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SPINAL AND BULBAR MUSCULAR ATROPHY: NEW INSIGHTS INTO THE DISEASE MECHANISM AND PROSPECTS FOR PHARMACOLOGICAL THERAPY

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Spinal and Bulbar Muscular Atrophy: New Insights into the Disease Mechanism and Prospects for Pharmacological Therapy

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Sigrun & Oliver

Gerade Tatsachen gibt es nicht, nur Interpretationen.

It is precisely facts that do not exist, only interpretations.

Friedrich Nietzsche

ABSTRACT

Expansion of a polyglutamine-encoding trinucleotide CAG repeat in the androgen receptor (AR) gene causes spinal and bulbar muscular atrophy (SBMA, or Kennedy's disease). SBMA is an adult-onset disease characterized by progressive muscle weakness and atrophy due to the degeneration of lower motor neurons in the brainstem and spinal cord. At present, effective disease-modifying treatment is not available for this disorder. Neuronal dysfunction in SBMA is at least in part due to a toxic gain of function of the mutant AR, however, the underlying mechanism in the pathogenesis is not known. Work in this thesis identified new disease features of SBMA (**Study I**), investigated the effect of the polyglutamine expansion in the context of normal AR function (**Study II**), and explored pharmacological strategies for reducing mutant AR as potential treatments (**Studies III** and **IV**).

In **Study I**, we describe a 29-year old SBMA patient with a 68 CAG repeat, the largest reported to date. The patient had an unusually early onset and novel clinical features, including developmental defects and autonomic dysfunction.

In **Study II**, we examined the effect of the polyglutamine expansion in the AR on androgen-induced differentiation of neuronal cells. We show that mutant AR expression in this model leads to aberrant neurite outgrowth and reduced cell cycle arrest. The expanded polyglutamine tract in the AR interferes with the activity of the ubiquitin ligase APC/C-Cdh1, a critical regulator of cell cycle exit and neuronal architecture. These findings suggest that cellular abnormalities due to the stabilization of APC/C-Cdh1-dependent substrates may contribute to the pathogenic mechanism in SBMA.

Augmentation of insulin-like growth factor (IGF)-1/Akt signaling was previously shown to promote the degradation of polyglutamine-expanded AR. In **Study III**, we tested the efficacy of exogenous IGF-1 administration in a transgenic mouse model of SBMA. We report that systemic delivery of IGF-1 reduces mutant AR accumulation and ameliorates disease manifestations in SBMA mice. We also tested a novel curcumin analog in cell and animal models of SBMA and investigated its mechanism of action. We show in **Study IV** that this compound enhances the clearance of mutant AR and mitigates the SBMA phenotype in *Drosophila melanogaster* and mice. The protective effect of the compound on mutant AR-induced degeneration in *Drosophila* is mediated through the Nrf1/Nrf2-dependent antioxidant response. Our results establish IGF-1 and curcumin analogs as candidates for therapeutic intervention in SBMA.

In summary, our findings extend the known phenotype of SBMA. We also provide evidence that the mutant AR alters ubiquitin-dependent degradation pathways that are

necessary for neuronal differentiation and function. Lastly, our results demonstrate the preclinical efficacy of IGF-1 and curcumin analogs in SBMA and warrant further investigation of these compounds in clinical studies.

LIST OF PUBLICATIONS

This thesis is based on the following articles and manuscripts:

I Grunseich C, Kats IR, **Bott LC**, Rinaldi C, Kokkinis A, Fox D, Chen KL, Schindler AB, Mankodi AK, Shrader JA, Schwartz DP, Lehky TJ, Liu CY, and Fischbeck KH (2014). Early onset and novel features in a spinal and bulbar muscular atrophy patient with a 68 CAG repeat. Neuromuscular Disorders, *24*, 978-981

II **Bott LC**, Salomons FA, Maric D, Fischbeck KH, and Dantuma NP. APC/C-Cdh1 dysregulation by the polyglutamine-expanded androgen receptor causes cell cycle reentry in spinal and bulbar muscular atrophy. *Manuscript in preparation*

III Rinaldi C*, **Bott LC***, Chen KL, Harmison GG, Katsuno M, Sobue G, Pennuto M, and Fischbeck KH (2012). Insulinlike growth factor (IGF)-1 administration ameliorates disease manifestations in a mouse model of spinal and bulbar muscular atrophy. Molecular Medicine, *18*, 1261-1268

IV **Bott LC**, Badders N, Chen KL, Harmison GG, Bautista E, Shih CCY, Taylor JP, Dantuma NP, Fischbeck KH, and Rinaldi C. A small-molecule activator of Nrf1 and Nrf2 mitigates polyglutamine toxicity in spinal and bulbar muscular atrophy models. *Manuscript in preparation*

* These authors contributed equally to the work

LIST OF PUBLICATIONS (CONTINUED)

Related publications not included in the thesis:

Beskow A, Grimberg KB, **Bott LC**, Salomons FA, Dantuma NP, and Young P (2009). A conserved unfoldase activity for the p97 AAA-ATPase in proteasomal degradation. Journal of Molecular Biology, *394*, 732-746

Tresse E, Salomons FA, Vesa J, **Bott LC**, Kimonis V, Yao TP, Dantuma NP, and Taylor JP (2010). VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. Autophagy, *6*, 217-227

Svensson C, Ceder J, Iglesias-Gato D, Chuan YC, Pang ST, Bjartell A, Martinez RM, **Bott L**, Helczynski L, Ulmert D, Wang Y, Niu Y, Collins C, and Flores-Morales A (2014). REST mediates androgen receptor actions on gene repression and predicts early recurrence of prostate cancer. Nucleic Acids Research, *42*, 999-1015

Grunseich C, Zukosky K, Kats IR, Ghosh L, Harmison GG, **Bott LC**, Rinaldi C, Chen KL, Chen G, Boehm M, and Fischbeck KH (2014). Stem cell-derived motor neurons from spinal and bulbar muscular atrophy patients. Neurobiology of Disease*, 70*, 12-20

Dantuma NP, and **Bott LC** (2014). The ubiquitin-proteasome system in neurodegenerative diseases: Precipitating factor, yet part of the solution. Frontiers in Molecular Neuroscience, 7:70

Rinaldi C*, **Bott LC***, and Fischbeck KH (2014). Muscle matters in Kennedy's disease. Neuron, *82*, 251-253

* These authors contributed equally to the work

CONTENTS

LIST OF ABBREVIATIONS

APC/C	anaphase-promoting complex/cyclosome
AR	androgen receptor
ASO	antisense oligonucleotide
CHIP	C-terminus of Hsp70-interacting protein
CBP	CREB-binding protein
CGRP1	calcitonin gene-related peptide 1
Hsf1	Heat shock factor 1
Hsp	heat shock protein
IGF-1	insulin-like growth factor 1
mTOR	mammalian target of rapamycin
NCOA4	nuclear receptor coactivator 4
NFE2L1	nuclear factor (erythroid-derived 2)-like 1
NFE2L2	nuclear factor (erythroid-derived 2)-like 2
PI3K	phosphoinositide 3 kinase
RNAi	RNA interference
SBMA	spinal and bulbar muscular atrophy
SCA	spinocerebellar ataxia
TFEB	transcription factor EB
UPS	ubiquitin-proteasome system
VEGF	vascular endothelial growth factor

