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Review

Neurotoxic effects of androgens in spinal and bulbar muscular atrophy

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ABSTRACT

Expansion of polyglutamine tracts in nine different genes causes selective neuronal degeneration through unknown mechanisms. Expansion of polyglutamine in the androgen receptor is responsible for spinal and bulbar muscular atrophy (SBMA), a neuromuscular disorder characterized by the loss of lower motor neurons in the brainstem and spinal cord. A unique feature of SBMA in the family of polyglutamine diseases is sex specificity. SBMA fully manifests only in males. SBMA is a disease triggered by the binding of polyglutamine androgen receptor to its natural ligand testosterone. Recent evidence has emerged showing that the expanded polyglutamine tract itself is not the only determinant of disease pathogenesis. There is evidence that both the native structure and function of the disease protein strongly influence the pathogenicity of mutant protein. Here, we review recent progress in the understanding of disease pathogenesis and advancements towards development of potential therapeutic strategies for SBMA.

1. Introduction

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, was first described in the nineteenth century by Hiroshi Kawahara and again nearly a century later by William Kennedy [51,52]. The gene coding for androgen receptor (AR) was cloned in 1988 by three independent groups [10,60,109] and linked to SBMA three years later [55]. The causative mutation in SBMA is the expansion of a CAG trinucleotide repeat, which encodes a polyglutamine (polyQ) tract, in the AR gene. The polyQ tract is polymorphic in length. In normal individuals, the polyQ stretch ranges between 9 and 36 residues, and expansion over 38 and up to 62 residues is pathogenic. SBMA is one of nine neurodegenerative disorders caused by expansion of polyQ tracts. These disorders are known as polyQ diseases and include Huntington's disease, dentatorubral–pallidoluysian atrophy, and six types of spinocerebellar ataxia (SCA), known as SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 [77,84]. The causative genes are huntingtin [61], atrophin-1 [54,70], ataxin-1 [76], ataxin-2 [39], ataxin-3 [50], CACNA1A [119], ataxin-7 [17], and the TATA-binding protein (TBP) [71], respectively. PolyQ diseases share several features. All of these diseases are neurodegenerative disorders with typically late onset, and all are inherited in an autosomal dominant fashion except SBMA, which is X-linked. There is a positive correlation between CAG repeat length and disease severity, and a negative correlation between repeat length and the age of disease onset. Similar to other repeat expansion disorders, polyQ diseases show genetic anticipation, a phenomenon in which one generation shows a more severe phenotype and an earlier onset of disease compared with the previous generation, due to the fact that the repeat tends to expand when it is passed down from one generation to the next. Despite several common features shared by polyQ diseases, expansion of polyQ tracts in the different proteins causes degeneration only in specific neuronal subpopulations in each disease. This selective neuronal vulnerability results in clinically distinct disease phenotypes. Recently, evidence has emerged suggesting that there is a strong relationship between the structure and function of polyQ proteins with toxicity [11,25,59,80]. A major limitation in the study of polyQ disease pathogenesis comes from the lack of information related to protein structure and, more importantly, protein function. SBMA represents an exception in the polyQ field, as a large body of information about AR protein structure and function is available to date. Pathological features of polyQ diseases are discussed elsewhere [6,77,94,101]. This review will focus on SBMA.

2. Clinical features of SBMA

SBMA is a neuromuscular disease. The prevalence of the disease is estimated to be 1–2/100,000 in the male population of Western European descent, although this is likely to be an underestimation, as patients can be misdiagnosed or undiagnosed [26,82]. SBMA typically has a late onset around the third to fifth decade of life and a relatively slow progression, although a family case with juvenile onset and fast progression has been reported [23]. SBMA is

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characterized by the degeneration and loss of lower motor neurons in the brainstem and spinal cord, together with progressive weakness, atrophy and fasciculation of proximal limb and bulbar muscles. Distal muscle weakness and atrophy are observed in the arms more than the legs. Cramps, hand tremor and fatigue often precede muscle weakness, sometimes by several years. As motor dysfunction progresses, patients may require the use of canes and wheelchairs. Patients show facial fasciculations with contraction of muscles around the mouth and chin, fasciculation of the tongue, and in some cases dysarthria and dysphagia. SBMA patients also show signs of mild subclinical sensory impairment. Neurologic examination can reveal diminished or absent deep tendon reflex. Other symptoms include endocrine abnormalities, such as diabetes mellitus, as well as signs of mild androgen insensitivity, including gynecomastia, reduced fertility and testicular atrophy. Androgen levels in the serum of SBMA patients are normal, or in some cases elevated. A unique feature of SBMA among polyQ diseases is sex specificity, as SBMA fully manifests only in males. Because the gene coding for AR is on the X chromosome, the disease was initially thought to be recessive. However, females homozygous for the mutation were described to have only subclinical disease manifestations, which could not be explained with a canonical recessive pattern of inheritance for an X-linked mutation [92]. This conundrum was elucidated with the development of animal models of SBMA. The sex specificity of SBMA is indeed well recapitulated in both vertebrate and invertebrate animal models of the disease. For instance, in transgenic mice expressing mutant AR, the disease fully manifests only in males [12,45,118]. Importantly, manipulation of androgen levels in animals dictates disease manifestations: treatment of transgenic female mice with testosterone induces disease manifestations, while castration of transgenic male mice prevents degeneration. Expression of mutant AR in *Drosophila melanogaster* causes degeneration only if the flies are reared in a hormone-containing food [102]. This evidence provides proof of principle that SBMA is a disease triggered by the binding of polyQ-AR to its natural ligands, testosterone and its more potent derivative dihydrotestosterone (DHT).

