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From gene to therapy in spinal and bulbar muscular atrophy: are we there yet?

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Abstract

Abnormal polyglutamine expansions in the androgen receptor (AR) cause a muscular condition, known as Kennedy's disease or spinal and bulbar muscular atrophy (SBMA). The disease is transmitted in an X-linked fashion and is clinically characterized by weakness, atrophy and fasciculations of the limb and bulbar muscles as a result of a toxic gain-of-function of the mutant protein. Notably, affected males also show signs of androgen insensitivity, such as gynaecomastia and reduced fertility. The characterization of the natural history of the disease, the increasing understanding of the mechanism of pathogenesis and the elucidation of the functions of normal and mutant AR have offered a momentum for developing a rational therapeutic strategy for this disease. In this special issue on androgens and AR functions, we will review the molecular, biochemical, and cellular mechanisms underlying the pathogenesis of SBMA. We will discuss recent advances on therapeutic approaches and opportunities for this yet incurable disease, ranging from androgen deprivation, to gene silencing, to an expanding repertoire of peripheral targets, including muscle. With the advancement of these strategies into the clinic, it can be reasonably anticipated that the landscape of treatment options for SBMA and other neuromuscular conditions will change rapidly in the near future.

Introduction

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is caused by expansions of a CAG tandem trinucleotide repeat, encoding glutamine, in the first exon of the androgen receptor (*AR*) gene (La Spada et al., 1991). SBMA is one out of nine neurological disorders caused by expansions of CAG repeats in the coding regions of specific genes. These disorders are known as polyglutamine diseases and include Huntington's disease, dentatorubral-pallidoluysian atrophy, and spinocerebellar ataxia type 1, 2, 3, 6, 7, and 17 (Fan et al., 2014; Orr and Zoghbi, 2007; Pennuto and Sambataro, 2010). Polyglutamine diseases are caused by CAG repeat expansions in the coding regions of the genes coding for huntingtin (Macdonald et al., 1993), atrophin-1 (Koide et al., 1994; Nagafuchi et al., 1994), ataxin-1 (Orr et al., 1993), ataxin-2 (Imbert et al., 1996), ataxin-3 (Kawaguchi et al., 1994), CACNA1A (Zhuchenko et al., 1997), ataxin-7 (David et al., 1997), and the TATA-binding protein (TBP) (Nakamura et al., 2001).

Polyglutamine diseases are all inherited in an autosomal dominant fashion, except for SBMA, which is X-linked. These diseases are progressive and have typically a late onset exordium, with a negative correlation between the length of the CAG repeat and the age at onset and a positive correlation with disease severity. Consistent with these features, polyglutamine diseases show the phenomenon of genetic anticipation, with the next generation more likely to inherit a longer polyglutamine tract and present a more severe phenotype. Although the polyglutamine-expanded proteins are expressed in several tissues and, in some cases, have housekeeping functions in the cells, neurons are primary targets of polyglutamine-expanded proteins. Even more intriguingly, specific populations of neurons degenerate in polyglutamine diseases, resulting in different clinico-pathological disease manifestations (Roselli and Caroni, 2015; Saxena and Caroni, 2011). The molecular basis of selective neuronal vulnerability remains obscure.

Polyglutamine diseases are caused by both toxic gain of function and loss of function mechanisms. Generation of knock out, knock in and transgenic animal models of polyglutamine diseases has allowed to better appreciate the mechanism of neurodegeneration in these disorders. Knock out or knock down of polyglutamine proteins generally is associated with phenotypes different from those caused by the polyglutamine-expanded proteins. For instance, loss of function mutations of AR in humans as well as ablation of AR expression in mice result in a phenotype that does not include a neuromuscular dysfunction, supporting a toxic gain of function model for SBMA. Moreover, overexpression of polyglutamine-expanded proteins in the presence of the wild type counterpart causes disease, indicating that polyglutamine diseases are not caused by pure loss of function mechanisms. Nevertheless, polyglutamine expansion also confers a loss of protein

function to the mutant protein, which contributes to disease pathogenesis. Indeed, SBMA patients present frequently with symptoms of partial androgen insensitivity, such as gynecomastia, reduced libido and impotence (Querin et al., 2015). Moreover, loss of endogenous AR has been shown to aggravate the phenotype caused by mutant AR, thereby showing a contribution of the loss of AR function in SBMA (Thomas et al., 2006).

