(SBMA), dentatorubral-pallidoluysian atrophy (DRPLA) and six forms of spinocerebellar ataxia (SCA) (Table 1). SBMA, also known as Kennedy's disease, is the first of the neurodegenerative diseases, for which the molecular basis was discovered to be the expansion of a trinucleotide CAG repeat in the gene of the causative gene.

Table 1. Classification of polyglutamine diseases.

Disease	Major clinical features	Affected regions	Causative protein	Gene (locus)
Huntington's disease (HD)	Chorea, cognitive deficits, psychiatric disturbances	Striatum, cerebral cortex	Huntingtin	*IT15* (4p16.3)
Spinal and bulbar muscular atrophy (SBMA)	Weakness, muscular atrophy, bulbar palsy	Spinal cord, brainstem	Androgen receptor	*AR* (Xq13-q12)
Spinocerebellar ataxia type 1 (SCA1)	Ataxia, bulbar palsy, pyramidal signs, muscular atrophy	Cerebellum, brainstem	Ataxin 1	*SCA1* (6p23)
Spinocerebellar ataxia type 2 (SCA2)	Ataxia, slow eye movement, neuropathy	Cerebellum, brainstem	Ataxin 2	*SCA2* (12q24.1)
Spinocerebellar ataxia type 3 (SCA3, Machado-Joseph disease)	Ataxia, bulging eye, parkinsonism, spasticity, fasciculations	Cerebellum, basal ganglia, brainstem, spinal cord	Ataxin 3	*SCA3/MJD* (14q32.1)
Spinocerebellar ataxia type 6 (SCA6)	Ataxia	Cerebellum	α1A-voltage-dependent calcium channel subunit	*CACNA1A* (19p13)
Spinocerebellar ataxia type 7 (SCA7)	Ataxia, retinal degeneration	Cerebellum, retina, brainstem, visual cortex	Ataxin 7	*SCA7* (3p12-p13)
Spinocerebellar ataxia type 17 (SCA17)	Ataxia, cognitive deficits, dystonia, parkinsonism	Cerebellum, striatum	TATA box binding protein	*TBP* (6q27)
Dentatorubral-pallidoluysian atrophy (DRPLA)	Ataxia, myoclonic epilepsy, choreoathetosis, cognitive deficits	Cerebellum, cerebral cortex, globus pallidus, red nuclei, subthalamic nuclei	Atrophin 1	*DRPLA* (12p13.31)

2. Clinical and genetic features of SBMA

In general, symptoms of polyglutamine diseases typically appear in mid-life and progressively deteriorate before death from fatal complications. Clinical features vary for each disorder, corresponding to the pathological distribution of neurodegeneration (Table 1).

Major symptoms of SBMA are weakness, atrophy and fasciculations of bulbar, facial and limb muscles [1]. Patients with SBMA occasionally demonstrate signs of androgen insensitivity such as gynecomastia, testicular atrophy, impaired erection and decreased fertility, some of which are detected before the onset of motor impairment. Female carriers are usually asymptomatic, but some express subclinical phenotypes including high amplitude motor unit potentials on electromyography. The progression of SBMA is usually slow, but life-threatening respiratory tract infection often occurs in the advanced stages of the disease, resulting in early death in some patients. The cardinal cause of death is aspiration pneumonia [2].

The molecular basis of SBMA is the expansion of a trinucleotide CAG repeat, which encodes the polyglutamine tract, in the first exon of the androgen receptor (AR) gene [3]. The CAG repeat within AR ranges in size from 11 to 35 in normal subjects, but from 40 to 62 in SBMA patients [3-5]. There is an inverse correlation between the CAG repeat size and the age at onset of motor impairments or the disease severity adjusted by the age at examination in SBMA [6,7] as well as in other polyglutamine diseases [8]. In a nerve conduction study of SBMA, the CAG repeat size and the age at onset were significantly different among the patients with motor- and sensory-dominant phenotypes, indicating that a longer CAG repeat is more closely linked to the motor-dominant phenotype and a shorter CAG repeat is more closely linked to the sensory-dominant phenotype [9].

3. Neuropathology and molecular mechanisms of SBMA

The fundamental histopathological finding in SBMA is loss of lower motor neurons in the anterior horn of the spinal cord as well as in the brainstem motor nuclei except for the third, fourth and sixth cranial nerves [10]. The number of nerve fibers is reduced in the ventral spinal nerve root, reflecting motor neuronopathy. Sensory neurons in the dorsal root ganglia are less severely affected, and large myelinated fibers demonstrate a distally accentuated sensory axonopathy in the peripheral nervous system. Muscle histopathology includes both neurogenic and myogenic findings: there are groups of atrophic fibers with a number of small angular fibers, fiber type grouping and clamps of pyknotic nuclei as well as variability in fiber size, hypertrophic fibers, scattered basophilic regenerating fibers and central nuclei.

In general, the abnormal polyglutamine protein forms inclusion bodies in affected neurons, which is a unifying histopathological hallmark of polyglutamine diseases [11]. These neuronal inclusion bodies are often detected in the nucleus, although they may be formed within the cytoplasm or neurites. The deposition of inclusion bodies is not only found in the postmortem neural tissues from patients, but has also been reported in animal models of polyglutamine diseases. The abnormal polyglutamine proteins in the inclusion bodies are often truncated, indicating that proteolytic cleavage appears to enhance the toxicity of the causative gene products [12]. The abnormal polyglutamine proteins are also expressed outside the nervous system, leading to non-neuronal pathology, such as diabetes mellitus, in some polyglutamine diseases [13,14].

In SBMA, nuclear inclusions (NIs) containing the pathogenic AR are found in the residual motor neurons in the brainstem and spinal cord as well as in non-neuronal tissues including the prostate, testes, and skin [15]. These inclusions are detectable using antibodies recognizing a small portion of the N-terminus of the AR protein, but not by those against the C-terminus of the protein. This

observation implies that the C-terminus of the AR is truncated or masked upon formation of NI. A full-length AR protein with an expanded polyglutamine tract is cleaved by caspase-3, releasing a polyglutamine-containing toxic fragment, and the susceptibility to cleavage is polyglutamine repeat length-dependent [16]. Thus, proteolytic cleavage is likely to enhance the toxicity of the pathogenic AR protein. Electron microscopic immunohistochemistry shows dense aggregates of AR-positive granular material without limiting membrane, both in the neural and non-neural inclusions, in contrast to the other polyglutamine diseases where NIs take the form of filamentous structures.

A number of studies have indicated that transcriptional dysregulation underlies the molecular mechanism of neuronal dysfunction in polyglutamine diseases. Transcriptional co-activators such as cAMP-response element binding protein-binding protein (CBP) have been shown to be sequestrated into the NIs through protein-protein interaction in mouse models and patients with SBMA [17]. It has also documented that the histone acetyltransferase activity of CBP is inhibited in animal models of polyglutamine diseases, and that histone acetylation level is decreased in a mouse model of SBMA [18]. Taken together, polyglutamine-mediated transcriptional dysregulation appears to play an important role in the pathogenesis of SBMA.