3. Structure–function relationship of AR

The *AR* gene maps on the long arm of the X chromosome (Xq11–12) and is composed of eight exons (Fig. 1). AR is a protein of approximately 919 amino acids with a molecular weight of about 110 kDa. AR belongs to the family of steroid hormone receptors, which includes the estrogen receptor, glucocorticoid receptor, progesterone receptor and mineralocorticoid receptor. Similar to the other members of the family, AR has a very well-defined domain structure. AR is composed of an amino-terminal domain, a DNA-binding domain, and a ligand-binding domain, which is linked to the DNA-binding domain by a hinge region (Figs. 1 and 2).

3.1. Amino-terminal domain

The amino-terminal domain of AR is encoded by exon 1 (amino-acids 1–555, NM_000044). In the family of steroid receptors, this domain is the less well-conserved portion of the protein compared to the other protein domains. The amino-terminal domain contains two highly polymorphic repeats, the polyQ and the polyglycine tracts. Although the functional role of these repeats remains to be elucidated, there is a strikingly tight correlation between AR function and polymorphic AR variants. For example, there is an inverse correlation between polyQ repeat length and AR transactivation: the longer the repeat, the lower the ligand-dependent AR transactivation [31,110,112]. In addition, the effect of polyQ tract polymorphisms on AR function correlates well with androgen-related disorders. Indeed, epidemiologic studies show that individuals carrying a polyQ tract shorter than 20 glutamine residues are at increased risk for prostate cancer compared to individuals with repeats longer than 26 residues [28,38,98]. Conversely, AR with a polyQ tract longer than 28 residues has been associated with male infertility [110]. Importantly, AR transactivation decreases with pathologic lengths of the polyQ tract, which may account for the signs of androgen insensitivity observed in SBMA patients [65]. Human AR has two other relatively short polyQ tracts in the amino-terminal domain, one composed of five (amino-acids 86–91) and the other of six (amino-acids 195–199) glutamine residues. Interestingly, these polyQ tracts also negatively regulate AR transactivation [31].

The polyglycine tract is encoded by the GGN trinucleotide repeat (amino-acids 451–473). While the effect of the length of the polyQ tract on AR function is well established, the effect of the polyglycine tract is less clear and may depend on cell context and on *cis*-elements on the promoter of target genes [20,27,36,115]. The impact of the polyglycine tract on the SBMA phenotype is not known.

In addition to the polyQ and the polyglycine stretches, the amino-terminal domain of AR contains a polyproline tract, which is composed of eight residues (amino-acids 374–381). The functional role of this amino acid sequence in AR is not known, although there is evidence that polyproline-rich sequences reduce toxicity in Huntington's disease. Huntingtin has two polyproline tracts separated by a proline-rich sequence. Presence of this amino acid tract on the

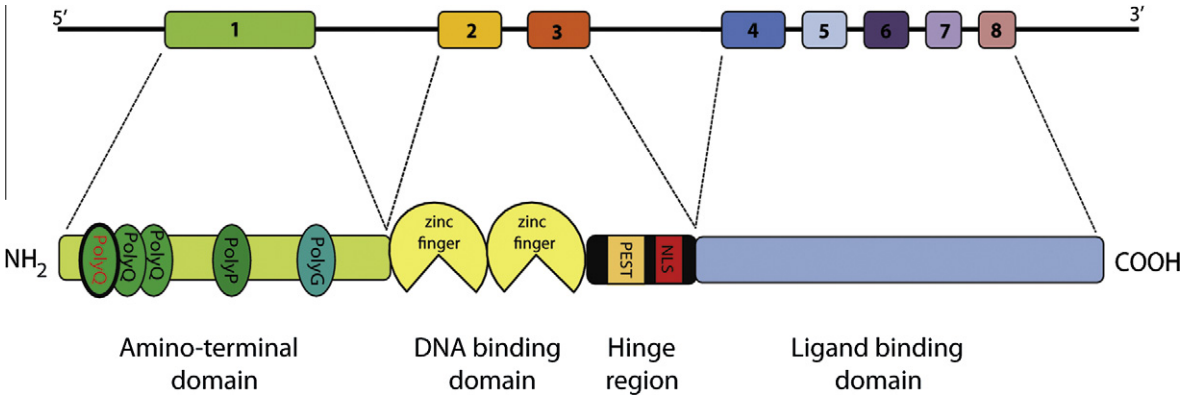

Fig. 1. Scheme of AR gene and protein. The AR gene is composed of eight exons. The first exon encodes the amino-terminal domain, which contains three polyQ tracts (polyQ), a poly-proline tract (polyP) and a poly-glycine tract (polyG). The first polyQ tract (red) is expanded in SBMA. Exons 2 and 3 encode the DNA-binding domain, which is formed by two zinc fingers, and the hinge region, which contains the PEST sequence and the nuclear localization signal (NLS). Exons 4 through 8 encode the ligand-binding domain.

