分裂させ四分子解析によりゲノムのβ５が破壊され、導入したプラスミド依存的に増殖が可能となっている株を選択した。選別後の株を 4ml のＹＰＤ液体培養地で培養して回収した後、pRS315 ベクターに WT もしくは変異型β subunit を組み込んだプラスミドを導入し、SD(-Leu)Plate にまいた。２－３日後、生えてきた colony から５つを選び、FOA(-Leu)Plate に植え継いだ。２６℃で一晩培養後、SD(-Ura), SD(-Leu)Plate に植え継ぎ、SD(-Ura)plate で生えず、SD(-Leu)plate で生える株を選別し、pRS316-β５WT を持たず pRS315 ベクターに WT もしくは変異型β５が組み込まれたプラスミドのみを持つ形質変換体を取得した。

３）ターゲティングベクターの作製：マウスβ５(Psmb5)遺伝子を含む BAC クローンより、以下に示す断片を単離し、pBluescript-SKII にサブクローニングすることでターゲティングベクターを作製した。2.0kb の SacI-SacI 断片を５'側相同部位、5.5kb の BamHI-BamI 断片を３'側相同部位とした。その間に exon2, exon3 のβ5cDNA 配列、pcDNA3-3×FLAG 由来の３×FLAG 配列およびポリ A 付加シグナル配列、そしてポジティブ選別のために MC1 プロモーターのネオマイシン耐性遺伝子(Neo)を、lox 配列に挟んだ配列をサブクローニングした。３'側 lox 配列と３'側相同部位との間には exon2, exon3 のβ5cDNA 配列にそれぞれ任意の変異を導入したもの、pcDNA3-3×HA 由来の３×HA 配列およびポリ A 付加シグナル配列をつなげた配列をサブクローニングした。さらにこれらの配列を、ERP 配列で挟み、その３'相同部位との間に exon2, exon3 のβ５cDNA 配列、pcDNA3-Venus 由来の Venus 配列およびポリ A 付加シグナル配列をつなげた配列をサブクローニングした。非相同組み替えに対するネガティブ選別のために 1.0kb 断片のジフテリア毒素遺伝子(MC1DT-A)を 3'相同部位の下流にサブクローニングした。

４）遺伝子ターゲティング：ターゲティングベクターを SalI により線状化した後、TT2 ES 細胞に GENE PULSERII を用いて 210V, 950uF にてエレクトロポレーション法による遺伝子導入を行った。ES 細胞はエレクトロポレーション48時間後から200ug/ml G418 により、６－８日間選別した。ネオマイシン耐性クローンは PCR 法により遺伝子型同定を行った。この PCR 法により、5.1kb 断片の増幅のみが見られるものを野生型アリル、5.1kb, 3.5kb 両方の断片の増幅が見られるものをノックインアリルとして相同組み替え ES 細胞を同定した。PCR は LA-Taq を用いて行った。

５）ペプチダーゼ活性の測定：100mM Tris-HCl pH8.0 の反応バッファーに終濃度 1mM の基質を加え、これに 0.1mg/ml のサンプルもしくは各グリセロールフラクションから１０ul を混合し、３７℃で４０分経過後、Twinkle LB にて測定した。ペプチダーゼ活性は 1nmol の蛍光基質を１分で分解する活性を１単位とした。Chymotrypsin 様活性（Sue-LLVY-AMC）、Trypsin 様活性（Boc-LRR-AMC)、Caspase 様活性（Cbz-LLE-AMC）を活性測定に供した。

（倫理面への配慮）

本年度の研究は、主として培養細胞およびマウスを用いた基礎的研究およびリコンビナント蛋白質を用いた生化学的研究である。従って、これらの実験の実施には、倫理面への配慮は不要であった。

C. D. 研究結果と考察

「プロジェクトＩ：SCF^{Fbs1} リガーゼの分子構造と作用機構の解明」

分泌蛋白質や膜蛋白質などの分泌系蛋白質は粗面小胞体上のリボソームで合成される。これらは翻訳と共役して小胞体膜上の膜透過装置のチャネルを通って内腔側へ送り込まれる。小胞体内腔もしくは小胞体膜に組み込まれた新生蛋白質は小胞体内の分子シャペロンと呼ばれる蛋白質群の助けを借りて、フォールディングやアセンブリーなどの高次構造形成が行われる。そこで正しい高次構造を獲得した蛋白質だけが、輸送小胞によりゴルジ体以降のコンパートメントに輸送される。