Polyglutamine expansion is associated with protein misfolding, aggregation and inclusion formation. Polyglutamine-expanded proteins aggregate into detergent-insoluble amyloid-like fibrils (Adegbuyiro et al., 2017). The length of the polyglutamine tract directly correlates with the propensity to form amyloid fibrils. Biochemically, micro-aggregates/oligomers can be detected as high molecular weight species that accumulate in the stacking gel by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) and by filter retardation assay (Palazzolo et al., 2009). In addition, polyglutamine proteins form inclusion bodies, larger structures composed of fibrillar aggregates. These inclusions form in both nucleus and cytosol, are a hallmark of disease, yet their role is disease pathogenesis is far from understood. While initially considered toxic species whose formation correlated with disease progression, more recently inclusions have been proposed to be protective species, and neurons capable of depositing misfolded polyglutamine proteins into inclusion bodies were shown to survive longer compared to neurons unable to form such structures (Arrasate et al., 2004; Palazzolo et al., 2010). Rather, diffused misfolded proteins and amyloid fibrils may be the toxic species that not only grow inside the neurons, but that can also be transmitted from one neuron to another in response to neuronal activity and by means of extracellular vesicles (Pecho-Vrieseling et al., 2014; Ren et al., 2009).

An intriguing aspect of polyglutamine diseases is that the same mutation in different genes causes the dysfunction and degeneration of specific populations of neurons in the central nervous system. This indicates that expansion of polyglutamine tracts is necessary, but not sufficient to dictate disease features. Rather, intrinsic protein features play a critical role in dictating the initiation and progression to cellular dysfunction and degeneration (Graham et al., 2006; Katsuno et al., 2002; Klement et al., 1998; Tsuda et al., 2005). A large body of evidence shows that both the structure and the native functions of polyglutamine-expanded proteins are important in the neurodegenerative process. This is particularly evident in SBMA. Indeed, the sex-specificity of SBMA indicates that the expanded polyglutamine tract is not the only causative determinant of motor neuron loss and skeletal muscle atrophy. Rather, protein domains outside the polyglutamine tract are expected to play a key role in disease pathogenesis. Moreover, recent findings support the idea that the native functions of polyglutamine-expanded proteins cause neurodegeneration through gain and loss of function mechanisms involving native protein-protein interactions and alteration of

the native functions of the disease proteins. AR is an ideal model protein to appreciate the contribution of protein structure and function to disease pathogenesis, as its structure and functions are quite well characterized.

Clinical features

SBMA is an X-linked neuromuscular condition where only males are fully affected. Female carriers are generally asymptomatic or may experience recurrent muscle cramps (Schmidt et al., 2002). Disease onset ranges from about ages 18–64, with most patients presenting in the fourth or fifth decade of life with tremor, cramping, proximal and distal weakness, and muscle atrophy, secondary to the lower motor neuron degeneration and primary muscle atrophy (Rhodes et al., 2009). Involvement of the bulbar muscles is a frequent finding, accounting for dysarthria and dysphagia, hypernasality, with decreased range of pitch and loudness, and perioral fasciculations. Weakness of the temporalis and masseter muscles with selective preservation of pterygoid muscles causes fatigue when chewing and occasionally jaw drop (Sumner and Fischbeck, 2002). Degeneration of the dorsal root ganglia often results in a loss of sensation in the lower extremities. Signs of androgen insensitivity include gynaecomastia, oligospermia and erectile dysfunction, as well as reduced risk of androgenetic alopecia in SBMA (Sinclair et al., 2007). SBMA subjects also often demonstrate metabolic alterations, including abdominal obesity, increased insulin resistance, and dyslipidemia, likely as a result of decreased androgen signal (Rhodes et al., 2009; Hashizume et al., 2012; Nakatsuji et al., 2017)). Although most patients have elevations in total testosterone, free testosterone and dihydrotestosterone, the levels of free testosterone and dihydrotestosterone may be reduced in some individuals. Low sensory nerve amplitudes, decreased compound motor action potentials and evidence of diffuse denervation are the characteristic features on electromyography and nerve conduction study. Motor unit nerve estimation (MUNE) is reduced to about half of healthy control values (Lehky et al., 2009). A muscle biopsy may show evidence of neurogenic and myogenic atrophy (Kennedy et al., 1968; Soraru et al., 2008). Female carriers do not usually develop weakness, although a minority may have muscle cramps (Ishihara et al., 2001).