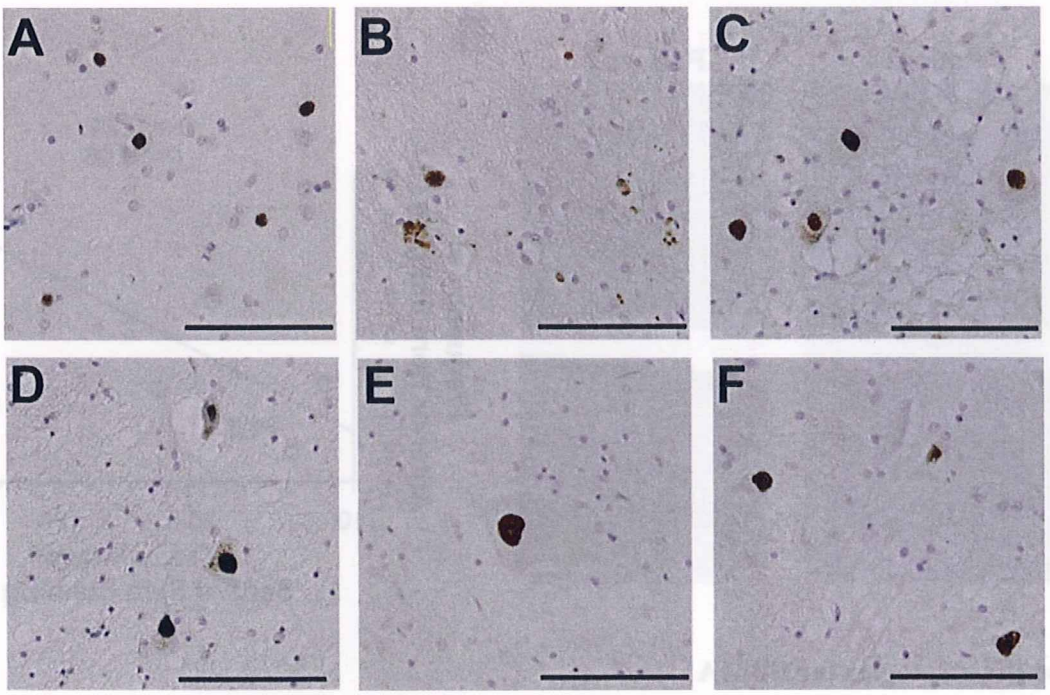
Mitochondrial impairment and oxidative stress have also been stipulated as a causative molecular event in polyglutamine diseases. Depolarization of the mitochondrial membrane and an elevated level of reactive oxygen species have been observed in a cellular model of SBMA [19]. Moreover, the pathogenic AR protein represses the transcription of the subunits of peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1), a transcriptional co-activator that regulates the expression of various nuclear-encoded mitochondrial proteins [19]. Similar finding have been reported in cellular and animal models of polyglutamine diseases, suggesting that mitochondrial dysfunction is a unifying molecular mechanism whereby abnormal polyglutamine proteins induce neuronal damage.

Obstruction of axonal transport has also gained attention as a cause of neuronal dysfunction in SBMA. The pathogenic AR has been shown to impair axonal transport through a pathway that involves activation of cJun N-terminal kinase (JNK) activity [20]. In a mouse model of SBMA, the nuclear accumulation of the abnormal AR protein induces transcriptional dysregulation of dynactin 1, an axonal motor protein that regulates axonal trafficking [21]. Given that a mutation in the dynactin 1 gene has been shown to cause motor neuron degeneration mimicking SBMA, a disrupted axonal transport is a potential molecular basis for SBMA [22].

4. Protein folding abnormalities in SBMA

Although NIs are a disease-specific histopathological finding, their role in pathogenesis has been heavily debated. Several studies have suggested that NIs may indicate a cellular response coping with the toxicity of abnormal polyglutamine protein [23]. Instead, the diffuse nuclear accumulation of the mutant protein has been considered essential for inducing neurodegeneration in polyglutamine diseases including SBMA (Figure 1). Recent data suggest that the toxic species of protein in polyglutamine diseases may be soluble mutant conformers, which can exist as oligomers or monomers containing beta-sheet conformation [24-26].

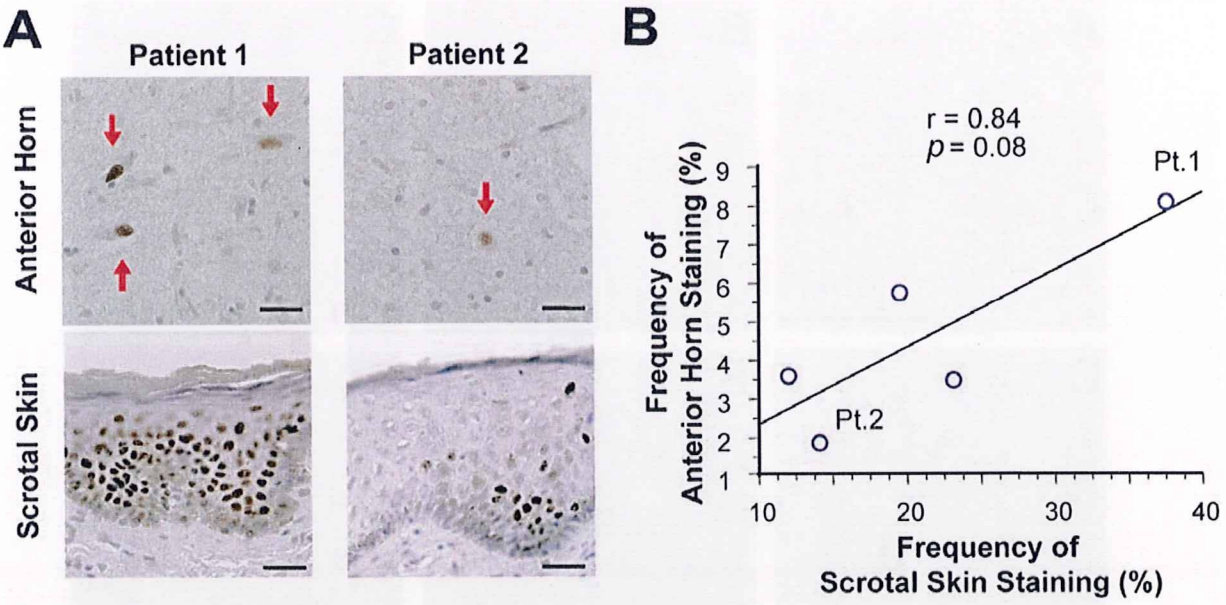
Figure 1. Accumulation of abnormal proteins in polyglutamine diseases. Immunohistochemistry of autopsy specimens from patients using an anti-polyglutamine antibody (1C2). (A) Cerebral cortex, HD; (B) Putamen, HD; (C) Dentate nucleus, DRPLA, (D) Globus pallidus, DRPLA; (E) Anterior horn of spinal cord, SBMA; (F) Pons, SBMA. Scale bar = 100 μm [27].



