Progress toward the development of treatment of spinal and bulbar muscular atrophy

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Abstract

Introduction: With greater longevity, the prevalence of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis becomes increasing, but these still remain intractable. This is also the case for a hereditary motor neuron disease, spinal and bulbar muscular atrophy or SBMA.

Areas covered: SBMA typically affects adult males, eliciting motor deficits due to progressive loss of lower motor neurons, while females do not manifest neurological signs. It results from a CAG-trinucleotide repeat expansion in the *androgen receptor* or *AR* gene which is translated into an expanded polyglutamine tract within the encoded protein. Ligand-dependent nuclear accumulation of the mutant AR is implicated to lead to transcriptional dysregulation and subsequent defects in pivotal cellular functions, causing motor neuron death.

Expert opinion: Even though the pathogenesis has not been fully understood, recent advances especially in the dissection of pathomechanisms associated with the pathogenic AR protein and aggregates accelerate the development of potential targeted therapeutics such as administration of leuprorelin acetate, a potent luteinizing hormone-releasing hormone analog, which has being successfully tested in clinical trials. Combination therapies antagonizing causative mutant AR and its downstream targets hold promise to further open a therapeutic avenue for the establishment of disease modifying drugs for SBMA.

Keywords

Androgen receptor; leuprorelin; polyglutamine disease; proteostasis; RNA; spinal and bulbar muscular atrophy

Introduction

Improvement of life circumstances including advanced dietary or sanitary conditions, and recent advances in medical care, contribute to incremental increases in life expectancy, which, in turn, may be a leading cause of morbidity and mortality in patients with neurodegenerative diseases [1-3]. Insoluble aggregate formation by a pathogenic protein in or around vulnerable neurons and glia cells is a common pathological hallmark of neurodegenerative diseases though they present a great diversity of clinical manifestations. Furthermore, a new paradigm of protein propagation, which is reminiscent of the transmission property of prion disease, has been recently established and is considered to promote spreading pathogenic aggregates [4]. This induces gain-of-function toxicity occasionally with a concomitant loss or augmentation of function of a respective native protein in specific cell populations. However, neither effective therapeutics nor a screening system to identify disease risk for prevention is currently established, warranting further investigation of the pathomechanisms. Polyglutamine (polyQ) diseases are caused by a CAG expansion within protein-coding regions of physically and functionally distinct genes whose products are widely expressed in mammalian tissues. Encoded polyQ-expanded proteins are inclined to misfold and form pathogenic cellular inclusion or aggregation that cannot be easily degraded [5]. Proteostasis, the blend words, protein and homeostasis, refers to cellular processing of the folding, trafficking and degradation of proteome. Imbalances of proteostasis result in the accumulation of misfolded proteins or excessive protein degradation, and can be associated with neurodegeneration. In addition to protein-mediated toxicity, however, toxic roles of

expanded repeat-containing RNA are recognized as emerging mechanisms in microsatellite expansion disorders as well as polyQ diseases [6].

Nine polyQ diseases with the CAG mutations in the distinct genes have been reported so far; SBMA, followed by Huntington's disease (HD), dentatorubralpallidoluysian atrophy and six types of spinocerebellar ataxia (SCA1, 2, 3, 6, 7 and 17). Importantly, these diseases share common phenotypes of adult-onset, progressive neuronal degeneration with accumulation of misfolded proteins in primarily affected neurons. SBMA, the first identified polyQ disease [7], causes lower motor neuron loss and uniquely exhibits myopathic features in a gender-dependent manner, and explicitly leaves cerebrum or cerebellum intact in contrast to other autosomal dominant polyQ diseases. AR is a ligand-activated nuclear receptor, and testosterone, a natural AR ligand, facilitates intranuclear accumulation of polyQ AR [8]. However, cell-specific pathogenesis such as the vulnerability of lower motor neurons and myocytes remains unclear. Recent efforts to develop novel therapies mainly focused on antagonizing the mutant protein and its functional property of nuclear translocation, or targeting effectors of downstream signaling pathways, which have been shown to be effective in the cell and animal models, though no single agent can rescue SBMA patients. However, there are several potential drug candidates such as leuprorelin which have been evaluated in clinical trials. Here, we review recent understandings of the pathomechanisms of SBMA and polyQ diseases, and summarize progress of therapeutic approaches and promises, and our research achievements for SBMA including the ongoing clinical studies.

Clinical and histopathological characteristics of SBMA

SBMA, or Kennedy's disease, is an X-linked motor neuron disease, manifesting late-onset, progressive neuromuscular phenotypes. The prevalence of SBMA is estimated at 1-2 per 100,000 individuals, though patients may be misdiagnosed as amyotrophic lateral sclerosis and 5q-spinal muscular atrophy, more common motor neuron diseases. A high incidence of SBMA was reported in the isolated region of Western Finland. No effective disease-modifying therapy is currently available. The causative mutations are the CAG repeat expansions in the first coding exon of X-chromosomal *AR* gene that is translated into the protein harboring an expanded polyQ tract [7]. Thus, SBMA is referred as polyQ diseases, and its identification has triggered serial discoveries of other polyQ diseases and microsatellite expansion disorders. SBMA affects only male, and female carriers remain asymptomatic, though subclinical neurogenic abnormalities shown by electromyogram are occasionally evident [9]. It causes progressive muscle weakness, atrophy and fasciculations in facial, bulbar and proximal muscles with motor neuron degeneration in the brainstem and spinal cord. Loss of lower motor neurons and cellular proteinaceous aggregates in the remaining neurons are the pathological hallmarks of SBMA, but degeneration does not occur uniformly across the motor neurons [10]. Skeletal muscle involvement should be also taken into consideration, since histopathological myopathic changes and elevation of serum creatine kinase (CK) levels, indicative of primary muscle degeneration, are among important SBMA signs. However, only few cellular aggregates are observed in muscle [11].