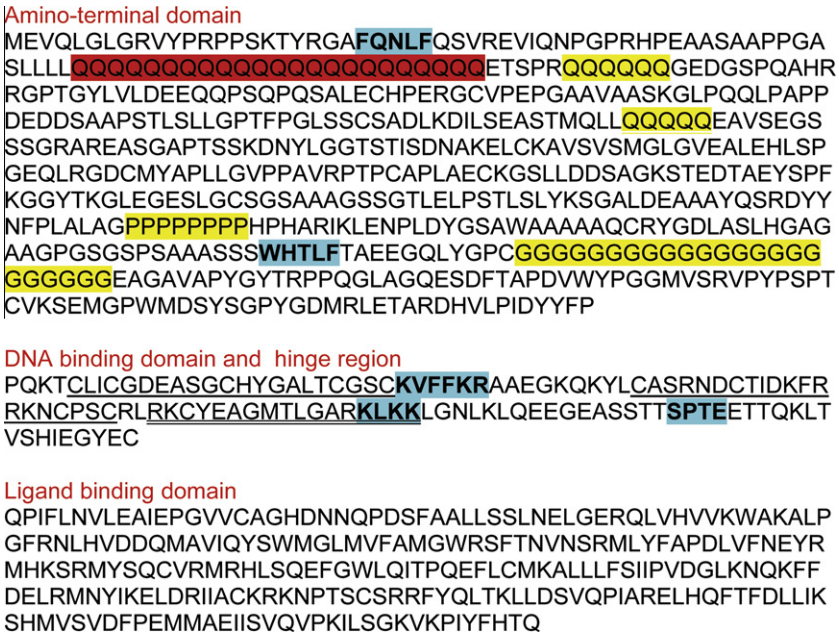

Fig. 2. AR sequence (NM_000044). The AR sequence has been subdivided in the amino-terminal domain, DNA-binding domain and hinge region, and ligand-binding domain. *Amino-terminal domain*: Amino-acids 1–555. The pathogenic polyQ tract is highlighted in red, while the other polyQ tracts, the poly-proline tract and the poly-glycine tract are in yellow. The FXXLF (FQNLF) and WXXLF (WHTLF) motifs are in bold-cyan. *DNA-binding domain and hinge region*: Amino-acids 556–670. The zinc fingers are underlined, and the nuclear localization signal is double-underlined. The calreticulin-binding site (KVFFKR), the acetylation consensus sequence (KLKK), and the PEST sequence (SPTE) are in bold-cyan. *Ligand-binding domain*: Amino-acids 671–920.

carboxy-terminus of a pathogenic polyQ tract reduces aggregation and toxicity both *in vitro* and *in vivo* [7,16,19,22]. Whether the polyproline tract of AR influences the toxic properties of mutant protein remains to be established.

In the amino-terminal domain, AR has two domains critical for AR function: activation function 1 (AF-1) and activation function 5 (AF-5). AF-1 spans amino-acids 51–211 and is critical for androgen-dependent regulation of gene transcription. This domain is ligand-dependent, and it is masked by the interaction of AR with heat shock proteins in the inactive state. AF-5 spans amino-acids 370–494. These two domains are required for interaction with co-regulators of transcription. The role of these domains, if any, in SBMA pathogenesis is unknown.

3.2. DNA-binding domain and hinge region

The DNA-binding domain and the hinge region of AR are encoded by exons 2 and 3 (amino-acids 556–670). The DNA-binding domain of AR is highly conserved and is composed of two zinc fingers. The first zinc finger contacts the major groove of DNA and is required for specificity in DNA binding, while the other zinc finger is required for stabilization of the DNA–protein interaction. The DNA-binding domain and hinge region contain a nuclear localization signal (amino-acids 617–634), which drives AR to the nucleus in response to ligand binding. The nuclear localization signal of AR is bipartite, as it is formed by two clusters of conserved basic residues separated by 10 amino acids [96]. Nuclear import of AR is mediated by the importin-α and importin-β systems, and binding of AR to importin-α mainly involves the second cluster of basic residues in the hinge region (residues 629–634) [15]. Additional nuclear localization signals in the amino-terminal domain as well as in the ligand-binding domain have been identified [43].

The hinge region (628–669) of AR contains a PEST sequence (AR sequence ^{651}SPTE^{654}, where P is proline, E glutamic acid, S serine, and T threonine), which targets proteins for degradation through the proteasome [88]. Deletion of this sequence results in increased accumulation of AR, which is further enhanced by proteasome inhibition, suggesting a role for this sequence in proteasome-mediated AR degradation [103].