Although it is difficult to determine the toxic protein species in human histopathology, diffuse accumulation of the causative gene products has been construed as an important finding. An immunohistochemical study on autopsied SBMA patients using an anti-polyglutamine antibody demonstrated that diffuse nuclear accumulation of the pathogenic AR is more frequently observed than NIs in the anterior horn of the spinal cord [28]. Intriguingly, the frequency of diffuse nuclear accumulation of the pathogenic AR in spinal motor neurons strongly correlates with the length of the CAG repeat in the AR gene. No such correlation has been found between NI occurrence and the CAG repeat length. A similar observation has been reported in DRPLA [29]. Taken together, it appears that the pathogenic AR containing an elongated polyglutamine tract principally accumulates within the nuclei of motor neurons in a diffusible form, leading to neuronal dysfunction and eventual cell death in SBMA. In support of this hypothesis, neuronal dysfunction is halted by genetic modulation preventing nuclear import of the pathogenic polyglutamine-containing protein in cellular and animal models of polyglutamine diseases [8].

Since the human AR is widely expressed in various organs, nuclear accumulation of the pathogenic AR protein is detected not only in the central nervous system, but also in non-neuronal tissues such as scrotal skin. The degree of pathogenic AR accumulation in scrotal skin epithelial cells tends to be correlated with that in the spinal motor neurons in autopsy specimens, and it is well correlated with CAG repeat length and inversely correlated with the motor functional scale [30]. These findings indicate that scrotal skin biopsy with anti-polyglutamine immunostaining is a good biomarker with which to monitor SBMA pathogenic processes (Figure 2).

Figure 2. Mutant AR nuclear accumulation in scrotal skin and spinal motor neurons. (A) Mutant AR accumulation was remarkable in both spinal motor neurons and scrotal skin of Patient 1, but less remarkable in both motor neurons and skin in Patient 2. Scale bar = 30 μm. (B) The extent of mutant AR accumulation in scrotal skin epithelial cells showed a tendency to correlate with that in anterior horn cells.



5. Therapeutic strategies for SBMA

For any given polyglutamine disease, more than one mechanism likely contributes to neuronal dysfunction and eventual cell death. They include: (i) misfolding of the disease protein resulting in altered function; (ii) deleterious protein interactions engaged in by the mutant protein; (iii) formation of toxic oligomeric complexes; (iv) transcriptional dysregulation; (v) mitochondrial dysfunction resulting in impaired bioenergetics and oxidative stress; (vi) impaired axonal transport; (vii) aberrant neuronal signaling including excitotoxicity; (viii) cellular protein homeostasis impairment; and (ix) RNA toxicity [31]. Although each of these molecular mechanisms could be subject to therapeutic interventions, upstream events are more plausible targets than secondary cellular changes.

There is no well-established disease-modifying therapy for SBMA. Potential therapeutics, however, have emerged from basic research using animal models. Among these therapeutic approaches, androgen deprivation has been translated into clinic [27]. Anti-androgen therapies have been developed taking advantage of the fact that the accumulation of the pathogenic AR proteins is dependent on the circulating level of testosterone [32,33]. Surgical castration has been shown to reverse motor dysfunction in mouse models of SBMA [34]. The luteinizing hormone-releasing hormone analogue, leuprorelin, prevents nuclear translocation of aberrant AR proteins, resulting in a significant improvement of disease phenotype in a mouse model of SBMA [35]. These results of animal studies were verified in a phase 2 clinical trial of leuprorelin, in which the patients treated with this drug exhibited decreased mutant AR accumulation in scrotal skin biopsy, significantly higher functional scores and better swallowing parameters than those receiving placebo (Figure 3). Autopsy of one patient who received leuprorelin suggested that androgen deprivation inhibits the nuclear accumulation and/or stabilization of mutant AR in the motor neurons of the spinal cord and brainstem

(Figure 4). These observations suggest that administration of leuprorelin suppresses the deterioration of neuromuscular impairment in SBMA by inhibiting the toxic accumulation of mutant AR [36].

Figure 3. Efficacy results of leuprorelin in SBMA patients. (A) The frequency of diffuse nuclear 1C2 staining (indicative of mutant AR) in the scrotal epithelial cells was significantly decreased after the 48-week administration of leuprorelin acetate. (B) Changes in the ALSFRS-R scores showed treatment duration-dependent improvements in the leuprorelin-treated groups. Scale bars = 50 μm. Data are expressed as means ± SEM. *p < 0.05; **p < 0.005; ***p < 0.001 with respect to Group D [36].