The age of onset of weakness ranges 30-60 years and occurs predominantly in the lower limbs, which is usually preceded by postural hand tremor and muscle cramps.

Patients typically become wheelchair-bound in 15-20 years once muscle weakness presents. At advanced stages, dysphagia develops, eventually leading to aspiration pneumonia and respiratory failure [12]. Patients may have peripheral sensory neuropathy which mildly impairs vibration sensation in the lower extremities, partly due to degeneration of dorsal root ganglia neurons [9]. Electromyography reveals decreases in both motor and sensory conduction velocities and compound nerve action potentials, consistent with axonal-neuropathy features [13]. Autonomic dysfunction has been reported in some severe cases [14, 15]. Signs of androgen insensitivity like gynecomastia, undermasculinization, testicular atrophy and reduced fertility occasionally appear later in the disease course. Metabolic complications of hyperlipidemia and impaired glucose intolerance, hepatic and cardiac complications, and lower urinary tract symptoms can be also observed [16-18]. Particularly, cardiomyopathy accompanied by Brugada-type electrocardiogram may lead to sudden death [17]. In short, male-specific, overt facial and tongue fasciculation and atrophy, gynecomastia and sensory disturbance are the distinguishing features of SBMA from other motor neuron diseases, while genetic testing is essential for its diagnosis.

Prognostic markers of SBMA

Although the age of onset is correlated with CAG repeat length in the *AR* gene, there is considerable variability in the onset age despite the same CAG repeat size, suggesting the presence of modifier genes other than *AR* in SBMA [12]. Such genes are identified in a fly model, but have not been confirmed in human [19]. In a small-scale prospective study, the urinary 8-hydroxy-2'-deoxyguanosine levels, an oxidative stress marker, at baseline are correlated with changes in the 6-minute walk test during

24-month follow-up [20]. This result indicates that oxidative stress may be a prognostic factor in SBMA, but it needs validation in large-scale studies. Other markers such as serum creatine levels and indices of insulin resistance are associated with the degree of motor impairment, though the relationship between those biomarkers and prognosis has yet to be determined [21-23].

Molecular mechanisms of SBMA

I. Pathogenic androgen receptor

• Causative repeat expansion mutations

AR belongs to a nuclear steroid receptor and transcription factor that contains N-terminal transactivation domain where trinucleotide repeats reside, DNA binding domain, and ligand binding domain. The N-terminal domain including activation function 1 region interacts with transcriptional coregulators, and possesses potent transcriptional regulatory activity. SBMA mutations would disrupt this interaction and influence gene expression levels. An inactive form of AR is localized in the cytoplasm, and interacts with heat shock proteins. Upon testosterone binding, AR translocates into the nucleus, undergoes a conformational change and dissociates heat shock protein 90 that mediates intermolecular interactions between N- and C-terminal domains of AR. AR subsequently binds to androgen response elements in the target promoter or enhancer regions and recruits transcription coregulators to regulate gene expression. The N/C interaction of mutant AR is required for its androgen-dependent neurotoxicity [24, 25]. Gender differences in the testosterone levels determine this ligand-dependent disease manifestation of SBMA.

AR functions physiologically through the effects of androgen, which is important for male sexual development including an increase in muscle strength. AR shows widespread expression in reproductive organs as well as nonreproductive organs, implicating a variety of functional and structural roles for cellular development as well as maintenance. This reflects defects in skeletal muscle and other peripheral organs observed in patients [18].

The repeat length of polymorphic CAG trinucleotides in AR normally ranges 6-36 and in contrast elongates to 38-68 in SBMA. CAG repeats exhibit tissue-specific somatic mosaicism [15], with the largest expansion in the severely affected central nervous system (CNS) [26]. In polyQ diseases, the length of CAG repeats is correlated inversely with onset age and directly with disease severity, but does not affect the speed of disease progression [21, 27]. In line with this, the repeat length inversely correlates with own transcriptional activation activity of AR [28].

The CAG repeat mutation is inherently unstable and undergoes somatic expansion in successive generations [27]. This occurs particularly in CNS cells that are susceptible to the pathogenesis. Unusual DNA structures might be erroneously processed by the mismatch repair system, which is implicated in the cause of CAG repeat instability, though the tissue-specific regulation is poorly understood. Mismatch repair genes such as *MSH2*, *MSH3*, *MLH1* and *MLH3* are identified as potent drivers of somatic instability for repeat expansion mutations [29].

• Polyglutamine-mediated proteotoxicity

The expanded polyQ stretch alters protein structure from a random coil to a β-sheet, dependent on a CAG repeat length, and leads to protein misfolding and misassembly.

The formation of a nucleus presumably composed of misfolded monomers or soluble oligomers is the first and rate-limiting step, which further entraps monomers for generation of protein aggregation. It is suggested that oligomeric intermediates drive pathology, rather than aggregation per se, and resulting misassembly induces proteotoxicity and neuronal degeneration. The nuclear inclusions are immunoreactive with antibodies to epitopes in N-terminus of AR, but not with those in the C-terminus, implying that the C-terminal domain is truncated or is not exposed on the surface [30]. Gain-of-function property of this nuclear inclusion sequesters proteasome components and basal transcription factors, and leads to transcriptional dysregulation and proteostasis declines. This would cause axonal transport impairments, mitochondrial dysfunction, and defects in DNA damage response, responsible for cellular dysfunctions [5]. In addition, post-translational modifications of AR may promote proteinaceous accumulation and thus increase proteotoxicity [31, 32], though signaling pathways downstream of polyQ AR remains largely unresolved.