1 INTRODUCTION

Spinal and bulbar muscular atrophy (SBMA, or Kennedy's disease) is an inherited, adult-onset degenerative disease of lower motor neurons in the brain stem and spinal cord. The clinical features and X-linked inheritance pattern of this disease were described by the physician William Kennedy in 1968 (Kennedy et al., 1968). In 1991, a trinucleotide CAG repeat expansion in the androgen receptor (AR) gene, a novel type of mutation at the time, was defined as the genetic cause of SBMA. While the length of this CAG repeat ranges from 9 to 36 in the general population, it is increased to 38 or greater in SBMA and leads to an expanded polyglutamine tract in the AR protein (La Spada et al., 1991). Polyglutamine expansions in unrelated proteins were subsequently found to cause eight different neurodegenerative disorders, including Huntington's disease, dentatorubral-pallidoluysian atrophy, and six spinocerebellar ataxias (SCA types 1, 2, 3, 6, 7, and 17) (Orr and Zoghbi, 2007). Today, the polyglutamine diseases constitute a common group of hereditary neurodegenerative disorders, and much progress has been made in understanding the underlying mechanisms.

Members of the polyglutamine disease family have several similarities that reflect their shared type of mutation. The length of the CAG repeats in the respective genes shows a similar pathological threshold, inversely correlates with the age of onset, and tends to increase with successive generations. Despite widespread expression of the causative proteins, neurons appear to be particularly vulnerable to the effects of the mutation. Moreover, the mutant proteins accumulate in the neuronal populations that are susceptible to degeneration. These findings have led to the hypothesis that the disorders also share a common pathogenic pathway. However, each disease affects different subsets of neurons, which results in distinct pathology and clinical features. In recent years there has been an emerging realization that the protein context may play an important role in defining the toxicity of expanded polyglutamine tracts. This has prompted the investigation of the polyglutamine expansion in relation to the normal functions of the host proteins (Gatchel and Zoghbi, 2005).

The identification of key pathways in the pathogenic mechanism, and the development of effective treatment are remaining challenges in the polyglutamine disease field. These two separate but interconnected themes form the basis of this thesis, focusing on the mutant AR protein in SBMA.

1.1 Clinical characterization

SBMA becomes manifest in mid-life as progressive weakness and atrophy of bulbar and extremity muscles in males. Symptoms and findings include dysarthria, dysphagia, fasciculations, cramps, tremor, gait disturbances, and mild sensory impairment (Atsuta et al., 2006; Kennedy et al., 1968; Rhodes et al., 2009). In addition to the neurological manifestations, affected individuals often show mild signs of androgen insensitivity, such as gynecomastia, testicular atrophy, and reduced fertility (Arbizu et al., 1983). Life span is often normal in SBMA, but the disease severity can cause premature death due to aspiration pneumonia or respiratory failure in severely affected individuals. With an estimated prevalence of 1 in 40,000 worldwide, SBMA is a rare disorder. However, individuals with SBMA are frequently misdiagnosed with other neuromuscular, such as spinal muscular atrophy and amyotrophic lateral sclerosis (Rhodes et al., 2009).

At the histopathological level, SBMA is characterized by degeneration and loss of motor neurons in the anterior horn of the spinal cord and brainstem motor nuclei, and of sensory neurons in the dorsal root ganglia (Sobue et al., 1989). Skeletal muscle biopsies of SBMA patients show features of both denervation and myofiber degeneration (Soraru et al., 2008). A hallmark of polyglutamine diseases is the presence of nuclear inclusions containing the mutant protein. In SBMA, nuclear inclusions containing mutant AR are present primarily in motor neurons, but also in other parts of the nervous system as well as in non-neuronal tissues including prostate, testis, liver, and skin (Adachi et al., 2005; Li et al., 1998a; Li et al., 1998b).

1.2 The androgen receptor

The AR is a ligand-activated transcription factor that mediates the biological actions of androgens, including testosterone and dihydrotestosterone. It belongs to the superfamily of nuclear receptors and consists of three functional domains: an amino-terminal regulatory domain, a central DNA-binding domain, and a carboxy-terminal ligand-binding domain. The polymorphic polyglutamine tract is located in the amino-terminal regulatory domain (**Figure 1**). In the absence of androgen, the AR localizes to the cytoplasm in complex with the heat shock proteins. Ligand binding causes the AR to dissociate from the heat shock protein complex and translocate in the nucleus, where it dimerizes and regulates the expression of target genes (**Figure 2**). Evidence suggests

that the AR also has non-canonical functions that are independent of its interaction with DNA and, instead, rely on association with signaling proteins (Matsumoto et al., 2013).

Although not essential for life, AR is required for the development and maintenance of the male sexual phenotype. Full or partial loss of AR causes androgen insensitivity syndrome, a spectrum of developmental abnormalities that range from mild virilization defects to varying degrees of feminized phenotypes (Quigley et al., 1995).

1.3 Disease mechanism

1.3.1 Lessons from animal models

Despite the finding of androgen insensitivity in SBMA, clinical and genetic evidence suggested that the causative mutation in the AR leads to an abnormal gain of function, since loss of the AR is not associated with a neurological phenotype or weakness (Quigley et al., 1992). With the identification of other members of the polyglutamine disease family, it became clear that the repeat expansion in otherwise unrelated proteins is a common denominator for neurodegeneration in these disorders. Studies addressing the pathogenic mechanism in polyglutamine diseases have therefore focused primarily on the expanded polyglutamine tract. These studies showed that peptides and protein fragments containing long stretches of glutamines have a tendency to form insoluble

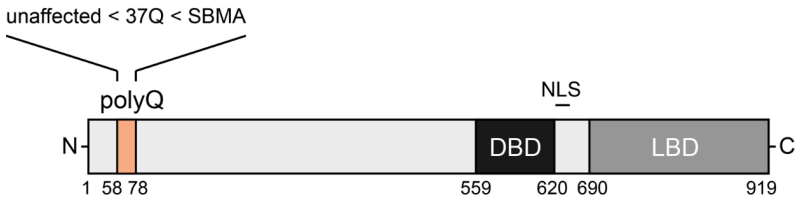

Figure 1: Domain structure of the AR. AR belongs to the superfamily of nuclear receptors and consists of three functional domains: an amino-terminal regulatory domain, a central DNA-binding domain (DBD), and a carboxy-terminal ligand-binding domain (LBD). The DBD and LBD are linked via a flexible hinge region, which contains a bipartite nuclear localization signal (NLS). The polymorphic polyglutamine tract (polyQ) is located in the amino-terminal regulatory domain. The length of this tract ranges between 9 and 36 in the normal population, and is 38 or greater in SBMA.

aggregates *in vitro* and *in vivo* (Marsh et al., 2000; Ordway et al., 1997; Scherzinger et al., 1997). The length threshold for aggregation correlates with the threshold for disease in humans, indicating that aggregation may be related to the pathogenic process.