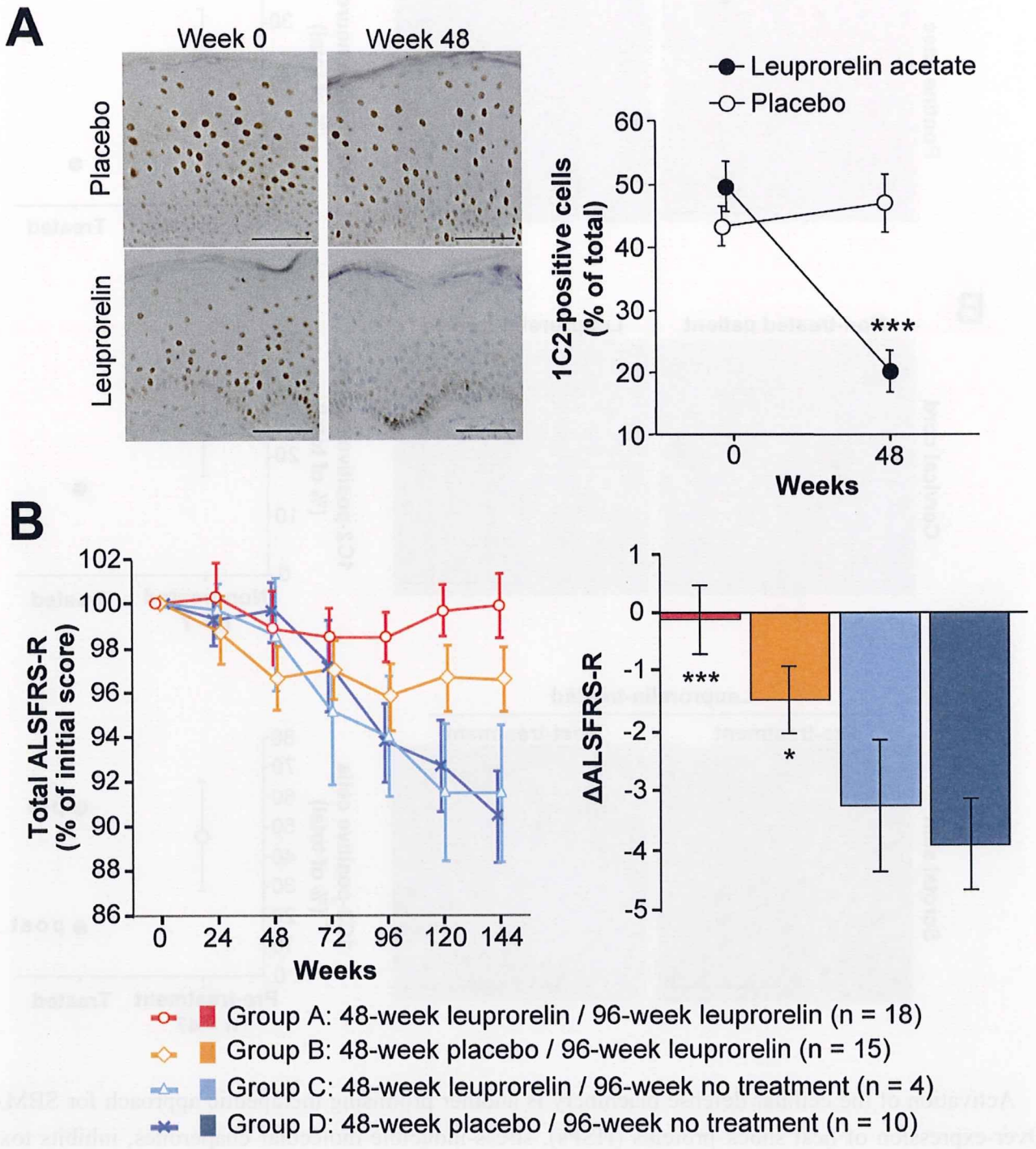
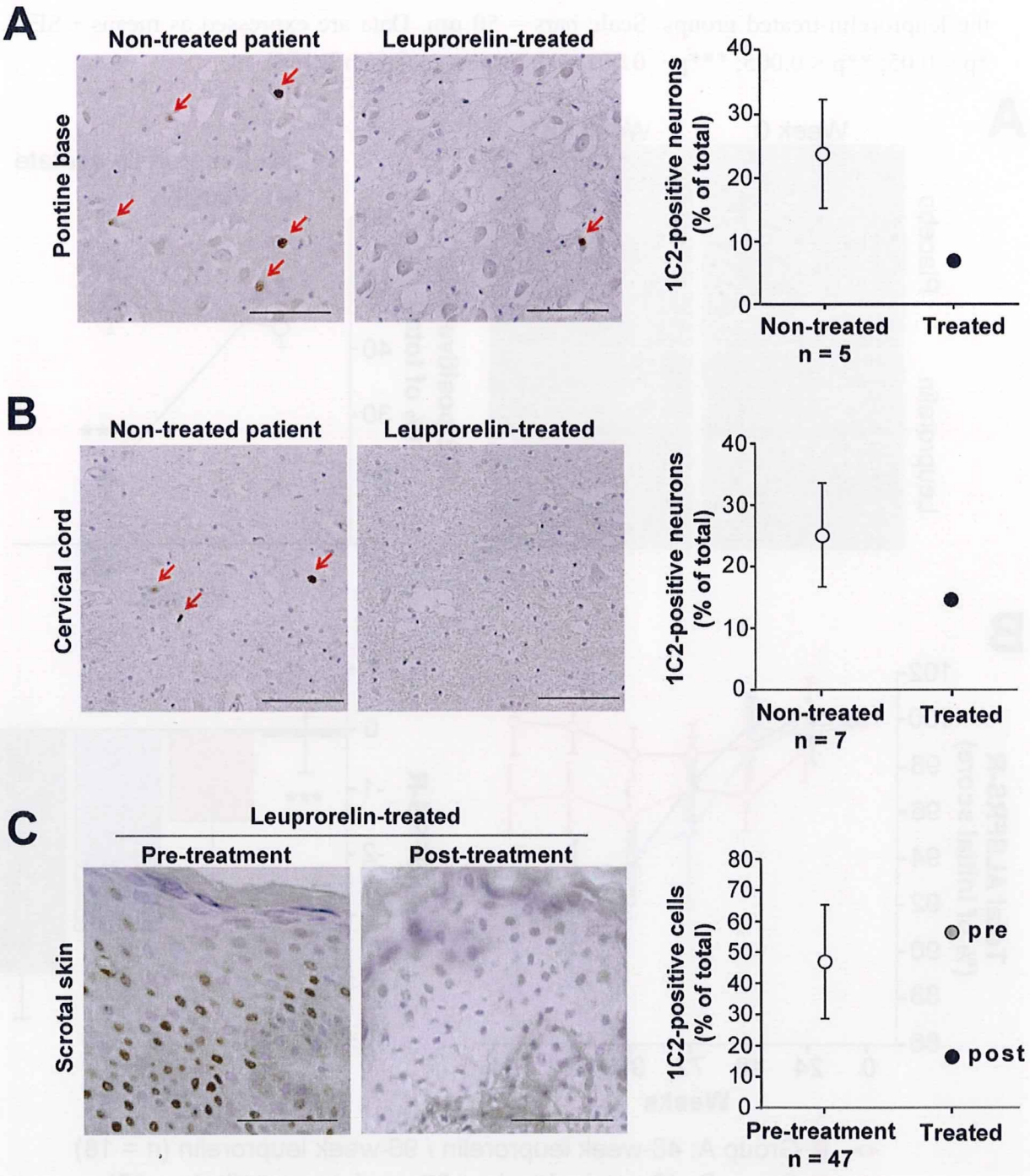


Figure 4. Effects of leuprorelin acetate on nuclear accumulation of mutant AR. (A, B) The accumulation of mutant AR in neurons was remarkable both in the pontine base and in the spinal anterior horn of all the control, non-treated autopsied patients, but the number of 1C2-positive neurons was relatively small in the leuprorelin-treated patient. Scale bars=100 μm. (C) Mutant AR accumulation in biopsied scrotal skin epithelial cells was markedly reduced by leuprorelin. Scale bars=50 μm. Data are expressed as means±SD [36].



Activation of the cellular defense machinery is another promising therapeutic approach for SBMA. Over-expression of heat shock proteins (HSPs), stress-inducible molecular chaperones, inhibits toxic accumulation of abnormal AR protein and suppresses neurodegeneration in a mouse model of SBMA

[37]. Similar beneficial effects have also been achieved by the pharmacological induction of HSPs [38]. On the other hand, inhibition of Hsp90 has been demonstrated to arrest neurodegeneration by activating the ubiquitin-proteasome system in SBMA. Treatment with 17-allylamino geldanamycin (17-AAG), a potent Hsp90 inhibitor, dissociated p23 from the Hsp90-AR complex, and thus facilitated proteasomal degradation of the pathogenic AR in cellular and mouse models of SBMA [39, 40]. Similar effects were observed in the SBMA mice being treated with an oral Hsp 90 inhibitor, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) [41].

Transcriptional dysregulation is another target for therapeutic intervention. Because suppression of histone deacetylase (HDAC) activities results in an augmentation of histone acetylation and a subsequent restoration of gene transcription, HDAC inhibitors have been considered to be of therapeutic benefit in polyglutamine diseases [42]. Butyrate was the first HDAC inhibitor to be discovered, and the related compound, phenylbutyrate, has been successfully employed in experimental cancer therapy. Oral administration of sodium butyrate ameliorates the symptomatic and histopathological phenotypes of a mouse model of SBMA through upregulation of histone acetylation in nervous tissues [18]. This compound has also been shown to alleviate neurodegeneration in a mouse model of DRPLA [43]. In mouse models of HD, the administration of HDAC inhibitors (sodium butyrate, suberoylanilide hydroxamic acid and phenylbutyrate) has been shown to alleviate polyglutamine toxicity and improve neuronal dysfunction [44-46].

5. Conclusions

Although the genetics of polyglutamine diseases were discovered as an abnormal expansion of a trinucleotide CAG repeat, detailed mechanisms of the diseases including SBMA have not been fully elucidated. The clinical trial of leuprorelin acetate suggests that androgen deprivation inhibits the nuclear accumulation and/or stabilization of mutant AR in the motor neurons, and thereby stabilizes the disease progression in SBMA patients. Future research approaches have to determine the main mechanisms which contribute to neuronal dysfunction and eventual cell death in SBMA.