The nuclear localization signal contains the acetylation motif KXKK (AR sequence ^{631}KLKK^{634}, where K is lysine and L leucine). Deletion or mutation of this sequence decreases nuclear translocation but enhances AR transactivation [29,79,104,106]. The DNA binding domain and hinge region of AR contain the calreticulin-binding motif KXFFKR (AR sequence ^{581}KVFFKR^{586}, where V is valine, F phenylalanine, and R arginine) [18]. Interestingly, calreticulin has been shown to interact with AR in the nucleus and to inhibit DNA binding and transactivation, thereby inhibiting neuronal differentiation *in vitro*. It would be interesting to determine whether this motif plays a role in SBMA pathogenesis.

3.3. Ligand-binding domain

The ligand-binding domain of AR is encoded by exons 4 through 8 (amino-acids 671–920). This domain is relatively well conserved in the family of steroid receptors and is formed by helix-1 to -12 and four β-strands assembled into a three-layer α-helical structure [63]. Upon ligand binding, this domain undergoes a conformational change that leads to the assembly of activation function 2 (AF-2). AF-2 is composed of helix-3, -4, -5 and -12 and consists of a hydrophobic surface flanked by two charged residues, K720 and E897. Helix-12 is particularly important, as it is repositioned differently depending on the nature of the ligand [113]. In the agonist-bound state, helix-12 works as a lid to close the pocket, while when in the antagonist-bound state, helix-12 leaves the pocket open. In most steroid receptors, AF-2 plays a major role in receptor transactivation by serving as the interaction surface for transcriptional co-regulators that contain an LXXLL motif. However, the AF-2 of AR has relatively weak activity *per se*, and preferentially binds to the FXXLF motif (AR sequence ^{23}FQNLF^{27}) and the WXXLF motif (AR sequence ^{435}WHTLF^{439}) in the amino-terminal domain of AR [34]. This results in an interaction between the amino-terminal domain

and the ligand-binding domain, which is known as the N/C interaction [56,57]. The N/C interaction can be intra-molecular or inter-molecular, the first likely occurring in the cytosol, while the other taking place upon transport to the nucleus [91].

4. Molecular mechanisms of disease pathogenesis

Does polyQ expansion in AR cause disease through a loss of function or a gain of function mechanism? The endocrine abnormalities observed in SBMA patients indicate that polyQ expansion leads to a partial loss of AR function. Genetic evidence in mouse models supports the idea that the loss of AR function caused by polyQ expansion contributes to disease pathogenesis [107]. However, a pure loss of function mechanism is difficult to reconcile with the observation that mutations that completely abolish AR function result in androgen insensitivity syndrome with no signs of neurodegeneration. Rather, expansion of polyQ is thought to confer a toxic gain of function to the mutant protein. The last decade has brought an extensive advancement towards the molecular characterization of the details through which polyQ expansion alters AR biology and function (Fig. 3). These findings are summarized in this section.

4.1. Interaction with heat shock proteins

In the cytosol, AR is bound to heat shock proteins (Hsps), including Hsp90, Hsp70, and Hsp40. AR exists in two different complexes, one containing p23, and the other not. The p23-containing complex stabilizes the Hsp90-client proteins. The other complex promotes protein folding and solubilization and targets proteins for proteasomal degradation. Overexpression of Hsps in animal models of SBMA and other polyQ diseases has been shown to decrease the toxicity of polyQ proteins by promoting protein degradation and reducing protein aggregation [1,3,37,40]. The effect of manipulation of Hsp levels on polyQ toxicity highlights this as a potential therapeutic avenue for SBMA.

4.2. Nuclear translocation: keep it out!

Ligand binding results in the dissociation of the receptor from Hsps and translocation to the nucleus. To determine whether ligand converts polyQ-AR into a toxic species because it brings the disease protein to the nucleus, several AR variants were generated with either mutations of the acetylation site KXKK, addition of a nuclear export signal, or deletion of the nuclear localization signal. These mutations block or reduce nuclear translocation without altering ligand binding. These AR variants suppress polyQ-AR toxicity, indicating that nuclear translocation is a prerequisite for toxicity [68,72,102]. Similar to SBMA, nuclear localization of other polyQ proteins has been shown to be critical for neurodegeneration [8,53,90]. To what extent is nuclear translocation important for toxicity? PolyQ-AR fused to a nuclear localization signal localizes to the nucleus in the absence of ligand, but fails to trigger neurodegeneration, indicating that nuclear translocation is necessary, but not sufficient for toxicity [68,72]. This observation implies that events beyond ligand-induced nuclear translocation are central to disease pathogenesis.