Motor neuronal viability relies upon growth factors that function as neurotrophic effectors. PolyQ AR affects the expression of genes important for neuron survival, and, for example, perturbs neuroprotective TGF-β- Smad2/3 pathway [33]. In addition to the direct toxicity of polyQ AR onto motor neurons, emerging evidence highlights that reduced trophic support from SBMA muscle to motor neurons may contribute to a non-cell-autonomous mechanism of neuronal degeneration (Fig. 1A) [34, 35] The pathogenic AR disrupts retrograde axonal transport via dysfunction of dynactin 1, an axonal motor-associated protein [36, 37]. Expression of trophic VEGF, NT-4, GDNF and BDNF are decreased in skeletal muscle of mouse models of SBMA [38-40]. In contrast, overexpression of VEGF, IGF-1 or HGF ameliorates disease manifestations in SBMA mice [31, 38, 41], as is observed in IGF-1 administration [42].

The acquired proteotoxic property of polyQ AR confers a key role in the pathogenesis, while loss of native AR function also contributes to the pathology of SBMA. Expansion of the polyQ tract induces partial loss of AR transactivation function, and thereby causes androgen insensitivity syndrome [28], though the motor deficits in SBMA is mainly attributed to a gain-of-function toxicity of polyQ AR. Indeed, complete androgen insensitivity syndrome conferring total loss of AR in patients or *Ar* knockout in mice does not elicit motor impairment, whereas introduction of a mutant *AR* transgene leads to SBMA-like motor phenotypes in mice holding endogenous *Ar* [8].

• Roles of post-translational modifications of AR

Expanded polyQ interferes with post-translational modification of AR protein itself [19, 32]. Post-translational modifications are important for the regulation of intrinsic transcription activity, nuclear translocation, and stability, so have relevance for the neurotoxicity of mutant AR. Phosphorylation of serine residues at Akt consensus sites of AR impairs the cognate ligand binding, and reduces nuclear translocation and polyQ AR toxicity shown in cultured neurons [43]. Augmentation of IGF-1/Akt pathway decreases mutant AR aggregation through the ubiquitin-proteasome system (UPS), and muscle-specific overexpression of IGF-1 increases phosphorylation of AR and ameliorates both muscle and spinal cord pathology in SBMA mice [31]. In contrast, specific phosphorylation of AR at serine 514 enhances caspase-3 activity and generates toxic N-terminal fragments through the proteolytic cleavage of AR [44]. The CDK2/cyclin E complex-mediated phosphorylation at serine 96 enhances polyQ AR stabilization and neuronal vulnerability, which is negatively regulated by the AC/PKA

signaling pathway [45]. A pituitary adenylyl cyclase activating polypeptide (PACAP) analog, an activator of the AC/PKA pathway, reduces the serine-96 phosphorylation, promotes polyQ AR degradation, and restores glycolytic myofiber levels in SBMA mice. Nemo-like kinase phosphorylates at serine 81/308, and also enhances aggregation and polyQ AR toxicity [46].

Arginine residues at the Akt consensus site are methylated by protein arginine methyltransferase 6, and this modification is mutually exclusive with the serine phosphorylation by Akt [32]. The arginine methylation enhanced by polyQ expansion increases polyQ AR transactivation and neurodegeneration shown in the cell and fly models of SBMA [32].

Hyperacetylation at lysine 630/632/633 stabilizes polyQ AR and enhances the nuclear aggregation and neurotoxicity [47]. Sirtuin 1, a NAD-dependent histone deacetylase (HDAC), ameliorates polyQ aggregation and toxicity in the motor neuron models by deacetylating these lysine residues. An AR antagonist may exert neuroprotection by deacetylating AR via recruitment of sirtuin 1 [48] and blocking the recruitment of transcriptional coactivators such as CBP/p300 which involves AR acetylation [49].

Sumoylation at lysine 386/520 in the N-terminal region impairs intrinsic transcriptional activation of polyQ AR [50]. Disruption of the sumoylation restores the transcriptional function, and rescues the type I fiber atrophy, exercise endurance and survival in mice, without alteration in polyQ AR expression or aggregation [51]. These findings improve the understanding of polyQ toxic properties associated with post-translational modifications of AR.

• RNA-mediated toxicity

In addition to the protein-mediated toxicity, recent works have highlighted a pathogenic role of mutant RNAs of causative genes or altered RNA metabolism in neurodegenerative diseases. The findings in microsatellite expansion disorders, where noncoding repeat expansions cause a toxic RNA gain-of-function mechanism, reveal a nuclear accumulation of RNA with expanded repeats which sequesters or dysregulate relevant splicing factors. In addition, expansion mutations produce bidirectional transcripts and homopolymeric proteins in all three frames by unconventional translation [6, 52]. Thus, both sense and antisense RNA and protein aggregates are proposed to be cooperatively responsible for neurodegeneration.

A genetic screen identified that transcription elongation factor Supt4h is specifically required for transcription of genes containing long trinucleotide repeats in either protein-coding or noncoding regions. Inhibition of Supt4h suppresses the synthesis of polyQ-expanded proteins, but not the unexpanded proteins. This potency is recapitulated against disease-associated, both sense and antisense transcripts derived from expanded hexanucleotide GGGGCC repeats in *C9orf72* that causes frontotemporal dementia and amyotrophic lateral sclerosis [53], highlighting a specific regulatory mechanism of transcription of repeat expansions [53, 54].