Acknowledgements

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Symposium: Clinicopathological aspects of neuromuscular disorders – A new horizon

Pathogenesis-targeting therapeutics for spinal and bulbar muscular atrophy (SBMA)

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Spinal and bulbar muscular atrophy (SBMA) is an hereditary, adult-onset, lower motor neuron disease caused by an aberrant elongation of a trinucleotide CAG repeat, which encodes the polyglutamine tract, in the first exon of the *androgen receptor* (*AR*) gene. The main symptoms are slowly progressive muscle weakness and atrophy of bulbar, facial and limb muscles. The cardinal histopathological findings of SBMA are an extensive loss of lower motor neurons in the anterior horn of the spinal cord as well as in brainstem motor nuclei and intranuclear accumulations of mutant AR protein in the residual motor neurons. Androgen deprivation therapy rescues neuronal dysfunction in animal models of SBMA, suggesting that the molecular basis for motor neuron degeneration in this disorder is testosterone-dependent nuclear accumulation of the mutant AR. Suppression of disease progression by leuprorelin acetate has also been demonstrated in a phase 2 clinical trial. In addition, the clarification of pathophysiology leads to appearance of candidate drugs to treat this devastating disease: heat shock protein (HSP) inducer, Hsp90 inhibitor, and histone deacetylase inhibitor. Advances in basic and clinical research on SBMA are now paving the way for clinical application of pathogenesis-targeting therapeutics.

Key words: androgen receptor, leuprorelin acetate, polyglutamine, spinal and bulbar muscular atrophy (SBMA), testosterone.

INTRODUCTION

Spinal and bulbar muscular atrophy (SBMA) is an hereditary lower motor neuron disease affecting adult males.[1–4] The cause of SBMA was identified as the expansion of a trinucleotide CAG repeat in the *androgen receptor* (*AR*) gene in 1991.[5] From 9 to 36 CAGs are observed in the *AR* gene in normal subjects, but from 38 to 62 CAGs are observed in SBMA patients.[6,7] In SBMA patients, there is an inverse correlation between the number of CAGs and the age at onset.[8,9] The main symptoms of SBMA are weakness and atrophy of the bulbar, facial and limb muscles.[10] The deep tendon reflex is diminished or absent with no pathological reflex. In addition to motor symptoms, sensory impairment such as vibratory sensory disorder is often observed.[11–13] Patients occasionally demonstrate signs of androgen insensitivity such as gynecomastia and testicular atrophy. The onset of muscle weakness is usually between 30 and 60 years, but is often preceded by nonspecific symptoms such as postural tremor and muscle cramps. The progression of SBMA is usually slow, but a considerable number of patients need assistance to walk in their 50s or 60s. The susceptibility for aspiration pneumonia increases as bulbar paralysis develops, and the most common cause of death is pneumonia.[9,14] Many patients also have hypertension, hyperlipidemia, liver dysfunction, and glucose intolerance. Serum creatine kinase level is elevated in the majority of patients. Electromyogram shows neurogenic abnormalities such as high amplitude potentials, reduced interference, and polyphasic potentials. Deceased compound muscle action potentials and prolonged distal latencies are often observed in motor nerve conduction studies. Both sensory nerve action potential and sensory evoked potential are reduced or absent.[13,15]

GENETICS AND ETIOLOGY OF SBMA

The cause of SBMA is expansion of a trinucleotide CAG repeat, which encodes the polyglutamine tract, in the first exon of the *AR* gene.[5] A similar gene mutation has been detected in Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), and several forms of

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spinocerebellar ataxia.[16] Since CAG is translated to glutamine, these disorders, including SBMA, are called polyglutamine diseases. Although causative gene products are unrelated outside the polyglutamine stretch, polyglutamine diseases share salient genetic features such as the correlation between CAG repeat size and the age of onset. In SBMA patients, there is an inverse correlation between the CAG repeat size and the age at onset, or the disease severity adjusted by the age at examination.[8,9] However, the age at onset and severity are also variable even among patients with the same CAG repeat size,[8,9] indicating that some unknown genetic or environmental factors may influence the development of clinical heterogeneity.[9] It has been reported that CAG repeat size of causative genes determines the clinical phenotype of polyglutamine diseases.[17–19] In SBMA, CAG repeat size of *AR* influences electrophysiological phenotypes.[13] Taken together, these observations strongly suggest that common mechanisms underlie the pathogenesis of polyglutamine diseases.

The expansion of a polyglutamine tract in AR has been implicated in the pathogenesis of SBMA in two different, but not mutually exclusive, ways: loss of normal AR function induces neuronal degeneration; and the pathogenic AR acquires toxic property-damaging motor neurons. Since AR holds trophic effects on neuronal cells, one can assume that loss of AR function may play a role in the pathogenesis of SBMA. Expansion of the polyglutamine tract mildly suppresses the transcriptional activities of AR.[20] Although this loss of function of AR may be a factor in the androgen insensitivity of SBMA, the pivotal cause of neurodegeneration in SBMA has been believed to be a gain of toxic function of the pathogenic AR due to expansion of the polyglutamine tract. This hypothesis is supported by the observation that motor impairment has never been observed in severe testicular feminization patients lacking AR function. Moreover, a transgenic mouse model carrying an elongated CAG repeat driven by human AR promoter demonstrated motor impairment, suggesting that the expanded polyglutamine tract is sufficient to induce the pathogenic process of SBMA.[21]

Aggregation of abnormal protein has been considered to be central to the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and prion disease.[22] It is now widely received that the accumulation of causative proteins in neurons is an important event in the pathogenesis of polyglutamine diseases. The rate-limiting step of aggregation has been proposed to be the formation of oligomeric nucleus, which may occur after a repeat length-dependent conformational change of polyglutamine monomer from a random coil to a parallel, helical β-sheet.[23] Several experimental observations indicate that formation of toxic oligomers, or intermediates, of abnormal polyglutamine-containing protein instigates a series of cellular events which lead to neurodegeneration.[24] This hypothesis is likely to be the case in SBMA.

PATHOLOGILAL FEATURE OF SBMA

The histopathological hallmarks of SBMA are an extensive loss of lower motor neurons in the spinal cord and brain stem.[2,25] The number of nerve fibers is reduced in the ventral spinal nerve root, reflecting motor neuronopathy. Sensory neurons in the dorsal root ganglia (DRG) are less severely affected, and large myelinated fibers demonstrate a distally accentuated sensory axonopathy in the peripheral nervous system. Muscle histopathology shows both neurogenic and myogenic findings; there are groups of atrophic fibers with a number of small angular fibers, fiber type grouping and clamps of pyknotic nuclei as well as variability in fiber size, hypertrophic fibers, scattered basophilic regenerating fibers and central nuclei.