Nuclear inclusions in autopsy material were found to stain positively only with antibodies with epitopes in the amino-terminal portion of the protein, including the polyglutamine tract (Li et al., 1998a). Similar observations were made in other polyglutamine diseases and suggested that pathology in these disorders might be caused by proteolytic products of the full-length proteins (DiFiglia et al., 1997). Caspase-mediated cleavage of mutant AR was demonstrated *in vitro* (Kobayashi et al., 1998), in line with proteolytic processing of other polyglutamine-expanded proteins (Goldberg et al., 1996; Wellington et al., 1998). Such fragments are more toxic than the full-length proteins in cultured cells and in the fruit fly *Drosophila melanogaster* (Chan et al., 2002; Merry et al., 1998), and form intracellular inclusions that closely resemble those found in patients (Taylor et al., 2003).

The presence of inclusions consisting of the mutant protein in vulnerable neuronal populations initially suggested that they are directly responsible for toxicity in polyglutamine diseases (Ross and Poirier, 2004). It was subsequently shown that inclusions are neither necessary nor sufficient for neuronal dysfunction animal models (Cummings et al., 1999; Klement et al., 1998). The regional distribution of inclusions

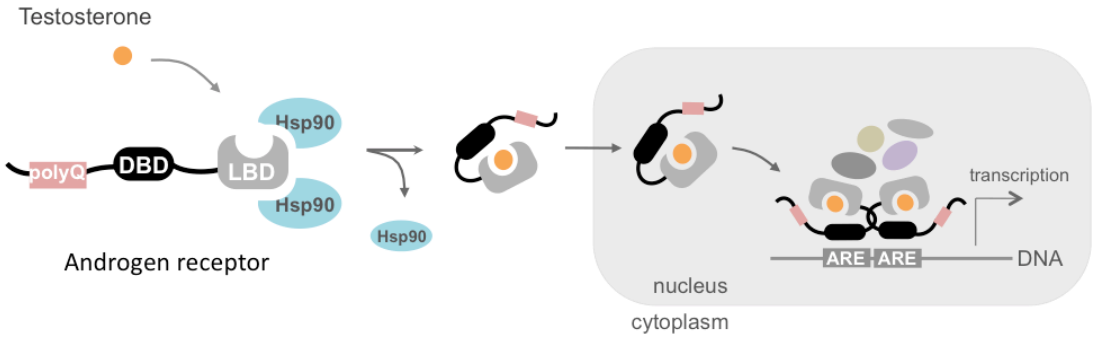

Figure 2: Function of the AR. In the absence of androgen, AR localizes to the cytoplasm in association with Hsp90. Binding of ligand to the LBD induces a conformational change, which causes AR to dissociate from the Hsp90 complex and translocate into the nucleus. Androgen-bound AR regulates the expression of target genes by binding to specific androgen response elements (ARE) in the DNA via the recruitment of transcriptional co-regulators.

in SBMA and other disorders does not always correspond to the selective pathology (Zoghbi and Orr, 2000). Detailed cellular analyses revealed a protective role for inclusions as part of an adaptive response that relocates toxic and aggregation-prone proteins to inert deposits (Arrasate et al., 2004; Taylor et al., 2003).

The link between repeat length, aggregation, and toxicity of the polyglutamine tract was not sufficient to account for the differential neuronal vulnerability in different polyglutamine diseases. Mice expressing an amino-terminal fragment of mutant AR develop widespread neuronal dysfunction, without the male-limited manifestations and motor neuron specificity of SBMA (Abel et al., 2001). Expression of an expanded polyglutamine tract under the AR promoter results in a similar phenotype in mice, indicating that cell type specificity is not determined by the expression level and pattern of the mutant protein alone (Adachi et al., 2001). The lack of gender effects and lower motor neuron specificity in mice expressing fragments of the mutant AR suggested that these disease features likely require the full-length protein.

The first attempts to model SBMA in mice using full-length human AR had transgenes with CAG repeat lengths ranging between 44 and 65. Although the repeat lengths of these constructs reflect the range seen in patients, the transgenic mice did not show a neurological phenotype (Bingham et al., 1995; Merry et al., 1996; La Spada et al., 1998). Since then, several SBMA mouse models have been developed that express full-length mutant AR with highly expanded CAG repeat lengths ranging from 97 up to 121 (Chevalier-Larsen et al., 2004; Cortes et al., 2014a; Katsuno et al., 2002; McManamny et al., 2002; Sopher et al., 2004; Yu et al., 2006). These mice show late-onset, progressive motor impairment and neuromuscular pathology, thus confirming that the full-length protein is needed to produce SBMA manifestations in this model system. The disease phenotype occurs only in males and can be rescued by castration, demonstrating a requirement for androgen in the pathogenesis (Chevalier-Larsen et al., 2004; Katsuno et al., 2002). Toxicity of the full-length mutant AR is also ligand-dependent in *Drosophila* models, with a requirement for exogenous androgen administration (Pandey et al., 2007; Takeyama et al., 2002). Subsequent studies have shown that the SBMA phenotype in mice and flies also requires nuclear localization of the mutant AR in addition to ligand binding (Montie et al., 2009; Nedelsky et al., 2010). These findings demonstrate that protein context and normal AR functions are required for the disease phenotype in SBMA.

1.3.2 Molecular mechanisms

The molecular mechanism in SBMA and other polyglutamine diseases has been subject to considerable debate. The polyglutamine expansion in AR leads to a slight loss of transactivation function *in vitro* (Chamberlain et al., 1994; Mhatre et al., 1993; Nakajima et al., 1996) but, as discussed above, this is unlikely to be the cause of neuronal degeneration in SBMA.

The tendency of expanded polyglutamine proteins to form aggregates indicates that they might affect the distribution and function of other cellular proteins. The presence of ubiquitin, chaperones, and proteasome subunits in inclusions suggested that the proteolytic pathway might be altered in affected neurons (Cummings et al., 1998; Li et al., 1998a). The ubiquitin-proteasome system (UPS) is a major pathway for the degradation of short-lived, misfolded, and damaged proteins in the nucleus and cytoplasm (Hershko and Ciechanover, 1998). The activity of the UPS mediates the turnover of factors involved in many different cellular processes, including cell cycle control, transcription, signaling, and apoptosis. It was proposed that expanded polyglutamine proteins might impair the UPS, either directly by blocking the proteasome or indirectly by sequestration of essential UPS components in inclusions (McKinnon and Tabrizi, 2014). Inhibition of this system leads to the stabilization of UPS substrates, which causes cellular dysfunction and death. Although polyglutamine proteins can impair the ubiquitin-dependent proteolysis in cultured cells (Bence et al., 2001; Bennett et al., 2005; Maynard et al., 2009), the UPS appears to be operative in mouse models of polyglutamine diseases, including SBMA (Bett et al., 2009; Bowman et al., 2005; Maynard et al., 2009; Tokui et al., 2009).