The miRNAs are 21-25-nucleotide noncoding RNAs which negatively regulate gene expression at the post-transcriptional level. In SBMA, miR-196a is upregulated in SBMA mouse spinal cord, and can enhance the decay of *AR* mRNA by silencing CELF2 that stabilizes *AR* mRNA. Viral delivery of miR-196a ameliorates the motor impairment of the mice [55]. MiR-298, whose levels are reduced in SBMA mouse muscle, downregulates *AR* mRNA and ameliorates disease manifestations in the mice [56].

II. Pathological processes

• Potential spreading of proteinaceous aggregates

Studies of cell and animal models as well as patient brains suggest that proteinaceous aggregation propagates from one neuron or glial cell, to another in neurodegenerative diseases of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. This progressive property of disease might partly account for the nature of later-onset neurodegeneration. Initially, template-directed misfolding of pathological proteins becomes fibrillar species which functions as seeds, and this triggers seeded aggregation and drives its cell-to-cell transmission, leading to sequential spreading of aggregates. The transmission would occur anatomically by diffusion and via axonal connections between adjacent cells, and transneuronal spread of a polyQ protein contributes to non-cell autonomous pathology in brain neurons [57]. Furthermore, extracellular nanovesicles such as exosomes could release disease proteins including polyQ proteins [58]. The propensity of aggregation and propagation depends on excessive concentrations and post-translational modifications of their own proteins, and mutations in familial cases of the disease [4]. Against this, exosome also promotes the clearance of intracellular pathogenic proteins [59], indicating the complex roles of exosome secretion. The mechanism of cell-type-specific propagation or its regulator, however, remains elusive.

• Motor neuron cell-nonautonomous pathology

The mechanism of the selective vulnerability of lower motor neurons in SBMA is still unclear, though the upregulation of a neuropeptide CGRP1 by polyQ AR induces

motor neuron degeneration via enhanced activation of the c-Jun N-terminal kinase (JNK) pathway [60]. SBMA also manifests pathology in the periphery such as skeletal muscle, neuromuscular junction and heart [11, 17, 61]. Despite only marginal aggregation formed in patients' muscle [11], pathogenetic roles of myopathy have been also considered pivotal, since defective retrograde supply of neurotrophic factors from muscle appears to affect the homeostasis of innervating neurons and cause non-cell autonomous neurodegeneration (Fig. 1A). In line with this, muscle-specific overexpression of IGF-1 attenuates disease in an SBMA mouse expressing mutant AR with 97 glutamines (AR-97Q) [31], whereas conditional expression of rat AR in mouse muscle induces motor neuron damage [37, 42]. Genetic inactivation of mutant *AR* in muscle rescues a mouse carrying AR-121Q (BAC fxAR121 mouse) [34]. Furthermore, antisense oligonucleotides (ASOs) that suppress mutant *AR* RNA levels in peripheral tissues ameliorate disease in BAC fxAR121 mice and a knock-in model with AR-113Q (AR113Q mouse) [35]. Recent studies show impaired glycolysis and mitochondrial dysfunction in the muscle of AR113Q mouse and patients [62-65]. Diminished PKA/AMPK signaling, which occurs in the denervated muscle, is restored by the ASO treatment [64]. These findings suggest a key role of the muscle pathology in SBMA, but the ASO treatment could only rescue the model-specific phenotype of these mice such as contentious marked myopathy [35].

III. Pathogenic events downstream of mutant AR

• Perturbed protein homeostasis

Cellular homeostasis maintains a delicate balance between protein synthesis and degradation. Misfolded proteins are degraded through distinct pathways of the UPS

and autophagy. In the UPS-mediated proteolysis, proteins are targeted as substrates by the ubiquitination machinery and sequentially unfolded and degraded by the proteasome [66].

The misfolded or processed mutant AR accumulates and forms inclusion bodies containing key components of the UPS and heat shock proteins (Hsps). These molecules serve as a chaperone involved in protein quality control, which recognizes misfolded proteins and assists their refolding. The soluble oligomer crucial for the neurotoxicity [66] is ubiquitinated, indicating altered proteostasis as well as excessive pathology beyond the degradable amount of proteasome in neurodegenerative diseases.

Hsp70 and Hsp90 chaperones, acting with their co-chaperones, play fundamental roles in maintaining protein homeostasis [67]. Hsp90 stabilizes the client proteins by forming heterocomplex assembly, and inhibits their ubiquitination and degradation. In contrast, Hsp70 with its co-chaperone Hsp40 facilitates protein degradation or redirects folding to regulate protein turnover. To improperly unfolded or misfolded proteins, substrate-bound Hsp70 recruits chaperone-dependent ligases such as C-terminus of Hsc70-interacting protein (CHIP) and Hsp70-interacting protein (Hip), for ubiquitination and following proteasomal degradation of these proteins. Both suppression of Hsp90 and overexpression of Hsp70, Hsp40, CHIP or Hip promote proteasomal degradation of polyQ AR, underscoring regulation of the AR expression by the Hsp90/Hsp70-based quality control [67]. Furthermore, proteotoxic insults activate a transcriptional factor Hsf1, a master regulator of the heat shock response, influences the nuclear accumulation of polyQ AR and distribution of the pathology in SBMA [68].