4.3. N/C interaction

Ligand binding results in a conformational change that leads to the AR N/C interaction. The ligand-induced N/C interaction has been shown to be critical for toxicity. Indeed, disruption of the N/C interaction through manipulation of the FXXLF motif reduces mutant protein aggregation and toxicity in cell models of SBMA [75]. The N/C interaction results in protein stabilization [32], and

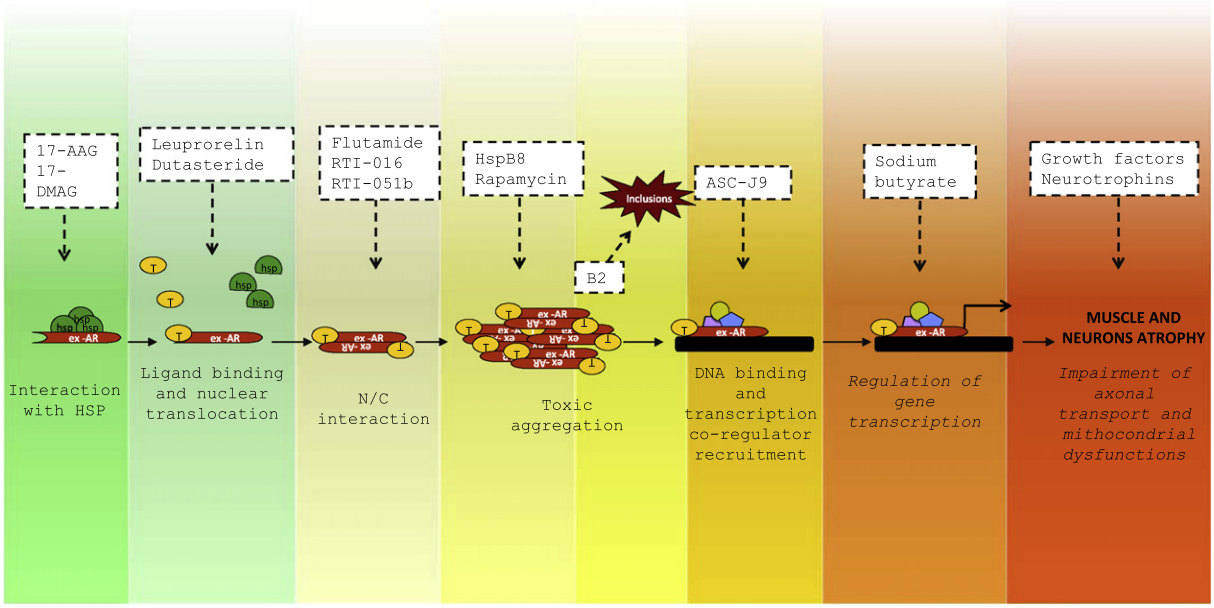

Fig. 3. Towards therapy for SBMA. In the inactive state, AR localizes to the cytosol in association with heat shock proteins (Hsps). Ligand binding leads to the dissociation of AR from Hsps and translocation to nucleus. Also, ligand binding induces a conformational change, which leads to intra- or inter-molecular AR amino/carboxy-terminal (N/C) interactions. Ligand induces polyQ-AR aggregation and inclusion formation. Nuclear translocation is followed by DNA binding, which in turn leads to co-regulator recruitment and regulation of expression of androgen-responsive genes. Pharmacologic intervention has been developed to target many of these steps. 17-AAG and its derivative compounds have been successfully used to increase the association of polyQ-AR with Hsps. Leuprorelin and dutasteride have been used to decrease testosterone levels in the serum and to inhibit the conversion of testosterone to its more potent derivative dihydrotestosterone, respectively. Flutamide and the SARMs RTI-016 and RTI-051b target polyQ-AR to reduce N/C interactions. Modulation of HspB8 expression reduces aggregation, while B2 increases the deposition of mutant AR into inclusions, with beneficial effects on toxicity. Activation of autophagy by rapamycin prevents neurodegenration. ASC-J9 targets co-regulator recruitment, while the histone deacetylase inhibitor sodium butyrate helps to reverse transcription dysregulation. Growth factor and neurotrophin modulation attenuates other aspects of disease, such as muscle atrophy and mitochondrial dysfunction.

this might represent the mechanism through which reduced N/C interaction attenuates toxicity. Protein stabilization is likely to play a role in disease pathogenesis, as modifications that hamper the effect of ligand on protein stabilization, i.e. phosphorylation of polyQ-AR by Akt, are associated with reduced toxicity *in vitro* and *in vivo* [78,80].

4.4. PolyQ aggregation and inclusion formation

Expanded polyQ tracts form anti-parallel beta-strands held together by hydrogen bonds formed between the main chain of one strand and the side chain of the adjacent strand [85]. This leads the polyQ protein to acquire a non-native beta-sheet conformation, which results in the accumulation of misfolded protein into micro-aggregates/oligomers and inclusions. Micro-aggregates are relatively small species that can be detected by biochemistry as the material that accumulates in either the stacking portion of SDS–polyacrylamide gels in Western blotting or in the acetate membrane of a filter retardation assay [79,80]. Inclusions are larger species detectable by immunocytochemistry and immunohistochemistry. The biological role of protein inclusions is not entirely clear. There is evidence that inclusions are protective species [4], and that diffuse nuclear mutant AR may be the toxic species [2].