Molecular chaperones are another branch within the cellular protein quality control machinery that have been implicated in polyglutamine diseases. Their activities are directed towards protein refolding, stabilizing non-native proteins, and in some cases, assisting proteolytic pathways such as the UPS and the lysosomal system (Gestwicki and Garza, 2012). Polyglutamine-expanded proteins likely place an increased demand on the chaperone machinery due to their tendency to aggregate. Overexpression of chaperones reduces the accumulation and toxicity of the mutant AR (Adachi et al., 2003; Stenoien et al., 1999), indicating that protein quality control might be affected in SBMA.

Substantial evidence suggests that the mutant AR also has effects on gene expression. Polyglutamine-expanded AR affects the cellular distribution of CREB-

binding protein (CBP), NF-Y, p300/CBP-associated factor, and nuclear receptor coactivator 1 into inclusions and thereby may cause transcriptional dysregulation in cells (Katsuno et al., 2010; McCampbell et al., 2000; Stenoien et al., 1999).

Although inclusions have been disconnected from toxicity, it is possible that oligomeric species may be responsible for the pathology in polyglutamine diseases. A study reported the presence of soluble oligomers consisting of amino-terminal fragments of the mutant AR in a mouse model of SBMA. The oligomers disappeared with castration, which halts disease progression (Li et al., 2007).

An alternative explanation for toxicity in polyglutamine diseases is that the polyglutamine expansion alters existing functions of the host protein, which may lead to enhancement or reduction of certain activities. This hypothesis is supported by the finding that normal AR functions, such as ligand binding and nuclear translocation, are necessary for the SBMA phenotype in animal models (Montie et al., 2009; Nedelsky et al., 2010). In *Drosophila*, mutant AR-induced degeneration also requires DNA binding and cofactor interactions via the activation factor 2 domain (Nedelsky et al., 2010). The expanded polyglutamine tract is necessary, but also not sufficient for toxicity for other polyglutamine diseases. For example, phosphorylation can modulate the toxicity of mutant ataxin-1, which causes SCA1, and huntingtin, which is responsible for Huntington's disease (Emamian et al., 2003; Gu et al., 2009; Humbert et al., 2002).

It was recently shown that AR regulates the activity of the transcription factor EB (TFEB), which coordinates the expression of autophagy related genes (Cortes et al, 2014b). While the non-expanded AR promoted TFEB transactivation, the mutant AR interfered with this function and thereby reduced autophagic flux. This result demonstrates that effects of the polyglutamine expansion on normal protein function can have wide-ranging cellular consequences.

1.3.3 Role of non-neuronal tissues

Most mechanistic studies to date have assumed cell-autonomous toxicity of mutant AR in motor neurons in SBMA. It is established that neurons critically depend on neighboring cells for proper function and survival. Accumulating evidence suggests that non-neuronal tissue is also affected by the mutant AR and may be important to the pathogenic process. Skeletal muscle provides trophic support to motor neurons and regulates synaptic activity and axonal function (Funakoshi et al., 1995). Muscle pathology in SBMA has features of both denervation and myofiber degeneration

(Soraru et al., 2008), and serum creatine kinase levels are higher than expected for a purely neurogenic disease (Guidetti et al., 1996). Furthermore, knock-in mice expressing AR with 113 glutamines develop myopathy in the absence of motor neuron death (Yu et al., 2006). Overexpression of non-expanded AR in skeletal muscle leads to a neuromuscular phenotype in mice that recapitulates key characteristics of SBMA, including gender delineation, androgen-dependence, and motor axon loss (Monks et al., 2007). The contribution of skeletal muscle to SBMA pathogenesis was recently addressed with a new mouse model based on the Cre-Lox system that allows cell type-specific excision of a mutant AR transgene. Transgene expression in this model is driven by the AR promoter and causes motor dysfunction and premature death in males. Muscle-specific abrogation of mutant AR in the mice prevented not only the behavioral phenotype but also degeneration of motor axons, despite the observation that levels of mutant AR in the spinal cord were unaffected (Cortes et al., 2014a). Together, these findings indicate that muscle is a primary target for mutant AR toxicity and contributes to motor neuron degeneration in SBMA.

1.4 Therapeutic strategies

Neuronal death in SBMA is at least in part due to a toxic gain of function of the polyglutamine-expanded AR. The ideal scenario for treating SBMA in the future is gene therapy, which involves replacement of the mutant AR gene with a healthy allele. Until this form of therapy becomes feasible, our next best bets are approaches that target the downstream consequences of the mutant AR gene. Studies in cell and animal models have led to important insights into the pathogenic mechanism of SBMA. Based on this knowledge, a number of different therapeutic approaches have been developed, as discussed below.

1.4.1 Androgen reduction

Androgen is critical in the pathogenesis of SBMA. Ligand binding to the AR induces dissociation from heat shock proteins and nuclear uptake, which are necessary steps for toxicity of the mutant protein (Katsuno et al., 2002; Montie et al., 2009; Nedelsky et al., 2010). This explains why the full disease becomes manifest only in men; females, even if homozygous for the mutation, are protected by having low levels of circulating androgens (Schmidt et al., 2002). In mice, the SBMA phenotype of males is rescued by castration, while testosterone administration triggers the disease in females (Chevalier-

Larsen et al., 2004; Katsuno et al., 2002). The close link between androgen and toxicity in SBMA indicates that the ligand-binding step of mutant AR toxicity could be a target for therapeutic intervention. A study in mice showed that the androgen-reducing agent leuprorelin, a lutenizing hormone-releasing hormone agonist that reduces testosterone release from the testis, prevents onset of the SBMA phenotype (Katsuno et al., 2003).

Androgen reduction therapy in SBMA patients has been tested in three randomized, placebo-controlled clinical trials. The agents used in the studies were leuprorelin and dutasteride, a 5-alpha-reductase inhibitor, which blocks the conversion of testosterone into the more potent dihydrotestosterone. Neither of the two androgen-reducing agents significantly improved primary clinical outcome measures in randomized, placebo-controlled studies (Fernandez-Rhodes et al., 2011; Katsuno et al., 2010). However, leuprorelin improved swallowing parameters and decreased nuclear accumulation of mutant AR in scrotal skin cells after 48 weeks in a phase 2 study with 50 patients (Banno et al., 2009). Although a subsequent phase 3 study in 200 patients showed no effect on swallowing function overall, there was a benefit on *post hoc* analysis in a sub-group of patients with disease duration less than 10 years (Katsuno et al., 2010). While the results from these studies overall were negative, there are indications that androgen reduction therapy reduces mutant AR accumulation and may modify the progression of SBMA at early disease stages.