細胞外に異常蛋白質が蓄積したり分泌されたりすると生体にとって有害になるので、分泌経路の入り口である小胞体には、高次構造形成に失敗した蛋白質を選別し、再生・破壊するための様々な機構が兼ね備えられている。このような異常蛋白質を送り出さない仕組みは「小胞体の品質管理」と呼ばれている。小胞体には沢山の分子シ

ャペロンが存在し高次構造形成に最適な条件を作り出しているにも関わらず、新生蛋白質の約30%以上は高次構造形成に失敗し、細胞内で分解を受けており、この分解機構は ERAD（小胞体関連蛋白質分解）と総称されている。

ERAD の基本経路は、小胞体内で ERAD の基質となる蛋白質の識別、小胞体から細胞質への逆行輸送、細胞質におけるユビキチン化とプロテアソームによる分解の３つのステップよりなる。その中で、分泌系蛋白質の多くは N-結合型糖鎖修飾を受けた糖蛋白質であるが、糖鎖が異常蛋白質の識別・ERAD へのターゲティング・分解の一連のタグとして機能していることが明確となってきている。近年われわれは、糖鎖を識別するユビキチンリガーゼとして“SCF^{Fbs1}”を発見し、この酵素が ERAD に関与していることを突き止めた（１）。

即ち、われわれは N 結合型糖蛋白質が結合する細胞内レクチンを探索する目的で、高マンノース糖鎖をもったフェチュインをリガンドとした親和性クロマトグラフィーを作製してウシ脳抽出液を解析した結果、Fbs1（別称 Fbx2/Fbg1）の分離に成功した。Fbs1 は F-box ファミリー蛋白質の一つであり、SCF 複合体 Skp1-Cullin1-F-box 蛋白質(略記：F-box)-Roc1 型ユビキチンリガーゼの標的識別サブユニットであった。SCF 型ユビキチンリガーゼは Skp1-Cullin1-F-box-Rbx1/Roc1 から構成された４分子複合体であり、標的識別サブユニットである F-box を変換することによって多様性を確保したユニークな蛋白質識別機構を持ったユビキチンリガーゼである。

試験管内再構成系を用いた解析から、本 SCF^{Fbs1} 複合体が N 型糖鎖依存的に糖蛋白質をポリユビキチン化するユビキチンリガーゼであることが判明した(1)。Fbs1 の発現は成体脳、それもニューロン特異的である。一方、最近、Fbs には少なくとも５種類のアイソフォームが存在し、これらが遺伝子ファミリーを形成していることを見出した。そして種々の組織にユビキタスに発現している Fbs2/Fbg2 が、SCF^{Fbs1} と同様に、SCF^{Fbs2} 複合体を形成、ERAD に作用するユビキチンリガーゼであることを見出した (2)。

更にわれわれは、Fbs1 単独分子及び Fbs1 とキトビオースとの複合体の X 線結晶構造解析による立体構造解析に成功し、Fbs1による糖鎖識別機構を原子レベルで解明した（3)。Fbs1 の糖鎖結合ドメイン(SBD)の立体構造は 10 本の逆平行 β 構造が二層に重なったβサンドイッチ構造をしており、その一端に位置するループ領域により糖蛋白質では糖鎖の還元末端に位置するキトビオース(GlcNAcβ1-4GlcNAc)を認識し結合している。レクチンの立体構造としてβサンドイッチ構造は一般的な構造であるが、これまでに立体構造の報告されたレクチンの糖鎖認識部位はβシート領域であったのに対し SBD ではループ領域で糖鎖と結合する新しい様式をとっていた。

標的となる糖蛋白質において修飾された糖鎖の根元部分は、通常自身のペプチド部分と相互作用しているためFbs1との結合は困難であると考えられる。しかしFbs1の標的となる糖蛋白質はERADにおいて細胞質に輸送された高次構造の崩れた蛋白質であることから、根本のキトビオース部分も溶媒に露出していると考えられるためにFbs1と相互作用が可能となると推定された。このことは同じN結合型糖蛋白質でも変性させた方が、Fbs1と高い親和性を示すという実験事実とも一致しており（４)、これらのことからFbs1が分子の先端で糖鎖と特異的に結合することは、アンフォールド状態にある糖蛋白質と不必要な相互作用することなく、ユビキチンを付加するために合理化された機構であると考えられる。また本年、Fbs1-Skp1の二量体のX線結晶構造解析にも成功、SCF^{Fbs1}全体の高次構造のモデル化にも成功した。さらに基質であるRNaseと結合したSCF^{Fbs1}全体の構造解析にも成功した（論文投稿準備中)。この結果、SCF^{Fbs1}のユビキチンリガーゼとしての作用機構が分子レベルで判明した。