Disease progression is relatively slow, particularly when compared to other motor neuron diseases, such as amyotrophic lateral sclerosis (ALS), with muscle strength, as measured by quantitative muscle assessment, declining by 2% per year (Fernandez-Rhodes et al., 2011). The majority of individuals with SBMA have a normal life expectancy although affected subjects are at risk of choking on food and aspiration pneumonia because of weakness of the bulbar muscles (Atsuta et al., 2006; Kennedy et al., 1968). As bulbar symptoms correlate with the length of the CAG repeat and affect the prognosis (Atsuta et al., 2006), videofluorography-assessed by barium

swallow has been suggested as a reliable and relevant outcome measure in SBMA clinical trials (Fernandez-Rhodes et al., 2011; Katsuno et al., 2010). Interestingly, a 29-year old patient with 68 CAG repeats –the largest reported so far- developed signs and symptoms of autonomic dysfunction and abnormal sexual development in addition to the typical clinical picture (Grunseich et al., 2014). The diagnosis of SBMA is often delayed due to limited awareness; therefore the estimated prevalence of 1-2 per 100,000 is likely to be an underestimation. Time to diagnosis after onset of weakness averaged 5.5 years, and the time from first medical evaluation to diagnosis averaged more than 3 years (Rhodes et al., 2009). SBMA patients are most often misdiagnosed with ALS, myasthenia gravis, polymyositis, metabolic myopathy and chronic inflammatory neuropathy. With recognition of the characteristic clinical features and the availability of confirmatory genetic testing, a diagnosis can be relatively straightforward.

From the *AR* gene to protein

The *AR* gene lies on the X chromosome and is composed of eight exons that encode a protein of about 110 kDa (about 920 amino acids depending on the length of several polymorphic trinucleotide repeats, NM_000044). In addition to the open reading frame, the 11 kb mRNA contains 1.1 kb 5' untranslated region (UTR), the longest among steroid receptors, and a 6.8 kb 3'UTR (Faber et al., 1991). AR is highly expressed in sexual organs in males as well as in motor neuron and muscle cells, which degenerate in SBMA patients. AR negatively regulates its own expression by binding to the second intron of the *AR* gene (Cai et al., 2011). The AR is a steroid hormone receptor composed of three main domains: Exon 1 encodes the amino-terminal domain (amino-acids 1-555), exons 2 and 3 encode the DNA binding domain and the hinge region (amino-acids 556-670), and exons 4-8 encode the ligand binding domain (amino-acids 671-920). The AR is a transcription factor activated by testosterone and dihydrotestosterone.

The amino-terminal domain of the AR is poorly conserved throughout evolution and is the less structured domain of the protein. This domain contains the polyglutamine tract whose expansion causes SBMA. The length of the pathogenic polyglutamine tract ranges between 5 and 36 residues in the normal population, with an average length of about 21 glutamines. Expansions of 38-to-68 residues cause SBMA. The size of this polyglutamine tract affects AR function, with longer repeat tracts associated with lower AR activity (Harada et al., 2010; Tut et al., 1997; Wang et al., 2004). In SBMA, pathogenic expansions of the polyglutamine tract reduce AR activity and may be responsible for partial loss of AR function. AR has two other short polyglutamine tracts, whose length also negatively affects AR function (Harada et al., 2010). AR has two more tandem repeats, a polyglycine tract and a polyproline tract that are polymorphic in size. The length of the

polyglycine tract does not correlate with severity of disease (Bertolin et al., 2016). The role of the polyproline tract in SBMA is not known. The amino-terminal domain of the AR is critical for its function. It contains two subdomains, namely activating function 1 (AF-1) and AF-5, which span amino acids 51-211 and 370-494, respectively. AF-1 is masked by heat shock proteins (HSPs) and functions in a ligand-dependent fashion. The relevance of these functional domains in SBMA pathogenesis remains to be elucidated.