Autophagy is a conserved lysosomal pathway that promotes degrading misfolded proteins and defective organelles such as damaged mitochondria, to maintain protein quality and normal cellular function. Neuronal autophagy also acts to regulate synaptic development and remodeling of neuronal connections required for its plasticity. Cytosolic aggregates of polyQ proteins sequester key autophagic proteins including an autophagy adaptor p62/SQSTM1 [69]. Genetic inactivation of autophagy in flies accelerates polyQ AR-mediated eye degeneration, and depletion of p62 in SBMA mice exacerbates the disease progression, while pharmacological activation of autophagy and p62 overexpression ameliorates the phenotypes in the flies and mice, respectively [69, 70]. AR interacts with and activates the transcription factor EB (TFEB), a master regulator of autophagy, whereas polyQ AR interferes with TFEB transactivation function and causes autophagic flux defects and accumulation of autophagic vacuoles in the motor neuron models [71]. In contrast, TFEB activation or an enhanced signaling pathway of mTOR, a suppressor of autophagy and an inducer of a master regulator of muscle metabolism PGC-1α, leads to muscle wasting and fiber-type switching in SBMA [63, 71], revealing tissue-specific roles of TFEB and mTOR. The autophagy and UPS function are coordinated and complement each other, mediated by HDAC6 and p62 [70]. Accumulating evidence emphasizes the pathogenic importance of defective protein quality control system or proteostasis against polyQ proteins.

• Mitochondrial dysfunction

Mitochondrial defects have been implicated in the pathogenesis of SBMA, through AR's direct interaction of mitochondria and dysregulation of nuclear-encoded

mitochondrial genes. An ectopic or even endogenous induction of mutant AR leads to mitochondrial membrane depolarization and elevation of reactive oxygen species (ROS) levels in neuronal cells [62, 71]. N-terminally truncated polyQ-AR activates JNK and Bax, and then induces caspase-9 and caspase-3 activation, which trigger the release of cytochrome c from mitochondria and cause apoptosis in neurons [62, 72]. AR interacts with and sequesters cytochrome c oxidase subunit Vb in aggregates in a hormone dependent manner, and leads to mitochondrial dysfunction [73]. Skeletal muscle of an SBMA knock-in mouse shows decreased expression of several mitochondrial genes and carbohydrate metabolic genes, and exhibits diminished glycolysis, morphological defects in mitochondria and decreased mitochondrial DNA copy number [51, 62, 64]. Such changes are accompanied by the reduced activity of regulators of glucose metabolism, such as PKA and AMPA [64]. PKA phosphorylates a COX subunit and the translocase of the outer membrane complex receptors, controlling mitochondrial activities, whereas AMPK phosphorylates the mitochondrial biogenesis factor PGC-1α. Transcriptional downregulation of PGC-1 and its downstream targets PPARγ and Tfam, master transcriptional regulators of mitochondrial biogenesis, also underlies mitochondrial dysfunction in SBMA [62, 74]. PPARs perform essential functions in glucose homeostasis and energy production, and are crucial for neural function. Levels of a mitochondrial antioxidant SOD2 are decreased, also consistent with increased oxidative stress [62].

Toward the development of disease-modifying therapies

Androgen deprivation

• Inhibition of aggregation in SBMA mice

A gain-of-function toxicity of causative mutant proteins represents a potential therapeutic target especially in monogenic diseases, even though a disease mechanism remains to be fully elucidated **(**Fig. 1B). In SBMA, polyQ AR as well as its toxic properties associated with androgen-dependent nuclear translocation can be prime target candidates. Useful approaches to reduce levels of androgen ligands or AR have been explored in animal studies and, in some cases, have been further evaluated in clinical trials. Leuprorelin is a potent agonist of luteinizing hormone-releasing hormone (LHRH) and inhibits the production and release of gonadotropins, luteinizing hormone and follicle-stimulating hormones, as well as testosterone. This drug has been used for various sex hormone-dependent diseases such as prostate cancer, breast cancer, endometriosis and precocious puberty. Leuprorelin effectively inhibits nuclear accumulation of mutant AR, with a pronounced reduction in circulating testosterone, and provides a significant improvement in neuromuscular pathologies and survival of AR-97Q mice (Fig. 2A-C) [75]. The treatment can reverse the pathological AR accumulation that once forms. In contrast, an antagonist flutamide whose affinity for AR competes with testosterone was found to be ineffective possibly due to the driven nuclear translocation of AR [8, 75]. However, though it stimulates an increase in androgen levels, a recent study has shown that flutamide ameliorates disease symptoms in SBMA mouse models, and another AR antagonist bicalutamide has beneficial effects in the cell model [24], arguing against their proposed therapeutic mechanism of action [76].

• Clinical studies of antiandrogen therapy

The high efficacies of the preclinical study of leuprorelin prompted us to proceed to investigator-initiated clinical trials for SBMA patients. Following the positive results from the phase 2 trial, the 48-week randomized phase 3 trial had been conducted in 204 patients [77, 78]. Leuprorelin reduced mutant AR accumulation on scrotal skin biopsies and serum CK levels as the pathogenic markers [79], and was proven well tolerated with a favorable safety profile and effective for improving swallowing function in patients with disease duration less than 10 years (Fig. 2D). However, there was no significant improvement in motor function, partly due to the antianabolic effects on muscle. Also, a 5-α-reductase inhibitor dutasteride that blocks the conversion of testosterone to potent dihydrotestosterone had no significant effect on muscle strength in the phase 2 trial, though it could potentially improve their quality of life and swallowing function [80]. Considering the efficient rescue of SBMA mouse symptoms by leuprorelin, these results underscore the difficulties in treating a slowly progressive disease whose severity varies under different disease duration, genetic and environmental factors. The lack of sensitive and relevant biomarkers and the insufficient observation period and enrollment of patients with optimal stages for trials appear to be attributed to the potentially inconclusive results from these studies. Highly effective therapeutics providing clinically meaningful improvements, however, would readily overcome these limitations even in a small cohort of subjects of rare diseases with chronic courses. Importantly, through the experience of clinical trials, several therapeutic approaches can be pursued to identify temporally and spatially targeted tissues and cell types for SBMA therapy.