1.4.2 Antisense therapy

Neuronal dysfunction in SBMA and other polyglutamine diseases is in large part due to a toxic gain of function of the repeat expansion in the respective proteins. Because the exact mechanism underlying the pathogenesis in these disorders remains unclear, reducing levels of the disease-causing proteins has emerged as a promising approach for treatment. Conditional mouse models of Huntington's disease and SCA1 have shown that switching off the expression of the polyglutamine-expanded proteins slows disease progression and even reverses the pathology (Yamamoto et al., 2000; Zu et al., 2004). These findings have prompted the investigation of gene silencing approaches using RNA interference (RNAi) and antisense oligonucleotide (ASO) technology in polyglutamine diseases. Although adverse effects with long-term gene suppression are a concern with this strategy, encouraging results have been obtained in mouse models of Huntington's disease, SCA1, and SCA7 (Harper et al., 2005; Kordasiewicz et al., 2012; Ramachandran et al., 2014; Xia et al., 2004), and clinical trials are planned.

A recent study found that AR-specific ASOs suppress mutant AR expression *in vitro* and *in vivo*. The ASOs reduced mutant AR accumulation and attenuated the disease phenotype in two different mouse models of SBMA (Lieberman et al., 2014). In another study, Miyazaki et al. described an indirect approach by which RNAi was used to regulate mutant AR transcript levels. The authors identified a microRNA (miR-196a) that enhances the decay of AR mRNA by silencing CELF2, an RNA-binding protein known to bind to, and stabilize, AU-rich sequences. Viral delivery of miR-196a reduced mutant AR accumulation and ameliorated the disease phenotype in SBMA mice (Miyazaki et al., 2012). These studies show that antisense approaches aimed at decreasing AR expression can modify SBMA manifestations in animal models.

1.4.3 Enhancing protein clearance

An alternative strategy for reducing levels of the mutant AR is to accelerate the clearance of the protein. The main route of degradation of the AR is via the UPS (Lieberman et al., 2002). It is not settled whether expanded polyglutamine tracts can be degraded by the proteasome, which preferentially cleaves after hydrophobic, basic, and acidic residues (Venkatraman et al., 2004; Michalik and Van Broeckhoven, 2004; Verhoef et al., 2002). However, there is substantial evidence in cell and animal models to suggest that enhancing UPS-mediated clearance of the mutant AR has a protective effect (Palazzolo et al., 2009; Tokui et al., 2009; Waza et al., 2005). Current pharmacological approaches for enhancing mutant AR degradation target primarily the protein quality control machinery.

The function and stability of the AR are controlled by the heat shock protein (Hsp) 90/Hsp70-based chaperone complex. In this complex, association with Hsp90 stabilizes AR, while Hsp70 regulates the degradation of client proteins through the recruitment of chaperone-dependent ubiquitin ligases such as C-terminus of Hsp70-interacting protein (CHIP) (Pratt et al., 2015). Activation of Hsp70, or inhibition of Hsp90, promotes UPS-mediated clearance of the mutant AR (Wang et al., 2013, Thomas et al., 2006). Overexpression of Hsp70, Hsp40, or CHIP reduces mutant AR toxicity in cell culture and *in vivo* (Adachi et al., 2003; Adachi et al., 2007; Bailey et al., 2002; Kobayashi et al., 2000). Small molecules that influence the activity of this chaperone complex include geranylgeranylacetone and Hsp90 inhibitors, such as the geldanamycin derivatives 17-AAG and 17-DMAG. All three compounds were shown to effectively reduce mutant AR accumulation and ameliorate the disease phenotype in mice

(Katsuno et al., 2005; Tokui et al., 2009; Waza et al., 2005). Unfortunately, geldanamycin derivatives and related compounds have toxic side effects that make them unsuitable for the long-term treatment of chronic diseases such as SBMA (Supko et al., 1995). Chemical modulators of Hsp70 function may be an alternative option for this purpose. A recent study reported a small molecule, YM-1, that stabilizes Hsp70 in its ADP-bound state and promotes binding to unfolded proteins. YM-1 accelerates the degradation of mutant AR and rescues its toxicity in *Drosophila* (Wang et al., 2013). The efficacy of such an approach in mice remains to be determined.

Association with co-regulators also influences the stability of AR. For example, AR is stabilized by interaction with the nuclear receptor coactivator 4 (NCOA4) (Hu et al., 2004). ASC-J9 and genistein promote the dissociation of AR and NCOA4 (Ohtsu et al., 2002; Qiang et al., 2013). Both compounds decrease levels of mutant AR and attenuate disease manifestations in mouse models of SBMA (Qiang et al., 2013; Yang et al., 2007).

1.4.4 Reversing cellular defects

Transcriptional dysregulation is an important downstream effect implicated in mutant AR toxicity in SBMA (Lieberman et al., 2002). Aberrant interactions between expanded polyglutamine proteins and transcription factors and coregulators have been described, including the histone acetylase CBP (McCampbell et al., 2000). Changes in the cellular acetylation status can be counteracted with histone deacetylase inhibitors, such as suberoylanilide hydroxamic acid and sodium butyrate, which have shown a benefit in various models of polyglutamine disease (Ferrante et al., 2003; Hockley et al., 2003; Ying et al., 2006). In SBMA mice, sodium butyrate improves the motor phenotype and delays disease progression, and leads to an overall increase in histone acetylation (Minamiyama et al., 2004). A recent report suggests that transcriptional dysregulation in SBMA can also be counteracted by triptans (Minamiyama et al., 2012). Mutant AR expression is associated with upregulation of calcitonin gene-related peptide 1 (CGRP1), which leads to neuronal damage through stress kinase activation. The serotonin receptor 1B/1D receptor agonist naratriptan was shown to prevent transcriptional induction of CGRP1 and to ameliorate the disease phenotype in SBMA mice.

In summary, a number of different approaches have been tested for therapeutic intervention in SBMA that target different stages of mutant AR toxicity. Androgen

reduction has failed to result in effective treatment in clinical trials. Therapeutics development would benefit from a better understanding of the pathogenic pathways in SBMA. The disease mechanism in SBMA, and new pharmacological strategies aimed at reducing the mutant AR protein are a major focus of this thesis.

2 AIMS

The overall goal was to study the disease mechanism and explore new approaches for therapeutic intervention in SBMA.

The specific aims addressed in this thesis were to:

- Characterize the disease features in an SBMA patient with a 68 CAG repeat (**Study I**)
- Investigate the effect of the polyglutamine expansion in the context of normal AR function (**Study II**)
- Test pharmacological strategies for reducing the mutant AR as potential treatment for SBMA (**Study III** and **IV**)

3 RESULTS AND DISCUSSION

This thesis attempts to address the disease mechanism of the polyglutamine disorder SBMA and potential treatment strategies. The studies described in this chapter span from the clinical examination of a patient (**Study I**) to experimental work in cell culture (**Studies II** and **IV**) and animal models (**Studies III** and **IV**). The techniques used range from live cell imaging, and biochemical and histological analyses of cells and tissues, to behavioural testing of SBMA mice.