Intranuclear accumulations of mutant AR protein in the residual motor neurons are another hallmark (Fig. 1).[26,27] Intranuclear accumulations are composed of not only the abnormal protein, but necessary protein for homeostasis maintenance of neuronal cells such as heat shock protein (HSP), the transcription factor, and ubiquitin. The frequency of diffuse nuclear accumulation of the pathogenic AR in spinal motor neurons strongly correlates with the length of the CAG repeat in the *AR* gene.[26] On the other hand, in primary sensory neurons within the DRG, mutant AR was detected immunochistochemically as punctuate aggregates in the cytoplasm, the number of which tended to be inversely correlated with the size of CAG repeat in the *AR* gene.[13,26] The differential accumulation pattern of mutant AR between motor and sensory neurons, and their differential correlation to CAG repeat size is likely to be the pathophysiological background for the elctrophysiological phenotypes.

Since human AR is widely expressed in various organs, nuclear accumulation of the pathogenic AR protein is detected not only in the CNS, but also in non-neuronal tissues such as the kidney, skeletal muscle, adrenal gland, and scrotal skin. The degree of pathogenic AR accumulation in scrotal skin epithelial cells tends to be correlated with that in the spinal motor neurons in autopsy specimens, and it is well correlated with CAG repeat length and inversely correlated with the motor functional scale.[28] These findings indicate that scrotal skin biopsy with anti-polyglutamine (1C2) immunostaining is a potent biomarker with which to monitor SBMA pathogenic processes. Since SBMA is a slowly progressive disorder, appropriate biomarkers would help improve the power and cost-effectiveness of longitudinal clinical treatment trials.[29]

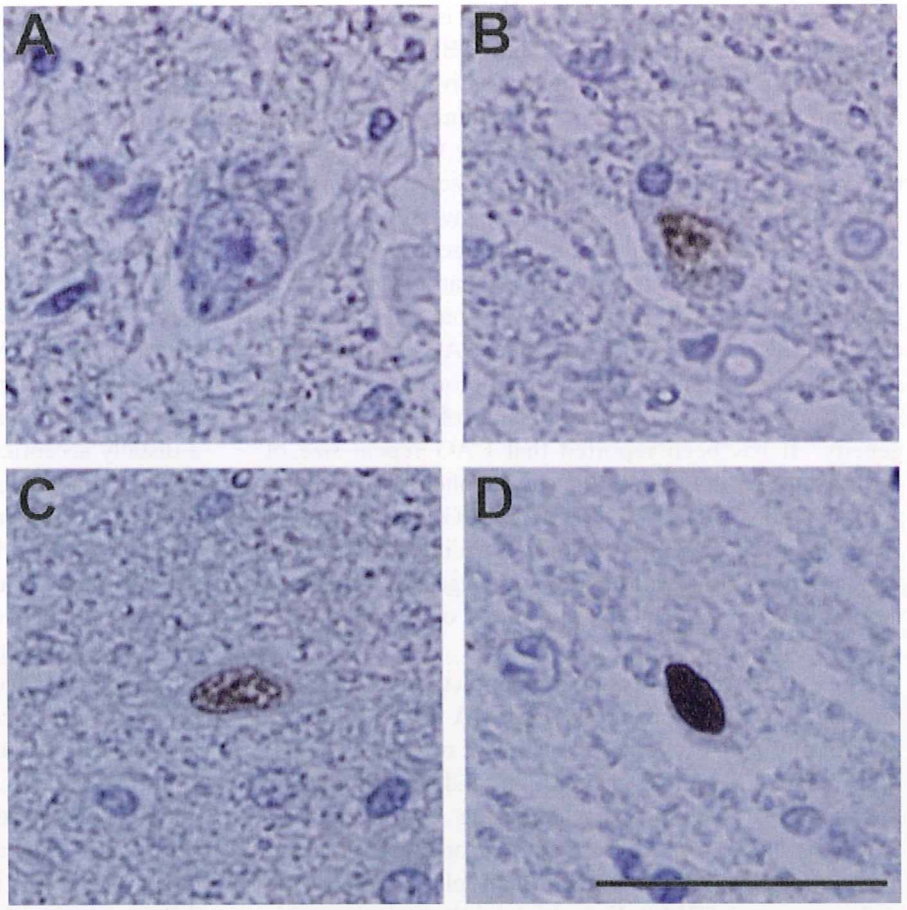

Fig. 1 Anti-polyglutamine immunostaining of motor neurons in a spinal and bulbar muscular atrophy (SBMA) patient. Mutant androgen receptor (AR) accumulates in the nucleus of lower motor neurons within the anterior horn of the spinal cord. Neurons with a densely stained nucleus are atrophic compared with those without staining or those showing intra-nuclear scattered aggregates. The neuron with a densely stained nucleus (D) is atrophic compared with the one without staining (A) or those showing intra-nuclear scattered aggregates (B,C). Scale bar = 100 μm.

TESTOSTERONE-DEPENDENT NEURODEGENERATION IN SBMA

SBMA is distinct among polyglutamine diseases in that the pathogenic protein, AR, has a specific ligand, testosterone, which alters the subcellular localization of the protein by favoring its nuclear uptake. The AR is normally confined to a multi-heteromeric inactive complex in the cell cytoplasm, and translocates into the nucleus in a ligand-dependent manner. This ligand-dependent intracellular trafficking of AR appears to play an important role in the pathogenesis of SBMA.

In order to investigate ligand effect in SBMA, we generated transgenic mice expressing the full-length human *AR* containing 24 or 97 CAGs under the control of a cytomegalovirus (CMV) enhancer and a chicken β-actin promoter.[30] Affected AR-97Q mice demonstrate small body size, short life span, progressive muscle atrophy and weakness as well as reduced cage activity, all of which are markedly pronounced and accelerated in the male AR-97Q mice, but either not observed or far less severe in the female AR-97Q mice. The 50% mortality ranged from 66 to 132 days of age in the male AR-97Q mice, whereas mortality of the female AR-97Q mice remained only 10–30% at more than 210 days. Male AR-97Q mice show markedly more abundant diffuse nuclear staining and nuclear inclusions than females. Western blot analysis revealed the transgenic AR protein smearing from the top of the gel in the spinal cord, cerebrum, heart, muscle and pancreas. Although the male AR-97Q mice had more smearing protein than their female counterparts, the female AR-97Q mice had more monomeric AR protein. These observations indicate that the testosterone level plays important roles in the sexual difference of phenotypes, especially in the post-transcriptional stage of the pathogenic AR (Fig. 2).