Antisense-targeting against the pathogenic AR

Though no single pathway has emerged as a prime mediator of SBMA pathogenesis, mutant AR expression can be targeted to ameliorate the disease. Advances in antisense drug technology improve *in vivo* potency, tolerance and stability of ASO chemistry, and contribute to the development of RNA therapeutics. ASO-mediated RNA cleavage enables to suppress both toxic CUG-expanded RNA and polyQ protein expression. Subcutaneous administration of ASOs decreasing peripheral expression of *AR* mRNA rescues muscle strength and survival in SBMA mice [35]. This result indicates the predominance of peripheral pathology, though it is unclear to what extent the efficacy observed in the mouse models can be expected for human SBMA. Profound nuclear inclusions in motor neurons and extensive neuronal loss are the fundamental SBMA pathology [10]. Neurogenic contraction fasciculation is among the initial SBMA symptoms, and neurogenic abnormalities are evident in electromyogram [9, 12]. Additionally, sensory and autonomic neuropathies are seen in patients [15, 81]. Indeed, mice expressing polyQ AR exclusively in motor neurons display secondary pathology in innervated muscle and motor dysfunction [82]. An intracerebroventricular injection of ASOs suppressing AR expression in the CNS, improves motor function and survival with the amelioration of neuronal histopathology, focusing on the neurotoxic AR as a therapeutic target [83]. ASOs help to address *in vivo* biological functions of a target gene product, and these studies support the applicability of antisense therapeutics for SBMA.

Boosting of proteostasis

Approaches that activate or restore a defensive proteostasis have been explored to promote degradation of misfolded, aggregate-prone proteins. Upregulation of the UPS,

autophagy machinery and heat shock proteins has therapeutic potentials of proteolysis of polyQ AR in SBMA.

The finding that components of the UPS and molecular chaperones colocalize with polyQ protein-containing inclusions, and their expression is decreased in polyQ disease models [84], implicates decline of intrinsic defense mechanisms underlying neurodegeneration. This supports the applicability of modulation of the chaperone activity against the burdens of misfolded proteins, since the UPS functions, revealed in SBMA mice [85]. Molecular chaperones can reduce polyQ-mediated toxicity through protein refolding and degradation. The unfolding polyQ proteins form soluble oligomers that presumably exert neurotoxicity and contribute to the pathology [86]. A soluble, monomeric species of aggregation-prone proteins are preferred substrates for the UPS, compared to assembly states including oligomers. Pharmacological alteration of the expression or activity of Hsps has emerged as a candidate approach of the UPS-mediated protein clearance. Hsp90 associates with a co-chaperone p23, and promotes protein stabilization. Since polyQ proteins are among Hsp90 clients, treatment of Hsp90 inhibitors induces proteasomal degradation of the proteins and alleviates neurotoxicity. Hsp70 and CHIP play vital roles in protein degradation by the UPS, and their overexpression enhances the efficacy. Thus, targeting the Hsp90/Hsp70-based chaperone machinery exerts neuroprotective effects in polyQ diseases [67].

Derivatives of an Hsp90 inhibitor geldanamycin, such as 17-AAG and 17-DMAG, dissociate p23 from the Hsp90 complex, promote polyQ AR degradation and reduce its toxicity in SBMA mice [85, 87]. A small molecule sulforaphane or ASC-JM17 acts on transcription factors such as Nrf1, Nrf2 and Hsf1, which increases refolding yields and enhances the UPS function, and mitigates the toxicity of polyQ proteins [88, 89].

In addition, an antiulcer drug geranylgeranylacetone binds to Hsp70 and leads to its dissociation from Hsf1. This, in turn, activates Hsf1 and promotes Hsp upregulation [90], and an Hsp inducer arimoclomol upregulates Hsp70 and VGEF [91]. These agents ameliorate an SBMA mouse phenotype. A synthetic co-chaperone YM-1 enhances the binding of Hsp70 to its substrates, and facilitates ubiquitination and degradation of polyQ AR in an SBMA fly model [92]. As Hsp90 interacts with numerous proteins, Hsp90 inhibitors cause defects in client protein degradation. Inhibition of Hsp70 may be preferable, to augment degradation of misfolded proteins via the UPS or induced solubilization.

Direct modulation of proteasome activity also stimulates the ubiquitin-dependent proteolysis. IU1, a small-molecule inhibitor of deubiquitination by USP14, enhances proteasomal degradation of polyQ proteins [93], though UPS potentiation for selective degradation of target proteins that retains the ability of protein clearance is still awaited.