STUDY I: Early onset and novel features in a spinal and bulbar muscular atrophy patient with 68 a CAG repeat

SBMA patients typically develop weakness in their thirties or forties and have an average CAG repeat length of 47 in the AR gene, with 62 the largest repeat reported to date (Atsuta et al., 2006; Rhodes et al., 2009). **Study I** describes a 29-year-old male SBMA patient with a 68 CAG repeat, who showed unusually early onset and new disease features. The patient has classical symptoms of SBMA such as weakness of facial and limb muscles and fatigue after exercise since age 18. The pronounced weakness and motor neuron involvement were confirmed on examination by quantitative muscle testing and magnetic resonance imaging of muscle. The patient also had gynecomastia, testicular atrophy, and sensory impairment. New findings included a congenital abnormality of the penis (chordee) and autonomic dysfunction.

An inverse relationship between CAG repeat length and age of onset has been demonstrated in SBMA and other polyglutamine diseases, with longer repeat expansions associated with earlier onset (Orr and Zoghbi, 2007). The long CAG repeat found in the AR gene of this patient likely accounts for the early onset. Mutant AR accumulation has been demonstrated in a wide range of neuronal and non-neuronal tissues (Adachi et al., 2005), and it is not surprising that a longer repeat may also result in additional manifestations. Certain signs of mild androgen insensitivity commonly found in individuals with SBMA include breast enlargement and reduced fertility, but the development of male sex organs is usually normal (Dejager et al., 2002). Defects in genital masculinization are typically associated with loss of function mutations in the AR (Quigley et al., 1995), but the coding region of the AR gene did not contain any known variants that could explain the chordee deformity in the patient. AR protein levels in fibroblasts derived from this patient were comparable to those in control fibroblasts with CAG repeats in the normal range, suggesting that the observed phenotypic effects are not due to reduced expression of AR. Undermasculinized genitalia have previously been associated with a 44 CAG repeat in the AR gene of an eleven-year-old

boy (Ogata et al., 2001), indicating that the repeat expansion in the AR may be responsible for defects in male sexual development.

While mutant AR toxicity in SBMA causes primarily lower motor neuron degeneration, other neuronal subtypes may also be affected in the disease. The patient had reduced sensory function, which has been documented in SBMA (Li et al., 1995; Rhodes et al., 2009; Sobue et al., 1989). Additional symptoms and findings include pain in the distal limbs, a decreased sweat response, and orthostatic tachycardia, indicating involvement of small unmyelinated nerve fibers. Mutant AR has been shown to accumulate in sympathetic ganglia and intermediolateral nuclei of the spinal cord on autopsy of SBMA patients (Adachi et al., 2005). Autonomic dysfunction in SBMA was also suggested by an earlier study that reported reduced epidermal nerve fiber density and autonomic skin denervation in skin biopsies from two patients (Manganelli et al., 2007). We did not find evidence for reduced epidermal nerve fiber density in this patient, which may be related to sampling location or fiber dysfunction without degeneration. Our findings extend the clinical picture of SBMA and suggest that the polyglutamine expansion can alter functions of AR that are important for male sexual development and neuronal physiology.

STUDY II: APC/C-Cdh1 dysregulation by the polyglutamine-expanded androgen receptor causes cell cycle reentry in spinal and bulbar muscular atrophy

The AR is expressed in motor neurons (Matsuura et al., 1993), where it promotes sex differences in neuronal organization and neuromuscular function during development (Morris et al. 2004). In addition, the AR has important roles in neuronal regeneration. Androgens attenuate neuron loss (Yu, 1989) and increase the rate of motor axon regrowth as well as functional recovery after injury (Kujawa et al., 1989, Kujawa et al., 1991). It was previously shown that the AR induces differentiation and neurite outgrowth in neuronal cells at physiological concentrations of androgen (Brooks et al., 1998; Marron et al., 2005). In **Study III**, we examined the effect of the polyglutamine expansion in the AR on androgen-dependent differentiation and neurite outgrowth in cultured cells. For this, we generated neuronal PC12 cell lines for the inducible expression of wild type or polyglutamine-expanded human AR. We found that cells expressing the mutant AR showed enhanced neurite outgrowth, but reduced arrest in the G0/G1 cell cycle phase, compared to cells expressing non-expanded AR in the presence of androgen. An earlier study suggested that androgen-dependent neurite outgrowth by the AR is mediated through transcriptional induction of neuritin (Marron et al., 2005). We found that the wild type and mutant AR did not differ in their ability to transactivate a luciferase-based reporter in this cell line. We also did not detect

any differences in neuritin expression between the two AR types in the presence or absence of androgen.

Although most functions of the AR are attributed to its role as a ligand-activated transcription factor (Matsumoto et al., 2013), there is evidence for non-canonical functions of AR in cell cycle control and neurite outgrowth through interaction with signaling proteins and components of the cell cycle machinery (Balk and Knudsen, 2008; Schindler et al., 2012). We found that the AR associates with the ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C)-Cdh1, which has important functions in proliferating and postmitotic cells. APC/C-Cdh1 acts in the nucleus to cell regulate cycle progression, mitotic exit, and neuronal morphogenesis and function. It maintains the G0/G1 phase by preventing the accumulation of S phase and mitotic cyclins. APC/C-Cdh1 also targets the transcriptional regulators Id2 and SnoN, which drive axon growth during neuronal differentiation (Puram and Bonni, 2011). We found that the mutant AR retains the ability to interact with APC/C-Cdh1. AR levels were not altered by inhibition of APC/C-Cdh1, indicating that the AR is not targeted for proteasomal degradation by the complex.

Expression of the polyglutamine-expanded AR stabilized an APC/C-dependent reporter substrate, suggesting that the mutant AR interferes with the activity of APC/C-Cdh1. Consistent with previous reports, we did not observe an effect of mutant AR on general UPS reporter substrates (Tokui et al., 2009). This indicates that stabilization of the APC/C substrate is specific to this ubiquitin-dependent degradation pathway and not due to global inhibition of protein turnover by the polyglutamine-expanded AR. Inhibition of the APC/C-Cdh1 complex in developing neurons leads to abnormalities in cell cycle exit, axon growth, and synaptic function (Lasorella et al., 2006; Konishi et al., 2004; van Roessel et al. 2004). APC/C-Cdh1 inhibition in mature neurons results in the accumulation of cell cycle factors, cell cycle reactivation, and induction of apoptosis (Almeida et al, 2005).

Live imaging of single cells expressing mutant AR revealed that accumulation of the APC/C reporter occurs concomitantly with neurite outgrowth, and is followed by neurite retraction and cell division. The results from the longitudinal analysis suggest that both the aberrant neurite phenotype and the mitotic behavior of the PC12 cell model are linked to the inappropriate stabilization of APC/C-Cdh1 substrates. Cell cycle reactivation has been implicated in a number of other neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Hernández-Ortega et al., 2011). In these diseases, increased immunoreactivity of mitotic cyclins and other cell cycle markers has been demonstrated in vulnerable neuronal populations.