The DNA binding domain of AR is composed of two zinc fingers, of which the first one dictates binding site specificity by contacting the major grove of DNA, and the other one stabilizes binding. The AR has a nuclear localization signal spanning residues 617-634, which works in a hormone-dependent fashion. This nuclear localization signal is bipartite and is composed of two clusters of conserved basic residues separated by 10 amino acids (Simental et al., 1991). Nuclear import of AR occurs through the importin-α and importin-β systems (Cutress et al., 2008). The hinge region contains a sequence known as PEST (where P is proline, E glutamic acid, S serine, and T threonine) (Sheflin et al., 2000), which targets proteins for degradation by the ubiquitin-proteasome system (UPS) (Rechsteiner and Rogers, 1996). Normal and polyglutamine-expanded AR are indeed rapidly and efficiently degraded mainly through the UPS in a process regulated through phosphorylation at the amino-terminal domain and the ligand-binding domain (Lin et al., 2001; Palazzolo et al., 2007).

The ligand-binding domain of AR is composed of 12 alpha-helices and 4 beta-strands (Matias et al., 2000). Hormone binding results in a conformational change that leads to formation of a transactivation domain, namely AF-2. AF-2 recruits co-regulators of transcription bearing the LXXLL motif (where L is leucine and X any amino acid). In the AR, the AF-2 preferentially binds to the FXXLF motif (where F is phenylalanine) and the WXXLF motif (where W is tryptophan) in the amino-terminal domain of the protein (He et al., 2000), resulting in an interaction between the amino (N)-terminal domain and the carboxy (C)-terminal domain, which is known as the N/C interaction (Langley et al., 1995; Langley et al., 1998). The N/C interaction can be intra-molecular and inter-molecular, the first likely occurring in the cytosol and the other in the nucleus (Schaufele et al., 2005).

Disease mechanisms

Since the discovery of polyglutamine expansions as the genetic cause of SBMA, a large body of evidence has been obtained to explain how binding of androgens to polyglutamine-expanded AR triggers SBMA. In the unbound/inactive state, AR forms complexes with heat shock proteins (HSPs) in the cytosol (**Fig. 1**). Binding to androgens results in dissociation from HSPs, a

conformational change that results in N/C interactions, translocation to nucleus, DNA binding, interaction with transcription co-factors and regulation of gene expression (Parodi and Pennuto, 2011). All these post-translational events triggered by hormone binding have been shown to play a role in disease pathogenesis. Moreover, hormone binding induces several post-translational modifications that play a key role in SBMA (**Fig. 1**). The relevance of post-translational modifications in SBMA pathogenesis is discussed elsewhere (Pennuto et al., 2009; Sambataro and Pennuto, 2017).

The interaction between polyglutamine-expanded AR and the HSPs has been extensively investigated in several in vitro and in vivo models of SBMA. Overexpression of HSPs, such as HSP40, HSP70, and HSP105alpha, in animal models of SBMA and other polyglutamine diseases reduces the toxicity of polyglutamine proteins by reducing protein aggregation and inducing protein degradation (Adachi et al., 2003; Adachi et al., 2007; Bailey et al., 2002; Howarth et al., 2007; Ishihara et al., 2003; Kobayashi et al., 2000). Recently, the heat shock protein B8 (HspB8), a member of the small heat shock protein family, has been shown to facilitate the clearance of polyglutamine-expanded AR through autophagy (Rusmini et al., 2013). AR is a HSP90 client protein that forms two types of complexes with opposite functions, one containing HSP70 and Hop, which drives proteins to degradation through the UPS, and the other containing Cdc37 and p23, which stabilizes proteins. Compounds like 17-AAG that promote the assembly of the HSP90/Hop/AR complex have been shown to have therapeutic potential in SBMA (Waza et al., 2005).

Binding of AR to androgens results in dissociation from the HSPs, an event followed by a change in conformation leading to intra- and inter-molecular N/C interactions. Recent evidence has emerged showing that the N/C interactions play a critical role in the pathogenesis of SBMA. Disruption of the N/C interactions by mutation of the FXXLF motif has been shown to reduce mutant protein aggregation and toxicity in cell models of SBMA (Orr et al., 2010). Since the N/C interaction is needed for protein stabilization in response to hormone binding (He et al., 2001), the beneficial effect derived from decreased N/C interactions may be the result of increased turnover, reduced protein accumulation and aggregation, and increased degradation by the UPS.