Stimulation of autophagy activity is another strategy to enhance the clearance of pathogenic proteins. In addition, autophagy plays a neuroprotective role in SBMA [94]. Genetic ablation of autophagy in an SBMA fly model promotes the eye degeneration phenotype [70] and depletion of p62, an adaptor protein to ubiquitin as well as an autophagosomal membrane LC3, exacerbates AR accumulation in SBMA mice [69]. Induction of autophagy mitigates polyQ AR toxicity in culture motor neurons [47], revealing a rationale for targeting cytoplasmic AR; degradation of the polyQ oligomers may reduce the nuclear accumulation and toxicity. Activation of autophagy using rapamycin suppresses neurodegeneration in flies, which depends on HDAC6 [70]. An autophagy activator trehalose increases p62 and LC3 expression [95] and 17-AAG

increases LC3 expression [96], which facilitates autophagic degradation of polyQ AR. Overexpression of TFEB or its pharmacological induction by paeoniflorin, contents of Paeonia plants, activates autophagy and has therapeutic effects in SBMA-derived neuronal cells or SBMA mice, respectively [71, 97]. In contrast, a genetic impairment of autophagy reduces muscle wasting and prolongs the lifespan of SBMA mice, suggesting the contribution of aberrant upregulation of autophagy to muscle pathology [98]. A β-agonist clenbuterol activates a signaling pathway of mTOR, a suppressor of autophagy, and reduces muscle pathology in SBMA mice [99], whereas a high-fat diet that decreases mTOR signaling activation but suppresses the expression of TFEB and PGC-1α, ameliorates the mouse phenotype [63]. These findings are consistent with the effects of IGF-1 in muscle through enhancing the autophagy repressor Akt [31, 42, 43]. Given that TFEB profile differs between myocytes and motor neurons in SBMA [71], these divergent results suggest cell-type- and stage-specific perturbation of autophagy control, and underscore the requirement of a refined strategy against local autophagy defects.

Modulating AR conformation

The interdomain N/C interaction in the AR stabilizes the androgen binding and contributes to the regulation of androgen-responsive gene expression. This conformational change is also required for the toxicity of polyQ AR, and pharmacological or genetic disruption of the N/C interaction prevents it in the cell models [24, 25]. Advances in cancer research have led to the discovery of drugs that disrupt the N/C interaction, including AR ligands as antagonists. The antagonist flutamide is shown to protect against the disease in SBMA mouse models [76]. In

addition, a genetic alteration that prevents the N/C interaction reduces the pathological features of SBMA mice, which depends on phosphorylation of AR at serine 16 [25]. Inhibitors of the N/C interaction and specific kinase modulators also represent potential therapeutic agents for the treatment of SBMA.

Therapies targeting downstream of AR

Steroid receptor coactivator-1 (SRC-1) interacts with AR and enhances the activity by recruiting additional coactivators CBP/p300 and coactivator-associated arginine methyltransferase 1 that can alter chromatin structure [49]. CBP sequestration in the nuclear inclusion compromises the SRC-1-mediated coactivation function, and the resulting decrease in histone acetylation leads to transcriptional dysregulation of genes such as *VEGF*, *TGFBR2* and *Calca* [33, 38, 60, 100]. Especially, increased expression of Calca induces toxicity via the activation of JNK pathway. Genetic depletion of *Calca* or its pharmacological suppression using an antimigraine drug naratriptan attenuates JNK activity and polyQ AR-mediated neuronal damage [60]. Furthermore, pharmacological augmentation of histone acetylation with HDAC inhibitors attenuates neurodegeneration in the cell and mouse models of SBMA [33, 101, 102].
AR coactivator ARA70 enhances the transactivation activity of the receptor. An AR degradation enhancer ASC-J9 dissociates ARA70 from AR, decreases polyQ AR aggregation, and ameliorates the mouse symptoms [103]. Likewise, an isoflavon, genistein, disrupts the interaction between AR and ARA70 and gives the similar effects [104], indicating that the interactions of AR coregulators can be therapeutic targets for SBMA.

Restoration of mitochondrial function

Cyclosporine A, an inhibitor of the mitochondrial permeability transition, mitigates mitochondrial depolarization, and an antioxidant co-enzyme Q10 or idebenone prevents ROS increases in the cell models of SBMA [62]. The autophagy regulator TFEB relieves autophagic flux blockage and mitochondrial depolarization in SBMA neuronal cells [71]. A PPARγ agonist, pioglitazone, alleviates oxidative stress and mitochondrial dysfunction in the cell and mouse models, as well as the neuromuscular phenotype of SBMA mice [74, 105]. Such agonists prove to be also effective in HD mice [105], revealing a potential mitochondrial therapy for polyQ diseases.

Management of muscle strength

Intramuscular uptake of creatine, a nitrogenous organic acid, is decreased in SBMA potentially due to downregulation of the creatine transporter SLC6A8, which is accompanied with amyotrophy [22]. Since creatine stimulates mitochondrial ATP production and cellular energy metabolism, the uptake impairment of creatine may account for the motor deficits. Thus, creatine supplementation may be useful as adjuvant therapy for SBMA.

Proper exercise training maintains or enhances muscle strength in both aging and disease populations. It increases myotrophic IGF-1 levels, which is beneficial for patients with SBMA [31]. Though the head lift exercise likely improves swallowing function in SBMA patients [20], neither aerobic training nor functional exercise akin to daily activities reveals significant effects on general muscle strength or function [106].

Conclusion

Cell and animal phenocopy models have enabled major advances in our understanding of the molecular mechanisms unique to SBMA as well as broadly relevant to polyQ diseases or motor neuron diseases. Recent progress in potential therapeutics for microsatellite diseases, such as ASOs for myotonic dystrophy, further probes and accelerates therapeutic development for polyQ diseases, especially for HD and SBMA. SBMA, however, has characteristic features of hormone-dependent pathogenesis as well as additive muscular pathologies, and may require distinctive targeted therapies. Since various therapeutic approaches have been investigated so far to target diverse molecules and molecular pathways (Fig. 1B; Table 1), a combination therapy may achieve a higher efficacy for the treatment of SBMA.