Since both expanded and non-expanded AR variants interact with APC/C-Cdh1 without being targeted for proteasomal degradation, it is possible that the AR regulates the ubiquitin ligase complex during androgen-dependent differentiation. A regulatory function has been demonstrated for the retinoblastoma protein, which interacts and functionally cooperates with APC/C-Cdh1 during cell cycle exit (Binné et al., 2007). Protein inhibitors of the APC/C-Cdh1 complex include Emi1 and Acm1, which compete with other substrates for binding to the Cdh1 adaptor (Choi et al., 2008; Enquist-Newman et al., 2008, Miller et al., 2006). Our ongoing work aims to characterize the interaction between AR and APC/C-Cdh1 in order to define the mechanism of mutant AR-mediated APC/C inhibition. We also plan to investigate the functional status of APC/C in other cellular models of SBMA, such as primary neuronal cultures and stem cell-derived motor neurons from patients. Our results indicate a non-transcriptional role for the AR in the regulation of APC/C-Cdh1, and suggest abnormal cell cycle reactivation as a pathogenic mechanism in SBMA.

STUDY III: Insulin-like growth factor (IGF)-1 administration ameliorates disease manifestations in a mouse model of SBMA

Posttranslational modifications are critical regulators of AR localization, function, and stability, and can modify the toxicity of the mutant protein. Phosphorylation of the AR at two Akt consensus sites, serine-215 and serine-792, blocks ligand binding, reduces nuclear translocation, and promotes UPS-mediated clearance of the AR (Palazzolo et al., 2007; Palazzolo et al., 2009). This effect can be stimulated through activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway by IGF-1. IGF-1 decreases mutant AR protein levels and toxicity in cultured cells (Palazzolo et al., 2007). Overexpression of a muscle-specific isoform of IGF-1 in SBMA mice was previously shown to reduce mutant AR accumulation and attenuate the disease phenotype (Palazzolo et al., 2009). These studies indicated that activation of the PI3K/Akt pathway could be a suitable therapeutic approach for SBMA.

In **Study III** we examined the therapeutic potential of exogenous IGF-1 administration in SBMA mice. Since IGF-1 has a short half-life *in vivo*, we used the compound mecasermin rinfabate (Iplex), which consists of human recombinant IGF-1 in complex with the IGF-1 binding protein 3 (INSMED Corporation, Richmond, VA, USA). Iplex has improved pharmacokinetics and reduced adverse effects compared to IGF-1 alone and was approved by the U.S. Food and Drug Administration for treatment of primary IGF-1 deficiency (Camacho-Hübner et al., 2006). We tested Iplex in a well-characterised mouse model of SBMA that expresses a transgene consisting of full-length human mutant AR with 97

glutamine residues (Katsuno et al., 2002). Toxicity of the mutant AR protein in these mice is androgen-dependent and associated with nuclear accumulation in motor neurons and skeletal muscle tissues. The disease phenotype includes progressive muscle weakness, weight loss, and premature death. To reflect the situation in symptomatic patients, treatment of the mice was started after disease onset. We found that systemic IGF-1 administration improved motor function, body weight, neuromuscular pathology, and survival of the SBMA mice. IGF-1 treatment increased Akt activation and reduced mutant AR accumulation in skeletal muscle of the mice. No changes in Akt activation and mutant AR protein levels were detected in the spinal cord, indicating that amelioration of the disease phenotype in the mice was mediated through effects of IGF-1 in peripheral tissues.

Accumulation of the mutant AR is widespread in neuronal and non-neuronal tissues in SBMA patients (Adachi et al., 2005; Li et al., 1998a; Li et al., 1998b). Recently it was demonstrated that systemic delivery of AR-specific ASOs improves the SBMA phenotype in mice more than intracerebroventricular administration (Lieberman et al., 2014), indicating that non-neuronal cell types contribute to neuronal degeneration in the disease. Increasing evidence suggests a role for skeletal muscle in the pathogenesis of SBMA (Cortes et al., 2014a; Soraru et al., 2008; Yu et al., 2006). Motor neurons rely on muscle for trophic support that is critical for neuronal survival, synaptic activity, and axonal function (Funakoshi et al., 1995). For example, brain-derived neurotrophic factor released from muscle can be taken up and transported in a retrograde manner by motor neurons (DiStefano et al., 1992). The expression of growth factors and neurotrophins in muscle is altered in SBMA mouse models and patients (Sopher et al., 2004; Yamamoto et al., 1999; Yu et al., 2006). Treatment with vascular endothelial growth factor (VEGF) was shown to reduce mutant AR toxicity in a neuronal cell model (Sopher et al., 2004). These findings suggest that exogenous IGF-1 restores trophic support to motor neurons by targeting mutant AR in muscle.

Activation of the PI3K/Akt pathway by IGF-1 also has other effects in muscle that are unrelated to mutant AR degradation. Akt promotes muscle hypertrophy through activation of mammalian target of rapamycin (mTOR) (Bodine et al., 2001; Rommel et al., 2001) and inhibition of glycogen synthase kinase 3 beta (Cross et al., 1995). Akt also prevents the expression of atrophy-related genes such as atrogin-1 and MuRF1 through inactivation of FOXO transcription factors (Sandri et al., 2004; Stitt et al., 2004; Zhao et al., 2007). In addition, IGF-1 has been shown to activate the proliferation of satellite cells, which are responsible for muscle regeneration (Musaro et al., 2001). It is likely that IGF-1 ameliorates the SBMA phenotype through both mutant AR-dependent and -independent effects on muscle.

IGF-1 can also act directly on motor neurons to promote sprouting, axonal growth, and survival (Caroni and Grandes, 1990). Approaches that increase the uptake of IGF-1 into the nervous system may therefore have an additional therapeutic benefit. Recently, it was shown that localized transfer of serum IGF-1 across the blood-brain barrier is stimulated by neuronal activity (Nishijima et al., 2010). This raises the possibility that the beneficial effect of IGF-1 in SBMA may be enhanced in combination with physical exercise. Our results helped to establish IGF-1 as a therapeutic strategy for SBMA and provided the basis for clinical studies that are now underway.

STUDY IV: A small-molecule activator of Nrf1 and Nrf2 mitigates polyglutamine toxicity in SBMA models

Curcumin is a naturally occuring polyphenol with pleiotropic biological properties, including anti-inflammatory and neuroprotective activities. Structural analogs of curcumin have been evaluated in SBMA due to their ability to disrupt AR-cofactor interactions (Ohtsu et al., 2002; Yang et al., 2007). ASC-J9, or dimethylcurcumin, was previously shown to ameliorate the disease phenotype of SBMA mice by promoting the degradation of mutant AR (Yang et al., 2007). In **Study IV** we showed that a new, orally available curcumin analog, ASC-JM17, enhances the clearance of AR by the UPS. Treatment with the compound reduced mutant AR accumulation and mitigated the SBMA phenotype in cell, fly, and mouse models.