Hormone binding results in AR nuclear translocation. Localization of polyglutamine proteins in the nucleus is a prerequisite for neurodegeneration (Bichelmeier et al., 2007; Klement et al., 1998; Saudou et al., 1998). In the case of SBMA, nuclear translocation is necessary, but not sufficient for toxicity. Indeed, either deletion of the nuclear localization signal (NLS) or addition of a nuclear export signal (NES) reduce hormone-induced nuclear translocation and attenuate neurodegeneration, indicating that nuclear localization is a key event in disease pathogenesis

(Montie et al., 2009; Nedelsky et al., 2010; Takeyama et al., 2002). Consistent with these findings, mutation of the acetylation motif, KXKK (where K is lysine), within the NLS reduces nuclear translocation and attenuates neurodegeneration in fly models of SBMA (Nedelsky et al., 2010). However, forced nuclear translocation of mutant AR in the absence of hormone binding failed to induce neurotoxicity, supporting the concept that nuclear localization is needed for toxicity, but is not sufficient to cause neuronal damage (Montie et al., 2009; Nedelsky et al., 2010). This evidence implies that events occurring in the nucleus and linked to AR biology and function are involved in neuronal damage and muscle atrophy.

Within the nucleus, activated AR binds to specific sequences, namely androgen-responsive elements (AREs), to regulate the expression of androgen-responsive genes (**Fig. 1**). DNA binding is therefore another androgen-induced post-translational event that is necessary for toxicity in SBMA. Mutations preventing DNA binding suppress toxicity, thereby indicating that DNA binding is a key event in the cascade of modifications triggered by androgen binding (Nedelsky et al., 2010).

DNA binding is followed by co-factor recruitment through the AF-2 surface (van Royen et al., 2007). Disruption of the AF-2 surface reduces co-factor recruitment and attenuates the toxicity of mutant AR (Nedelsky et al., 2010). One of the co-factors recruited through the AF-2 surface is protein arginine methyltransferase 6 (PRMT6), a co-activator of AR whose structural and functional interaction with AR is enhanced by polyglutamine expansion (Scaramuzzino et al., 2015). Recruitment of co-factors through AF-2 aberrantly enhances mutant AR function, thereby contributing to neurodegeneration. These observations provide evidence to the concept that native protein-protein interactions and functions of mutant AR are fundamental aspects in the neurodegenerative process. Whether the AF-1 and AF-5 in the amino-terminal domain of AR play a role in SBMA remains to be established.

As a transcription factor activated by androgens, the AR regulates (activates and represses) expression of androgen responsive genes in a tissue- and time-specific fashion. AR function is altered in SBMA, and dysregulation of gene expression has been reported in neuronal and peripheral tissues, such as skeletal muscle (Lieberman et al., 2002; Rocchi et al., 2016). Changes in gene expression can be primary, due to altered binding site specificity of mutant AR, or secondary, due to changes in the expression and function of other transcription factors and epigenetic regulators of gene expression. In skeletal muscle, expression of polyglutamine-expanded AR results in aberrant expression of hundreds of genes. Notably, several independent studies carried out on different rodent models of SBMA have led to identification of genes that are upregulated and genes whose expression is downregulated in SBMA muscle (Giorgetti et al., 2016; Mo et al., 2010; Rocchi et al., 2016). Gene ontology analysis revealed alterations in several pathways, including

glycolysis, lipid metabolism, mitochondrial genes, cell adhesion, and of course muscle atrophy and myogenesis. These gene expression analyses in muscle revealed altered muscle energy balance and metabolism and mitochondrial dysfunction as leading mechanisms underlying SBMA skeletal muscle atrophy.

Genes whose expression is altered in SBMA are peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) and genes encoding mitochondrial proteins (Borgia et al., 2017b; Ranganathan et al., 2009; Rocchi et al., 2016). PGC-1α controls mitochondrial biogenesis and function. Interestingly, mutant AR localizes to mitochondria and causes mitochondrial membrane depolarization both in SBMA motor neurons and muscle cells (Borgia et al., 2017a; Ranganathan et al., 2009). Amino-terminal fragments of mutant AR induce Bax-dependent cytochrome c release and apoptosis in primary cortical neurons (Young et al., 2009), as well as accumulation of reactive oxygen species, which can be reduced by the antioxidants co-enzyme Q and idebenone (Ranganathan et al., 2009). Importantly, muscle pathology is associated with increased clearance of the damaged mitochondria by mitophagy in SBMA patients (Borgia et al., 2017a). This evidence supports the idea that polyglutamine-expanded AR causes muscle atrophy by altering the energy balance and mitochondrial function.