The dramatic sexual difference of phenotypes led us to hormonal interventions in our mouse model. First, we castrated male AR-97Q mice in order to decrease their testosterone level. Castrated male AR-97Q mice showed profound improvement of symptoms, histopathologic findings, and nuclear localization of the pathogenic AR compared with the sham-operated male AR-97Q mice. Body weight, motor function, and lifespan of male AR-97Q mice were significantly improved by castration. Western blot analysis and histopathology revealed diminished nuclear accumulation of the pathogenic AR in the castrated male AR-97Q mice. Next, we administered testosterone to the

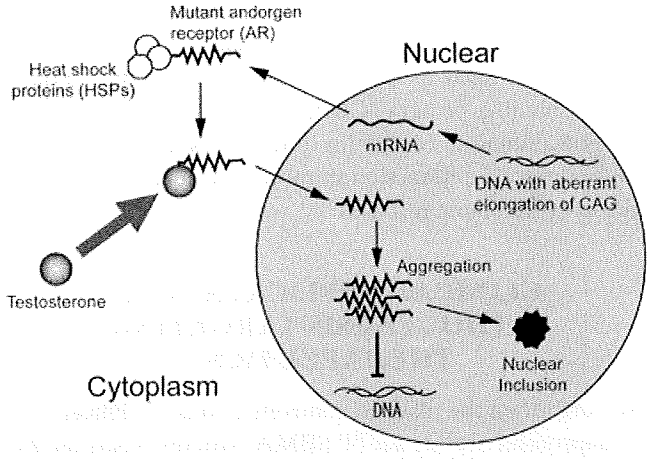

Fig. 2 Putative molecular pathogenesis of spinal and bulbar muscular atrophy (SBMA). In the absence of ligand, pathogenic androgen receptor (AR) is confined to a multi-heteromeric inactive complex with heat shock proteins (HSPs) in the cell cytoplasm. Ligand-binding facilitates its dissociation from this complex and translocation into the nucleus. In the nucleus, pathogenic AR aggregates through protein-protein interaction, and forms nuclear inclusions as a consequence.

female AR-97Q mice. In contrast to castration of the male mice, testosterone caused significant aggravation of symptoms, histopathologic features, and nuclear localization of the pathogenic AR in the female AR-97Q mice. Since the nuclear translocation of AR is ligand-dependent, testosterone appears to show toxic effects in the female AR-97Q mice by accelerating nuclear translocation of the pathogenic AR. On the contrary, castration prevented the nuclear localization of the pathogenic AR by reducing the testosterone level.

It thus appears logical that reducing testosterone levels would improve phenotypic expression by preventing nuclear localization of the pathogenic AR. In support of this hypothesis, ligand-dependent neurodegenaration has also been revealed in other animal models of SBMA.[31,32] Furthermore, a female carrier of SBMA, even if homozygous, does not express disease phenotypes.[3,33] A 85-year-old woman with 37/51 CAGs was also asymptomatic in another case report.[34]

TESTOSTERONE BLOCKADE THERAPY FOR SBMA

Successful treatment of AR-97Q mice with castration inspired us to test testosterone blockade therapies, luteinizing hormone-releasing hormone (LHRH) analogue and AR antagonist, used in the treatment of prostate cancer.[35] Leuprorelin acetate, LHRH analogue reducing testosterone release from the testis, initially increased the serum testosterone level by agonizing the LHRH receptor, but subsequently reduced it to undetectable levels. The leuprorelin-treated AR-97Q mice showed marked improvement of lifespan, muscle atrophy and reduced body size as well as motor impairment assessed by rotarod task and cage activity compared with the vehicle-treated AR-97Q mice.[35,36] In the western blot analysis and anti-polyglutamine immunostaining, the leuprorelin-treated male AR-97Q mice had markedly diminished mutant AR in the nucleus, suggesting that leuprorelin successfully reduced nuclear AR accumulation. Testosterone, which was given from 13 weeks of age, markedly aggravated neurologic symptoms and pathologic findings of leuprorelin-treated male AR-97Q mice. Since leuprorelin suppresses testicular testosterone production, this drug appears to improve neuronal dysfunction by preventing ligand-dependent nuclear translocation of mutant AR in the same way as castration. Leuprorelin thus appears to be a promising therapeutic agent for SBMA.

By contrast, flutamide, an AR antagonist, did not ameliorate symptoms, pathologic features, or nuclear localization of the mutant AR in the male AR-97Q mice, although there was no significant difference in the androgen blockade effects between flutamide and leuprorelin acetate.[35] Flutamide, the first discovered androgen antagonist, has highly specific affinity for AR, and competes with testosterone for binding to the receptor. It has been used for the treatment of prostate cancer, usually in association with an LHRH agonist, in order to block the action of adrenal testosterone. Although flutamide suppresses the androgen-dependent transactivation, it does not reduce the plasma levels of testosterone. Furthermore, flutamide does not inhibit, but may even facilitate, the nuclear translocation of AR.[37,38] This may be the reason why flutamide demonstrated no therapeutic effect in our transgenic mouse model of SBMA. Flutamide also promotes nuclear translocation of mutant AR containing expanded polyglutamine in a cell and fly model of SBMA.[31,39] Flutamide is not likely to be a therapeutic agent for SBMA.

MANIPULATION OF HEAT SHOCK PROTEINS

Many components of ubiquitine-proteasome and molecular chaperones are known to co-localize with polyglutamine-containing nuclear inclusions, implying that failure of cellular defense mechanism underlies neurodegeneration in polyglutamine diseases. HSP, a stress-inducible molecular chaperone, is another key to elucidation of the pathogenesis of SBMA. Hsp70 and Hsp90, essential components of AR-chaperone complex in the cell cytoplasm, regulate function, nuclear translocation, and degradation of AR.[40] Expression levels of HSPs are decreased in the brain lesion of an animal model of HD

and in that of the SBMA mouse.[41,42] Not only are HSPs implicated in the pathogenesis of neurodegeneration in polyglutamine diseases,[23,43] they are also potent suppressors of polyglutamine toxicity.[23,24] Overexpression of Hsp70, together with Hsp40, inhibits toxic accumulation of abnormal polyglutamine-containing protein and suppresses cell death in a variety of cellular models of polyglutamine diseases including SBMA.[44]

Over-expression of the inducible form of human Hsp70 markedly ameliorated symptomatic and histopathological phenotypes of our transgenic mouse model of SBMA.[25] Oral administration of geranylgeranylacetone (GGA) upregulates the levels of Hsp70, Hsp90, and Hsp105 via activation of heat shock factor-1 in the CNS and inhibits nuclear accumulation of the pathogenic AR protein, resulting in amelioration of polyglutamine-dependent neuromuscular phenotypes of SBMA transgenic mice.[42] Inhibition of Hsp90 is also demonstrated to arrest the neurodegeneration in SBMA mice.[45] Treatment with 17-allylamino geldanamycin (17-AAG), a potent Hsp90 inhibitor, dissociated p23 from the Hsp90-AR complex, and thus facilitated proteasomal degradation of the pathogenic AR in cellular and mouse models of SBMA. Overexpression of C terminus of Hsc70 (heat shock cognate protein 70)-interacting protein (CHIP), which interacts with Hsp90 or Hsp70 and ubiquitylates and degrades unfolded proteins trapped by molecular chaperones, ameliorates the phenotypes of SBMA mice by reducing nuclear-localized mutant AR via an enhanced degradation of the protein.[46] These results strongly suggest that facilitation of protein degradation through molecular chaperones and ubiquitin proteasome system is an important therapeutic strategy for neurodegenerative diseases including SBMA.[47,48]

RESTORATION OF TRANSCRIPTIONAL ACTIVITY

Disruption of transcriptional machinery has also been hypothesized to underlie the pathogenesis of polyglutamine diseases.[49] Histone acetylation level is determined by interplay between histone acetyltransferase and histone deacetylase (HDAC). Recruitment of HDAC to target genes represses transcription, leading to aberrant cellular function. Since cancellation of HDAC activity results in augmentation of histone acetylation and subsequent restoration of gene transcription, HDAC inhibitors have been considered to be of therapeutic benefit in polyglutamine diseases.[50,51] Butyrate is the first HDAC inhibitor to be discovered, and the related compound, phenylbutyrate, has been successfully employed in experimental cancer therapy. Oral administration of sodium butyrate ameliorates symptomatic and histopathological phenotypes of our mouse model of SBMA through upregulation of histone acetylation in nervous tissues.[52] Transcriptional co-activators such as c-AMP response element binding protein-binding protein (CBP) are also sequestrated into the polyglutamine-containing nuclear inclusions through protein-protein interaction in mouse models and patients with polyglutamine diseases.[53]

CLINICAL APPLICATION OF PATHOGENESIS-TARGETING THERAPEUTICS

We performed the placebo-controlled phase 2 clinical trial of leuprorelin acetate for 50 SBMA patients based on the results of the animal studies. Compared with placebo, the subcutaneous administration of leuprorelin acetate for 48 weeks improved the level of serum creatine kinase and the function of swallowing assessed by videofluorography.[54] We also identified a significant decrease in mutant AR accumulation using scrotal skin immunohistochemistry. Furthermore, the patients treated with leuprorelin acetate for 144 weeks showed higher motor function score than those without medication in an open-labeled follow-up trial (Fig. 3).