Recently, curcumin analogs have emerged as regulators of the protein homeostasis network (Alavez et al., 2011; Calamini et al., 2012), which regulates protein synthesis, folding, transport, and degradation. This network is controlled by several signaling pathways that help to alleviate damage in response to extrinsic and intrinsic stressors (Balch et al., 2008). The antioxidant response and heat shock response are important regulators of the protein homeostasis network. The antioxidant response is mediated by Nrf1/nuclear factor (erythroid-derived 2)-like 1 (NFE2L1) and Nrf2/NFE2L2, which belong to the cap'n'collar family of basic leucine zipper transcription factors. Nrf1 and Nrf2 regulate the expression of proteasome subunits and antioxidant enzymes, respectively (Radhakrishnan et al., 2010; Kensler et al., 2007). The heat shock response, mediated by heat shock factor 1 (Hsf1), leads to the induction of molecular chaperones (Pirkkala et al., 2001). We found ASC-JM17 to be a potent activator of the antioxidant response and heat shock responses in various cell lines. It increased the expression of proteasome subunits, antioxidant enzymes, and molecular chaperones in cell culture and in the SBMA mice. Treatment with ASC-JM17 reduced mutant AR accumulation, increased proteasome activity, and improved resistance to

oxidative stress. These findings establish ASC-JM17 as a small-molecule activator of cellular systems controlling protein folding, degradation, and redox balance.

Our results showed that overexpression of the *Drosophila* Nrf1 and Nrf2 ortholog CncC or Hsf1 rescued mutant AR-induced eye degeneration in flies, indicating that both the antioxidant pathway and heat shock response are protective in this model. Levels of Hsf1 were previously shown to influence the extent and distribution of mutant AR accumulation in SBMA mice (Kondo et al., 2013). Several Hsf1 targets, such as Hsp70, Hsp40, and Hsp27, reduce mutant AR protein levels and toxicity in cell and animal models (Adachi et al., 2003; Adachi et al., 2007; Bailey et al., 2002; Kobayashi et al., 2000). Chemical activators of the heat shock response ameliorate SBMA manifestations in mouse models (Katsuno et al., 2005; Tokui et al., 2009; Waza et al., 2005). Interestingly, knockdown of CncC, but not Hsf1, blocked the protective effect of ASC-JM17 on AR-induced degeneration in flies.

Two recent reports indicate that increasing proteasome activity promotes the degradation of aggregation-prone proteins. A recent study reported reduced aggregation of a polyglutamine protein in the nematode worm *Caenorhabditis elegans* overexpressing the proteasome subunit Rpn6/Psmd11, which increases proteasome activity (Vilchez et al., 2012). Inhibition of Usp14, a proteasome-associated deubiquitylating enzyme, accelerates proteasomal degradation of disease-linked proteins, such as polyglutamine-expanded ataxin-3 (Lee et al., 2010). These findings suggest that Nrf1 may be a promising therapeutic target in neurodegenerative diseases due to its ability to enhance proteasome function in cells (Radhakrishnan et al., 2010). Increased expression of antioxidant enzymes via Nrf2 may also contribute to the cytoprotective effects of ASC-JM17 in SBMA, since our results indicate that the Nrf2 pathway is altered in the SBMA mouse model. However, It will be important to dissect the individual contributions of Nrf1 and Nrf2 in SBMA.

Several lines of evidence suggest that the protective effect of the antioxidant pathway is not limited to SBMA. Activation of CncC was recently shown to reduce manifestations in a *Drosophila* model of alpha-synuclein toxicity (Barone et al., 2011). Furthermore, the cellular response to oxidative stress is impaired in models of Huntington's disease and amyotrophic lateral sclerosis (Jin et al., 2013; Kirby et al., 2005). Overexpression of Nrf2 prevented motor neuron loss in a mouse model of amyotrophic lateral sclerosis (Vargas et al., 2008). These findings suggest that small-molecule activators of the Nrf1/Nrf2 pathway may be broadly applicable for the treatment of neurodegenerative disorders. Our findings identify the antioxidant pathway as a modifier of mutant AR toxicity and highlight the therapeutic potential of the new curcumin analog ASC-JM17 in SBMA.

4 CONCLUDING REMARKS

A polyglutamine expansion in the nuclear hormone receptor AR causes SBMA, an adult-onset progressive neuromuscular disorder. The key pathways leading to neuron and muscle degeneration in SBMA are still unknown, and effective disease-modifying treatment is currently not available. Work in this thesis (i) characterized new disease features, (ii) investigated molecular mechanisms in SBMA, and (iii) tested pharmacological approaches for reducing mutant AR in animal models.

The results of **Studies I** and **II** link the polyglutamine expansion in the AR to alterations of normal functions. The 68 CAG repeat not only became manifest in early onset in the SBMA patient in **Study I**, as expected for such an unusually long repeat, but also in a more severe phenotype. New disease features included abnormal sexual development and autonomic dysfunction. While the additional neurological findings suggest a toxic gain of AR function, the developmental defect in this patient is consistent with a loss of function (Quigley et al., 1995). The polyglutamine expansion might cause a conformational change in the AR protein that enhances both gain and loss of function. In **Study II**, we identified a new role of the AR in the regulation of the ubiquitin ligase complex APC/C-Cdh1. The mutant AR retained the ability to associate with this complex, but negatively impacted its function. A recent study showed that the expanded polyglutamine tract in AR interferes with TFEB in a similar manner (Cortes et al., 2014b). These observations are consistent with the idea that the polyglutamine expansion leads to changes in existing protein interactions. The findings of reduced APC/C-Cdh1 and TFEB activity in SBMA models also indicates that the mutant AR has pleiotropic effects on cellular protein homeostasis.

In **Studies III** and **IV** we demonstrate the preclinical efficacy of two agents that promote the degradation of mutant AR, IGF-1 and a curcumin analog, in an SBMA mouse model. Our observations also indicate that peripheral tissues can be targeted in the disease. The significant amelioration of the phenotype in SBMA models with these agents warrants further investigation in clinical studies. **Studies II** and **IV** also revealed new potential therapeutic targets in SBMA. The finding of cell cycle reactivation in a cellular model of SBMA suggests that cell cycle inhibitors may counteract mutant AR toxicity in this disease (**Study II**). However, further work in other model systems is needed to evaluate the contribution of this phenomenon to the pathogenic mechanism in SBMA. The identification of the Nrf1/Nrf2-mediated antioxidant response as a modifier of mutant AR toxicity (**Study IV**) opens the possibility to repurpose existing drugs that are known activators of this pathway for the treatment of SBMA. Dimethyl fumarate, used clinically for the treatment of multiple

sclerosis, is a small-molecule activator of the antioxidant response (Ashrafian et al., 2012; Kappos et al., 2008). Dimethyl fumarate was recently shown to be protective in a mouse model of Huntington's disease (Ellrichmann et al., 2011). Our results in cell culture and animal models indicate that this compound might be effective in SBMA.

In conclusion, this thesis provides new insights into how the polyglutamine expansion alters normal AR functions and it offers new opportunities for therapeutic intervention in SBMA.

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