration.\textsuperscript{16} In addition to nuclear translocation and DNA binding, testosterone mediates the interdomain interaction of the amino- and carboxyl-terminal domains (N/C interaction) of AR, which is also required for the toxicity of pathogenic AR.\textsuperscript{17} The ligand-dependent interactions between the carboxyl-terminal AF-2 domain and coregulators are another requisite for the toxicity.\textsuperscript{16}

**Transcriptional Dysregulation**

Transcriptional dysregulation due to a decrease in histone acetylation has been implicated in the pathogenesis of polyglutamine diseases. Acetylation of the nuclear histone H3 is significantly diminished in the spinal cord of a transgenic mouse model of SBMA.\textsuperscript{18} Pathogenic AR-induced transcriptional dysregulation leads to a decreased expression of several genes, eg, vascular endothelial growth factor and type II transforming growth factor–β receptor, that are required for neuronal survival.\textsuperscript{16,20}

**Mitochondrial Dysfunction**

The polyglutamine-expanded AR represses the transcription of subunits of peroxisome proliferator-activated receptor γ coactivator 1, a transcriptional coactivator that regulates the expression of nuclear-encoded mitochon-
drial proteins, in cellular and mouse models of SBMA. Expression of pathogenic AR in the presence of ligand results in mitochondrial membrane depolarization and an elevated level of reactive oxygen species, which is blocked by treatment with antioxidants. The amino-terminal fragment of polyglutamine-expanded AR also stimulates the Bax-dependent apoptotic pathway via the activation of c-Jun N-terminal kinase.

**Myopathy**

Although neurons are a distinct target of polyglutamine-mediated toxicity, pathological changes in nonneuronal cells also play a role in the development of neurodegeneration. Myopathic changes in muscle biopsy specimens and elevated serum creatine kinase levels in patients with SBMA imply a direct involvement of the skeletal muscle. Morphologic and molecular changes associated with myopathy are detectable before the initiation of neurodegeneration in a knock-in mouse model of SBMA. Additionally, the targeted overexpression of wild-type rat AR in skeletal muscle induces motor axon loss in mice. These observations suggest that AR-mediated myopathy contributes to noncell autonomous degeneration of spinal motor neurons.

**Disruption of Axonal Transport**

Pathogenic AR suppresses kinesin-1 microtubule-binding activity and eventually disrupts anterograde axonal transport via c-Jun N-terminal kinase activation. Decreased messenger RNA levels of dynactin 1, a motor protein that regulates retrograde axonal transport, and eventual impairment of axonal trafficking were observed in a transgenic mouse model of SBMA. The disruption of retrograde axonal transport was also documented in a knock-in mouse model of SBMA and mice overexpressing wild-type AR in muscle, although this is not the case in another mouse model.

**POTENTIAL THERAPIES FOR SBMA**

**Ligand-Targeted Interventions**

On the basis of the ligand-dependent toxicity of the polyglutamine-expanded AR, several approaches that ma-
nipulate androgens have been developed and tested in animal models and clinical trials. Leuprorelin is a potent luteinizing hormone-releasing hormone analog that suppresses the release of testosterone and has been used for the treatment of a variety of sex hormone–dependent diseases including prostate cancer and prepuberty. In a transgenic mouse model of SBMA, leuprorelin inhibits nuclear accumulation of pathogenic AR, resulting in a marked improvement of the neuromuscular phenotype.\(^29\) In a phase 2 clinical trial, 12 months' treatment with leuprorelin significantly diminished the serum level of creatine kinase and suppressed the nuclear accumulation of pathogenic AR in the scrotal skin of patients. The frequency of I C2-positive neurons in the anterior horn and brainstem of an autopsied patient, who received leuprorelin for 2 years, was less than in non-treated patients with SBMA.\(^30\) Nevertheless, a randomized placebo-controlled multicentric clinical trial of this drug showed no definite effect on motor functions, although there was the improvement of swallowing function in a subgroup of patients whose disease duration was less than 10 years.\(^31\) Neither was the effectiveness of dutasteride, a 5α-reductase inhibitor that inactivates testosterone, clearly shown in a phase 2 clinical trial.\(^32\) Although the results of these studies are inconclusive, their findings do not exclude the possibility that ligand-targeted therapies slow the progression of SBMA. Given the strong evidence shown in basic studies, this hypothesis needs to be further verified in clinical trials with a rigorous and efficient design.

The AR coregulators, including ARA70, are alternative therapeutic targets, because they control the function and cellular distribution of AR. The curcumin-related compound 5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one dissociates AR and ARA70, resulting in the suppression of pathogenic AR aggregation and the amelioration of neuromuscular symptoms in a mouse model of SBMA.\(^33\) One dissociates AR and ARA70, resulting in the suppression of pathogenic AR aggregation and the amelioration of neuromuscular symptoms in a mouse model of SBMA.\(^34\) The AR coregulators, including ARA70, are alternative therapeutic targets, because they control the function and cellular distribution of AR. The curcumin-related compound 5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one dissociates AR and ARA70, resulting in the suppression of pathogenic AR aggregation and the amelioration of neuromuscular symptoms in a mouse model of SBMA.\(^33\)

**Activation of the Protein Quality-Control Systems**

The ubiquitin-proteasome system and autophagy constitute the most important cellular defense machinery against the accumulation of misfolded proteins.\(^34\) Treatment with 17-allylamino-17-demethoxygeldanamycin, a potent Hsp90 inhibitor, facilitates the proteasomal degradation of pathogenic AR and thereby ameliorates the motor deficits in a mouse model of SBMA.\(^35,36\) Pathogenic AR retained in the cytoplasm is subjected to protein degradation via the autophagic/lysosomal pathway.\(^37\) Pharmacological activators of autophagy, including rapamycin, also suppress neuronal damage in cellular and fly models of SBMA.\(^38,39\)

Heat shock proteins mitigate polyglutamine-mediated cytotoxicity by refolding and solubilizing the pathogenic proteins. Overexpression of the inducible form of human Hsp70 facilitates the proteasomal degradation of abnormal AR protein and markedly ameliorates the symptomatic and histopathological phenotypes in SBMA mice.\(^37\) Favorable effects are also exerted by a pharmacological induction of molecular chaperones.\(^38\)

**Other Experimental Therapies**

Butyrate, a histone deacetylase inhibitor, restores the transcriptional machinery and thereby ameliorates the neuromuscular phenotype of SBMA mice through the up-regulation of histone acetylation.\(^18\) Muscle-restricted overexpression of insulinlike growth factor 1 augments Akt-mediated AR phosphorylation, leading to suppression of AR accumulation and eventual amelioration of neuromuscular deficits in a transgenic mouse model of SBMA.\(^33\) Vascular endothelial growth factor is also neuroprotective for SBMA.\(^19\) Modulation of mitochondrial function is another potential therapeutic approach, although there is currently insufficient evidence of its effectiveness from animal studies.\(^21\)

**FUTURE DIRECTIONS**

During the 2 decades since the discovery of the AR gene mutation in SBMA, basic and clinical research have deepened our understanding of the disease phenotype and pathophysiology (Figure 2). However, despite positive results in animal studies, no therapy has proved to be effective in clinical trials, suggesting a need to elucidate the entire disease mechanism, the early initiation of therapeutic intervention, and sensitive outcome measures to evaluate drug effect. In addition, the efficacy of nonpharmacological approaches, including physical therapy, should also be rigorously tested.\(^2\) Further basic and clinical studies are needed to elucidate the pathogenesis of SBMA and develop effective therapies.

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