Another gene whose expression is dysregulated in SBMA is dynactin 1, which is a central regulator of axonal transport (Katsuno et al., 2006). Mutant AR has been shown to inhibit fast axonal transport through activation of cJun N-terminal kinase (JNK), which in turn phosphorylates kinesin-1 heavy chains and inhibits its microtubule-binding activity (Morfini et al., 2006). Although axonal transport defects were not detected in an animal model of SBMA (Malik et al., 2011), defects in motoneuronal retrograde axonal transport have been described in knock in SBMA mice and in mice overexpressing normal AR solely in skeletal muscle, suggesting that these alterations are consequence of expression of mutant AR in muscle and occur in a non-cell-autonomous fashion in the motor neuron (Halievski et al., 2016; Kemp et al., 2011).

Polyglutamine expansion results in the accumulation of mutant AR in the forms of micro-aggregates/oligomers and inclusion bodies (**Fig. 1**). Deposition of AR aggregates is increased in tissues that degenerate in SBMA, such as spinal cord and skeletal muscle (Katsuno et al., 2002; Palazzolo et al., 2009). By atomic force microscopy, polyglutamine expansion has been shown to shift the deposition of AR from annular oligomers to fibrillar oligomers, which may be toxic species (Jochum et al., 2012). Notably, activation of signaling pathways that increase the propensity of polyglutamine-expanded AR to form annular oligomers have been shown to exert beneficial effects on the phenotype of knock in SBMA mice (Polanco et al., 2016). Moreover, compounds that enhance inclusion formation also suppress polyglutamine-expanded AR toxicity in vitro and in vivo

(Palazzolo et al., 2010). Although the role of fibrils and inclusions in SBMA remains to be fully understood, it is possible that aggregates/fibrils represent toxic species, whereas inclusion are protective species, as established in other polyglutamine diseases (Arrasate et al., 2004).

Therapeutic perspectives

No disease-modifying treatment is currently available for SBMA. During the last few years, a number of potential therapeutic strategies for SBMA have emerged, some of which are already starting to be tested in clinical trials, as a result of a deeper understanding of the mechanisms of disease pathogenesis (**Fig. 1**). Several studies in animal models have demonstrated that SBMA pathogenesis depends on high circulating levels of testosterone in males, since surgical castration (Chevalier-Larsen et al., 2004; Katsuno et al., 2002) and androgen deprivation (Katsuno et al., 2003) are sufficient to reverse the phenotype in mice. These findings have prompted researchers to test anti-androgen therapies in clinical trials in SBMA. Promising results in preclinical (Katsuno et al., 2003) and phase 2 clinical trials (Banno et al., 2009) using leuprorelin, a potent luteinizing hormone-releasing hormone analogue that suppresses the release of gonadotropins, luteinizing hormone and follicle-stimulating hormone, led to the establishment of a larger, multicentre, placebo-controlled phase 3 clinical trial of leuprorelin in SBMA, where the primary endpoint was pharyngeal barium residue, measured by videofluorography (Katsuno et al., 2010). A total of 199 SBMA male patients were assigned to receive either leuprorelin or placebo subcutaneous injections every 3 months for 12 months. The treatment did not show significant effects on swallowing function in SBMA patients, unless treatment was initiated in early-stage patients (disease duration<10 years). A more recent placebo-controlled, phase 2 trial using dutasteride, a potent inhibitor of the enzyme 5-α-reductase, which mediates the conversion of testosterone to DHT, also failed to show a significant effect on the progression of muscle weakness (Fernandez-Rhodes et al., 2011).

Increasing protein degradation represents another promising attractive therapeutic strategy in disorders such as SBMA, where protein aggregation is a key pathological feature. Several approaches have been undertaken by targeting various components of the proteostasis network to enhance mutant AR clearance (Adachi et al., 2007; Bott et al., 2016; Katsuno et al., 2005; Rinaldi et al., 2016; Tokui et al., 2009; Waza et al., 2005). Recently, Wang et al. identified a small molecule that allosterically promotes HSP70 binding to unfolded substrates, alleviating toxicity in an SBMA Drosophila model (Wang et al., 2013). Trehalose stimulates autophagy and induces HSPB8 expression, suggesting therapeutic potential in SBMA (Rusmini et al., 2013). Although several of these strategies are effective in increasing HSP expression, non-specific upregulation of HSP levels

can potentially have deleterious consequences. However, one agent that can upregulate HSP expression yet avoid the potential problems of non-specific elevation in HSPs, is arimoclomol, a co-inducer of the heat shock response (HSR) only in stressed cells (Kieran et al., 2004) and that has been shown to be effective in an SBMA mouse model (Malik et al., 2013),.