One SBMA patient who received leuprorelin acetate in a phase 2 trial died 118 weeks after initiation of the treatment. Autopsy of the patient indicated acute cardiac failure due to cardiac arrhythmia as a possible cause of death. Autopsied specimens were assessed by anti-polyglutamine (1C2) immunohistochemistry, and were compared with the findings of previously autopsied SBMA patients who had not been treated with leuprorelin acetate. The frequencies of 1C2-positive neurons in the anterior horn and brainstem of a patient treated with leuprorelin acetate were less than those in non-treated SBMA patients (Fig. 4). Indeed, the pre-treatment frequency of 1C2-positive cells in the biopsied scrotal skin of this patient was a little higher than the mean value of other study participants, but decreased after 48 weeks of leuprorelin treatment. These observations suggest that leuprorelin acetate suppresses nuclear accumulation of mutant AR, the principle of pathogenesis of SBMA, in motor neurons. The results of the phase 2 trial have now been verified in a large-scale, phase 3 clinical study.

CONCLUSION

The causative gene of SBMA is *AR*, in which the length of CAG repeat extends in patients. Animal models have clarified that testosterone, the ligand of AR, plays a pivotal role in the pathogenesis of neurodegeneration in SBMA. Success in elucidation of pathogenesis has been translated into clinical trials with leuprorelin acetate.

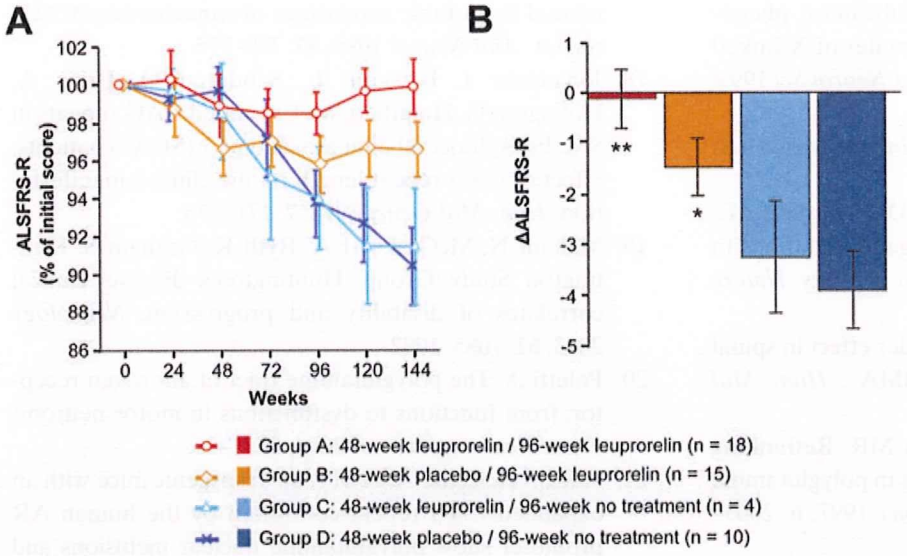

Fig. 3 Results of a phase 2 clinical trial of leuprorelin acetate for spinal and bulbar muscular atrophy (SBMA). The administration of leuprorelin acetate for 144 weeks inhibited the decline in motor function score (ALSFRS-R: amyotrophic lateral sclerosis functional rating scale – revised) of SBMA patients (A,B). Changes in the ALSFRS-R scores showed treatment duration-dependent improvements in the leuprorelin-treated groups. Data are expressed as mean ± SEM. $*P < 0.05$; $**P < 0.001$ with respect to Group D. This figure is reconstructed from a previous report.[54]

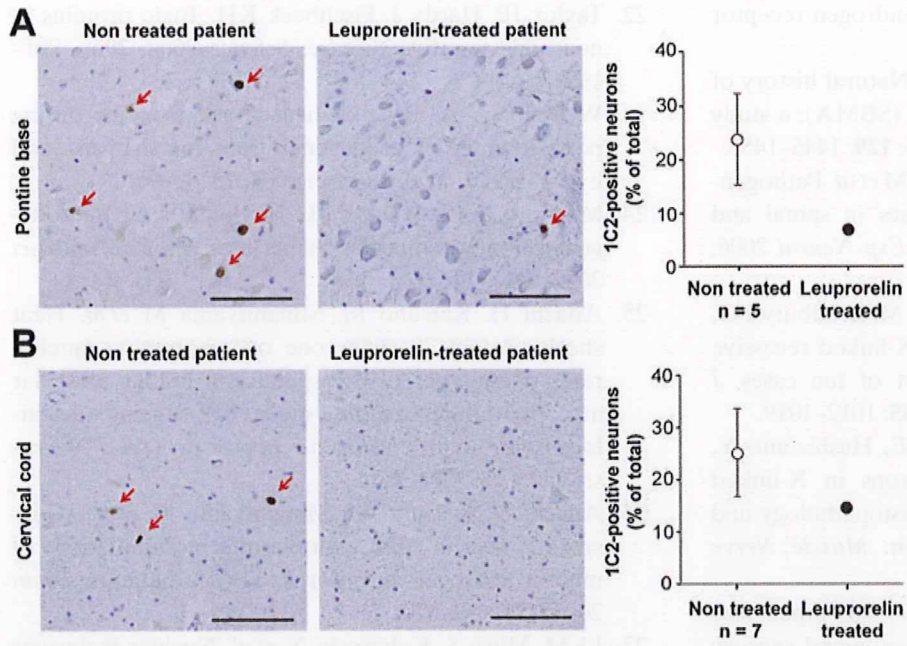

Fig. 4 Autopsy study of a spinal and bulbar muscular atrophy (SBMA) patient treated with leuprorelin acetate. Effects of leuprorelin acetate on mutant androgen receptor (AR) accumulation in neurons were assessed using immunohistochemistry. The nuclear accumulation of mutant AR in neurons was remarkable both in the pontine base (A) and in the spinal anterior horn (B) of all the control, non-treated autopsied patients, but the number of anti-polyglutamine (1C2)-positive neurons was relatively small in the leuprorelin-treated patient. Scale bar = 100 μm. Data are expressed as mean ± SD. This figure is reconstructed from a previous report.[54]

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