Expert opinion

Recent studies using the animal models have underscored the potential pathogenesis of skeletal muscle in SBMA, though autopsy findings in patients reveal massive degeneration and loss in lower motor neurons, as well as marked proteinaceous aggregates in residual neurons, contrasting with the muscle pathology that only shows minimal aggregates. Several mouse model systems exhibit salient myopathic phenotypes including lethal urinary retention, and do not develop motor-neuronal loss, casting the uncertainty in the direct relevance of the mouse SBMA-like disease to human SBMA. Furthermore, a deficient trophic supply from muscle to innervating motor neurons is thought to critically cause non-cell autonomous neurodegeneration, but still remains inconclusive *in vivo*.

Despite ubiquitous expression of the pathogenic AR, the mechanism of vulnerability of lower motor neurons in contrast to relative sparing of cortical motor neurons has yet to be elucidated. In addition, the relative contribution of motor neuron and muscle pathology, the primary pathogenic region, or the pathogenesis of other organs needs to be addressed, which stands out as important therapeutic targets.

Considering the suboptimal outcome obtained in the phase 3 clinical trial of leuprorelin, the experimental therapies being extensively explored to antagonize androgen or AR might not provide efficient rescue for SBMA. Besides the mutant protein, deciphering the downstream signaling pathways and key molecules that trigger or drive the pathology in SBMA, or those common in polyQ or motor neuron diseases may further provide insights into the mechanisms of genesis and progression of SBMA and in turn lead to the development of targeted therapies. The possibility of pharmacological prevention should also be explored, as done in HD [107].

A therapeutic ASO designed to restore *SMN2* pre-mRNA splicing in spinal muscular atrophy, another motor neuron disease, showed an acceptable safety profile and a promising clinical response in the phase 3 clinical studies, and has been recently approved for the drug by the U.S. FDA. This promotes the establishment of a novel disease modifying therapy for neurodegenerative disease, and the preclinical ASO studies in SBMA mouse models also demonstrate the applicability of antisense therapeutics for SBMA. In addition to the mutants of RNA and protein, ultimately, CAG-expansion mutations in polyQ diseases including SBMA could be corrected *in vivo*, such as by targeting genetic drivers of the repeat instability or using genome-editing technologies like TALEN and CRISPR/Cas9 systems. Furthermore, a novel device of robot medical suits for regeneration treatment has been recently developed and approved for neuromuscular diseases including SBMA, and is expected to

enhance clinical mobility and function in patients. Lastly, a discovery of useful prognostic biomarkers is still awaited for SBMA, which will hopefully lead to efficacious or even preventive therapeutic intervention that begins early or prior to the disease onset.

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Table 1. Potential drugs for SBMA.

Molecular target	Agent	Mechanism of action	Final study subject	Reference
Androgen	Leuprorelin	LHRH agonistic analogue	SBMA mice, patients	[75]
	Dutasteride	5α-reductase inhibitor	Patients	[80]
	Flutamide	AR antagonist	SBMA mice	[76]
	Bicalutamide	AR antagonist	SBMA cell	[24]
Androgen receptor	Antisense oligonucleotide	AR knockdown	SBMA mice	[35, 83]
UPS	Geldanamycin	Hsp90 inhibitor	SBMA mice	[85, 87]
	ASC-JM17	Nrf2 activator	SBMA mice	[89]
	Geranylgeranylacetone	Hsp70 inducer	SBMA mice	[90]
	Arimoclomol	Hsp70 inducer	SBMA mice	[91]
	YM-1	Hsp70 activator	SBMA fly	[92]
	IU1	Proteasome activator	Cell line	[93]
Autophagy	Rapamycin	Autophagy inducer	SBMA fly	[70]
	Trehalose	Autophagy activator	SBMA cell	[95]
	17-AAG	Autophagy inducer	SBMA cell	[96]
	Paeoniflorin	Autophagy inducer	SBMA mice	[97]
	Clenbuterol	mTOR activator	SBMA mice	[99]
Transcription regulation	Sodium butyrate	HDAC inhibitor	SBMA mice	[102]
	Trichostatin A, SAHA	HDAC inhibitor	SBMA cell	[101]
	ASC-J9, genistein	AR degradation enhancer	SBMA mice	[103]
	Genistein	AR degradation enhancer	SBMA mice	[104]
Mitochondria	Cyclosporine A	MPT inhibitor	SBMA cell	[62]
	Co-enzyme Q10, idebenone	Antioxidant	SBMA cell	[62]
	Pioglitazone	PPARγ agonist	SBMA mice	[74]
Phosphorylation	PACAP analog	AC/PKA activator	SBMA mice	[45]
JNK pathway	Naratriptan	5-HT1B/1D receptor agonist	SBMA mice	[60]
IGF-1/Akt pathway	IGF-1	Trophic factor	SBMA mice	[42]

Figure Legends

Figure 1. Molecular pathomechanisms of SBMA. A) Potential motor neuron cell-nonautonomous degeneration due to deficiency of neurotrophic factors from muscle to neurons. B) Targeted therapeutics in SBMA.

Figure 2. Leuprorelin treatment in SBMA. A, B) Rescue of an SBMA mouse phenotype [75]. C) Suppression of formation of nuclear inclusions in mouse spinal motor neurons [75]. D) Swallowing improvement in patients with disease duration less than 10 years [78].