Post-translational protein modifications, such as phosphorylation, methylation, SUMOylation and acetylation, modulate AR toxicity and therefore represent another promising target for therapeutic intervention (Chua et al., 2015; Montie et al., 2011; Palazzolo et al., 2007; Palazzolo et al., 2009; Pennuto et al., 2009; Scaramuzzino et al., 2015). Based on preclinical work showing therapeutic potential of IGF1-mediated phosphorylation of AR (Palazzolo et al., 2009; Rinaldi et al., 2012), a phase 2 clinical trial using an analogue of the insulin-like growth factor 1 (IGF-1) has been performed in a cohort of SBMA (ClinicalTrial.gov, Identification number: NCT02024932); its results will be soon available.

Among all viable approaches, a RNA interference strategy to target AR for suppression is currently gaining increasing interest, particularly in light of recent clinical success in other neuromuscular diseases (Finkel et al., 2016): reduction of polyglutamine-expanded AR expression was recently achieved in a mouse model of SBMA using miRNAs targeting AR either directly (Pourshafie et al., 2016) or indirectly (Miyazaki et al., 2012) delivered via recombinant adeno-associated virus (rAAV), and an antisense oligonucleotide targeting AR exclusively in either the periphery (Lieberman et al., 2014) or spinal cord after subcutaneous or intrathecal administration (Sahashi et al., 2015). This option holds great potential as a therapeutic strategy for SBMA and other diseases caused by a mechanism of toxic gain-of-function, as it allow to reduce the expression of the mutant protein before it can cause its deleterious effects. Nevertheless, translation of this approach into clinical setting may be hampered by the potential of exacerbation of signs and symptoms of loss of androgen function, given that affected patients only have one copy of the gene.

Outstanding questions and concluding remarks

Since the discovery of the causative gene in 1991, much work has been done to unravel the pathophysiology of SBMA. A tremendous advancement in knowledge has been achieved toward understanding the molecular details of disease pathogenesis. Today, we know that polyglutamine expansion causes neuronal dysfunction because it leads to protein misfolding and aggregation, and it hampers several cellular processes occurring in the nucleus, cytosol, and neurites. Polyglutamine expansion alters DNA, RNA and protein processing, stabilization, repair and function. Moreover, we know that polyglutamine expansion confers a toxic gain of function to the mutant protein, which involves amplification of native protein function, as well as a loss of protein function. Nevertheless,

despite these recent advancements, still a number of critical issues remain unsolved, which might at least partially account for why therapeutic strategies that work well in preclinical models have not quite yet been translated into a cure for patients. Here we have identified the following open questions in SBMA:

1. What are the molecular mechanisms underlying the tissue-specific toxicity?
2. What are the relative contributions to the disease pathogenesis of the proteotoxic gain of function and the intrinsically altered transcriptional activity of mutant AR?
3. How solely targeting muscle for therapy can attenuate disease severity and improve motor neuron pathology?
4. What are the non-neuromuscular features of SBMA and what is their burden on the disease phenotype?
5. Are therapeutic strategies simply aimed at reducing AR protein levels sufficient to treat the disease in affected patients?

Answering those questions not only will advance our understanding of the underlying molecular mechanisms in SBMA and other diseases of the motor unit, but it may also improve our ability to identify therapeutic targets with highly translational potential. We are optimistic that the increasing knowledge about the molecular mechanisms of polyglutamine disease pathogenesis will lead to the development of new and effective therapy for patients.

Figure Legend

Fig.1. Disease mechanisms and therapeutic targets for SBMA. The current understanding of disease pathogenesis has led to the identification of four main possible therapeutic strategies to treat SBMA: i) Androgen deprivation, ii) Therapies aimed at improving the protein quality control system in the cell, iii) Modulation of AR function (e.g. by targeting disease-specific post translational modifications or interaction with co-factors), and iv) Gene silencing (e.g. via antisense oligonucleotides or AAV-delivered miRNAs).

COMPETING INTERESTS

The authors have no conflict of interest to declare.