4.5. DNA binding

AR binds to DNA following ligand-induced nuclear translocation and a significant conformational change. Importantly, a mutation that abolishes the binding of polyQ-AR to DNA without interfering with ligand binding, i.e. substitution of alanine 574 with aspartate, suppresses toxicity in a *Drosophila* model of SBMA, indicating that DNA binding is a prerequisite for toxicity in this system [72]. This finding also indicates that the native function of the disease protein and events occurring in response to DNA binding are important for toxicity.

4.6. Co-regulator recruitment

The binding of AR to DNA is followed by the recruitment of transcriptional co-regulators, both co-activators and co-repressors of transcription [111]. The interaction between AR and transcription co-regulators is ligand-dependent. Substitution of K720 with alanine (K720A) disrupts the LXXLL-mediated interactions with AF-2 and partially alters the FXXLF-mediated interactions, while substitution of E897 with lysine (E897K) disrupts both the LXXLL- and the FXXLF-mediated interactions [21,33]. Importantly, K720A and E897K substitutions in polyQ-AR have been shown to prevent toxicity in flies, indicating that binding of mutant AR to co-regulators is a critical event for pathogenesis *in vivo* [72]. These findings imply that the mechanism underlying polyQ disease pathogenesis involves modification of the normal function of the disease protein, and this is supported also from studies on other polyQ diseases [25,59].

4.7. Regulation of gene transcription

AR is a transcription factor that specifically regulates the expression of hormone-responsive genes. Transcription dysregulation is central to polyQ disease pathogenesis [35], and polyQ expansion in AR leads to transcription dysregulation in cell and fly models of SBMA [58,72]. Although the mechanism through which polyQ expansion affects AR function is not known, chronic transcription dysregulation in motor neurons is likely to lead to neuronal dysfunction and death.

4.8. Axonal transport

Transcription dysregulation in SBMA mice results in altered expression of several genes, including dynactin 1, which is a central regulator of axonal transport [47]. Fast axonal transport has been shown to be altered in SBMA cells in a process that depends on c-Jun N-terminal kinase activation [69]. Because neurons rely on fast axonal transport for neurotrophic support, the effect of polyQ expansion on this process is likely to play an essential role in disease pathogenesis. However, axonal transport defects were not detected in an animal model of SBMA [62].

4.9. Mitochondrial dysfunction

Another gene whose transcription is dysregulated in SBMA cells is peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) [87]. PGC-1 is a transcription factor essential for mitochondrial biogenesis and function. Mutant AR has been shown to alter mitochondrial function, as amino-terminal polyQ-AR fragments induce Bax-dependent cytochrome c release and apoptosis in primary cortical neurons [117]. Full-length polyQ-AR causes mitochondrial membrane depolarization and accumulation of reactive oxygen species, an effect that can be attenuated by treatment of the cells with the antioxidants co-enzyme Q and idebenone [87]. Recently, abnormalities in the mitochondria of leukocytes derived from SBMA patients and carriers were reported [99]. These findings suggest a role for mitochondrial dysfunction in SBMA pathogenesis.

Ligand binding induces several other post-translational modifications, including phosphorylation and others. The impact of these modifications on protein function and polyQ toxicity is discussed elsewhere [83].

5. Clinical perspectives for SBMA

Although there is currently no treatment available to arrest or attenuate the progression of SBMA, several promising therapeutic strategies developed in animal models have emerged from basic research.

5.1. Anti-androgen therapy

The ligand-dependent nature of SBMA offers the unique opportunity to develop a therapy based on the reduction of androgens in the serum. Leuprorelin is a luteinizing hormone-releasing hormone analog that reduces testosterone levels in the serum by decreasing release from testis. Leuprorelin showed tremendous benefits in SBMA transgenic mice, reversing symptoms and histopathological phenotype [44]. Treatment of SBMA mice with leuprorelin decreased the accumulation of insoluble polyQ-AR in the nucleus in spinal cord as well as skeletal muscle. Importantly, leuprorelin reduced both polyQ-AR aggregation and inclusion formation in SBMA mice. These results were translated into a phase II clinical trial, which proved a beneficial effect of leuprorelin associated with amelioration of swallowing together with a reduction of polyQ-AR stabilization and nuclear accumulation [5]. More recently, a larger randomized, placebo-controlled, multi-center clinical trial confirmed that leuprorelin treatment reduces accumulation of polyQ-AR, but did not show any significant effect on swallowing function in patients with SBMA [48]. Leuprorelin decreased the levels of creatine kinase in the serum and reduced the accumulation of polyQ-AR in the nucleus of scrotal skin biopsies of treated patients. Moreover, leuprorelin inhibited the nuclear accumulation of polyQ-AR in the spinal cord and brainstem autoptic specimens of an SBMA patient that had been under treatment for two years [5].