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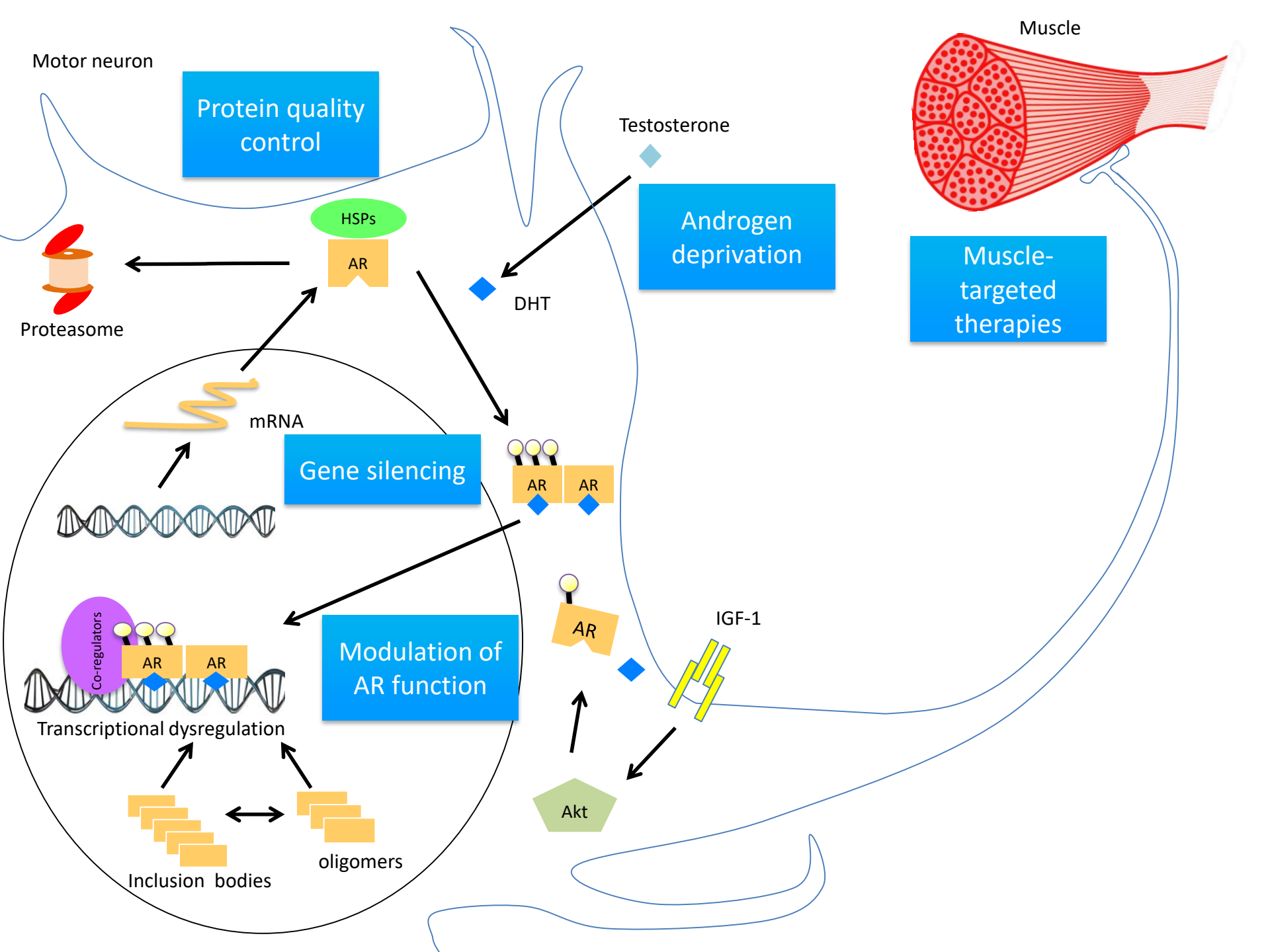
Motor neuron
Protein quality control
Testosterone
Androgen deprivation
Muscle
Muscle-targeted therapies
HSPs
AR
Proteasome
DHT
mRNA
Gene silencing
AR
AR
Co-regulators
AR
AR
Transcriptional dysregulation
Modulation of AR function
AR
IGF-1
Akt
Inclusion bodies
oligomers

Review highlights

- Polyglutamine expansions in the androgen receptor cause spinal and bulbar muscular atrophy, also known as Kennedy's disease
- Clinical features: genotype/phenotype correlation
- From gene to protein: Molecular pathways to neurodegeneration
- Development of novel therapeutic approaches