The observation that motor neurons degenerating in SBMA express high levels of 5-alpha-reductase suggests that the conversion of testosterone to DHT represents a potential therapeutic target for disease. Consistent with this idea, a 2-year double-blind placebo-controlled trial with the 5-α-reductase inhibitor dutasteride showed an improvement, although not significant, on muscle strength and a benefit in physical quality of life [24].

5.2. Clearance of mutant protein

Cells have evolved two mechanisms for the degradation of misfolded proteins: the ubiquitin–proteasome system (UPS) and lysosome-mediated autophagy. The observation that Hsp overexpression attenuates polyQ-AR toxicity indicates that enhancing protein quality control systems might be a viable therapeutic strategy for SBMA. Hsp synthesis can be induced by oral administration of the acyclic isoprenoid geranylgeranylacetone (GGA). In SBMA cells, GGA increased the levels of expression of Hsp70, Hsp90, and Hsp105, leading to inhibition of cell death. In SBMA mice, treatment with GGA resulted in amelioration of the neuromuscular phenotype, which was associated with increased expression of several Hsps via activation of heat shock factor-1 and reduction of nuclear accumulation of polyQ-AR [49]. Another approach to activate the heat shock response is the use of the benzoquinone ansamycin geldanamycin, which promotes the degradation of Hsp90 client proteins through the UPS [93]. Unfortunately, this compound is highly toxic and cannot be used for prolonged therapy in humans. A less toxic derivative of geldanamyin is 17-allylamino-17-demethoxygeldanamycin (17-AAG). PolyQ-AR has higher affinity for p23-Hsp90 complex than wild type AR. The major pharmacological effect of 17-AAG, a potent Hsp90 inhibitor, is to promote the dissociation of p23 from the Hsp90-AR complex, leading to enhanced proteasomal degradation of the disease protein. Administration of this compound markedly reduced motor neuron degeneration and increased survival in SBMA mice through increased degradation of monomeric and aggregated mutant AR [89,114]. Interestingly, in motor neuron-derived cells 17-AAG has been shown to protect from toxicity by promoting polyQ-AR degradation through autophagy rather than proteasome [89]. Recently, Sobue and colleagues described the therapeutic efficacy of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), a more potent and water-soluble Hsp90 inhibitor, on the phenotype of SBMA mice [108]. In cell cultures and transgenic mice, the protective effect of 17-DMAG was associated with enhanced degradation of pathogenic AR through the UPS.

PolyQ-AR is also degraded via autophagy. In cultured motor neurons, pharmacologic induction of both mTOR-dependent and mTOR-independent pathways of autophagy leads to the degradation of cytoplasmically retained polyQ-AR, which in turn results in the reduction of the toxic effects of mutant protein [68]. Autophagy can be induced by rapamycin, an inhibitor of the autophagy-negative regulator TOR. Treatment with rapamycin suppressed the neurodegeneration caused by polyQ-AR in SBMA flies [81]. Recent studies in models of amyotrophic lateral sclerosis and polyQ diseases suggest that the HspB8 removes misfolded proteins by activation of the autophagosome-lysosome pathway, highlighting HspB8 as a novel therapeutic target for these disorders [13,14].

5.3. Modulation of N/C interaction

The new evidence showing a role of the N/C interaction in disease pathogenesis highlights this ligand-induced post-translational modification as a novel therapeutic target for SBMA. Flutamide is a non-steroidal anti-androgen known to reduce or prevent the N/C interaction, and is already used for prostate cancer treatment. In SBMA mice, flutamide had no effect on disease progression and manifestations [44]. However, flutamide showed beneficial effects when administered at the prenatal stage in transgenic mice that develop muscle atrophy due to overexpression of normal AR solely in muscle, suggesting a potential beneficial effect when administered in very early developmental stages [42,67]. Another anti-androgen that reduces the AR N/C interaction is bicalutamide, which has recently been shown to reduce polyQ-AR aggregation and prevent DHT-dependent toxicity in PC12 cells expressing an AR with 112 glutamine residues and in primary motor neurons obtained from SBMA mice [75]. The effect of these anti-androgens suggests that selective AR modulators (SARMs) with the ability to inhibit the AR N/C interaction may be effective in SBMA. Consistent with this idea, the two SARMs RTI-016 and RTI-051b that inhibit AR N/C interaction were shown to induce nuclear translocation of AR in the absence of aggregation and toxicity [75]. Whether these SARMs will be effective *in vivo* is not yet known.

5.4. Targeting nuclear inclusion formation

Another potential therapeutic target for SBMA and other polyQ diseases is inclusion formation. The finding that inclusion formation can be associated with protection, rather than toxicity, has provided the rationale for identification of compounds that promote inclusion formation [4]. One such compound is B2, which has been shown to increase inclusion formation and reduce toxicity in cell models of Huntington's disease [9]. Recently, we showed that treatment of cell and fly models of SBMA with B2 results in increased accumulation of mutant AR into inclusions and reduced toxicity, further supporting the idea that accumulation of polyQ-expanded protein into inclusions is protective [79]. Interestingly, we found that the effect of B2 on inclusion formation and toxicity was associated with decreased AR transactivation, suggesting that AR function is important for pathogenesis. Amplification of the native function of polyQ protein has been proposed to be a critical component of polyQ disease pathogenesis [59,72]. The protective effect of B2 in SBMA cells may be the result of a primary effect on the accumulation of polyQ-AR into inclusions, which as a consequence may lead to reduced interaction with transcription co-regulators, reduced AR transactivation and attenuation of toxicity.

5.5. Targeting the interaction of AR with transcriptional co-regulators

The observation that the interaction of polyQ-AR with critical co-regulators (co-repressors and co-activators of transcription) is pathogenic highlights the relevance of this pathway as a therapeutic target [72]. This concept is supported by the observation that treatment of SBMA mice with the curcumin-related compound 5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one (ASC-J9) disrupted the interaction between AR and its co-regulator ARA70 and improved disease symptoms by decreasing nuclear aggregation and increasing mutant AR clearance [116]. Treatment of the mice with ASC-J9 did not alter the levels of testosterone in the serum of SBMA mice, thereby avoiding side effects on sexual activity and fertility. As new curcumin analogs have been identified, it would be interesting to determine whether any of them has beneficial effects in SBMA [95].

5.6. Targeting native AR function

Transcription dysregulation is a primary pathogenic process in polyQ diseases. Altered gene expression is a consequence not only of sequestration of transcription factors and co-regulators, but also of altered chromatin remodeling. One of the major modifications of chromatin involves histone acetylation, which is associated with gene expression, and histone deacetylation, which is associated

with gene silencing. Histone acetylation is dependent on two opposing classes of proteins: the histone acetyltransferases (HATs) and the histone deacetylases (HDACs). Disruption of the normal balance between histone acetyltransferase and deacetylase activities has deleterious consequences on proper gene expression patterns. PolyQ proteins sequester some HAT proteins, such as CREB-binding protein (CBP) [64,105]. Overexpression of CBP rescued histone acetylation and neurodegeneration in fly and mouse models of polyQ diseases. These studies suggest that inhibition of HDAC activity may be of therapeutic value. Indeed, the HDAC inhibitor sodium butyrate has been shown to ameliorate the neurological phenotype of SBMA transgenic mice, and this effect was associated with increased acetylation of nuclear histones in neural tissues [66].

5.7. Growth factors and neurotrophins

Altered gene expression as well as disruption of axonal transport may contribute to polyQ disease pathogenesis by decreasing trophic support for neurons. Among the genes that have been shown to be down-regulated in mouse models of SBMA are the vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), glial cell line-derived neurotrophic factor (GDNF), transforming growth factor-beta, and neurotrophin-4 [46,97,118]. Activation of VEGF and IGF-1 signaling has been shown to be beneficial in SBMA mouse models, indicating this as a novel therapeutic strategy to attenuate disease manifestations [80,97]. Interestingly, we have previously shown that muscle-restricted overexpression of a muscle-specific isoform of IGF-1 (mIGF-1) in SBMA mice protects from the toxicity of polyQ-AR via a mechanism that involves direct modification of the disease protein [80]. mIGF-1 activated Akt in muscle of SBMA mice, which then in turn stimulated the phosphorylation of polyQ-AR and its turnover through proteasome. Activation of the IGF-1/Akt signaling in muscle not only resulted in a remarkable amelioration of muscle, but also of spinal cord pathology together with amelioration of motor dysfunction and increased survival. These findings support the idea that muscle represents a critical tissue target of polyQ-AR toxicity and highlight that intervention in muscle may be therapeutically relevant for SBMA.

5.8. Physical exercise

Frequent, moderate-intensity aerobic training in patients with muscular dystrophies and metabolic myopathies has shown beneficial effects [30,41,73,74,100]. Most of these patients have clinical characteristics similar to patients with SBMA, such as muscle weakness and wasting along with a sedentary lifestyle. Moreover, physical exercise can increase the production of IGF-1 and other growth factors from muscle, which may have beneficial effects on muscle and spinal cord. Preisler and colleagues tested the effect of aerobic training for three months in eight SBMA patients [86]. There was an amelioration of certain aspects of disease manifestations, but the effect of exercise was limited. It remains to be established whether prolonged exercise training is of any therapeutic efficacy in SBMA patients.

6. Concluding remarks

The discovery of polyQ expansion in the gene encoding AR as the mutation responsible for SBMA dates to the early 1990s [55]. Two decades later, however, there is no effective therapy for SBMA and the other neurodegenerative diseases caused by expanded polyQ. Ligand binding to polyQ-AR converts the disease protein into a toxic species by altering several cellular processes, including protein folding, transcriptional regulation and mitochondrial function. From a therapeutic point of view, each of these pathways may represent a worthwhile avenue to pursue for development of therapy for SBMA. It is possible that effective therapy will come from combination therapy. For instance, a therapy based on the use of anti-androgens together with agents that reduce any of the ligand-induced modifications of mutant protein might be worthwhile to pursue.

Conflict of interest

The authors have no conflict of interest to declare.

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