ERADはすべての細胞・臓器で普遍的に見られる現象であることから、Fbs1がニューロンに特異的に発現していることの理由は、これまで推測の域を出ず大きな謎であった。当初われわれはニューロンのような非分裂細胞では、蛋白質の品質管理機構を厳格に維持することが細胞の生存戦略として極めて重要であり、これらの細胞では、共通のFbs2に加えてFbs1を特異的に発現させ、神経細胞における品質管理を強化するための進化的に獲得したと考えていた。こ

の仮説を検証するために、Fbs1やFbs2を含むその他のファミリー分子群が正確にSCF複合体を形成しているか否かを検証した。その結果、Fbs2を含む他のユビキタスな分子群はほとんど全てが、Cullin1と結合してSCF^{Fbs2}などの４分子複合体を形成していたが、Fbs1の大部分はCullin1と結合していなかった。しかし、小胞体膜結合型のFbs1はCullin1と結合しSCF^{Fbs1}の複合体として存在し、ERADに関与していることが示唆された。そこで、遊離のFbs1の役割を解析した結果、変性・凝集したClient N型糖蛋白質をフォールディングして正常な立体構造を持った分子に再生させる分子シャペロン作用をもつことが判明した。この結果、Fbs1はニューロンでは、Cullin1と結合しSCF^{Fbs1}の複合体を形成してERADに関与するのが主目的でなく、寧ろその大部分は、糖蛋白質に特異的な分子シャペロンとして細胞質で働いていることが判明した。小胞体内腔においてはカルネキシンなど糖蛋白質に対するシャペロン分子は存在するが、細胞質では、Fbs1が初めてである。なぜニューロンにおいてこのような特殊なシャペロン分子を造成したかは、大きな謎であるが、その解明は、運動ニューロン疾患を含む神経変性疾患の発症機構解明に大きなヒントを与えると思われる。

「プロジェクト II：変異導入によるプロテアソームの活性低下モデルの作製」

Chymotrypsin 様活性の低下した酵母の変異体作製：マウスをもちいた実験を行う前に、導入しようとしている変異が適切であるかどうかを、遺伝学的解析が容易である出芽酵母を用いて検討を行った。内在性プロモーターで発現する活性変異型β5 発現ベクターを導入した株に対して、野生型β5 の遺伝子を欠失させ、20S プロテアソームが完全に活性変異型β5 サブユニットに置き換わる株をβ5(A20T), β5(M45K), β5(M45R)について樹立した。

樹立した酵母を用いて、プロテアソームの活性をCaspase, Trypsin, Chymotrypsin 様活性の三種について測定したところ、野生株に比べてそれぞれ 30%, 40%, 70%の Chymotrypsin 様活性の低下を認めたが、Caspase, Trypsin 様活性は変化していなかった。これらの株は通常の生育条件(YPD 培地、26℃)では、野生株と同等の生育速度を示した。アミノ酸アナログであるCanavanineにおいても、生育に大きな影響はなく、M45R（最も Chymotrypsin 様活性が低下している株）のみがごく弱い感受性をしめした。

次に酵母に導入したものと同じ変異を哺乳類細胞に起こさせるベクターを作製し、293T 細胞にトランスフェクションした。細胞から蛋白質を抽出後、沈降速度解析にかけ、グリセロール濃度勾配によってサンプルをフラクションに分けた。Suc-LLVY-AMC を用いた活性測定の結果から、哺乳類細胞においても酵母と同様の変異を持たせることで Chymotrypsin 様の活性が低下することが確認できた。また、ウェスタンブロット法の結果より、活性測定の結果から 20S プロテアソーム、２６S プロテアソーム画分であると予想されたフラクションに、過剰発現させたβサブユニットのバンドが現れ、プロテアソームの複合体に正常に組み込まれていることが確認できた。

ターゲティングベクターをエレクトロポレーション法により ES 細胞に導入した。この細胞を、G418(GIBCO)を添加した ES 培地で遺伝子が導入された細胞を選択した。さらに PCR 法により 5.1kb 断片の増幅のみが見られるものを野生型アリル、5.1kb, 3.5kb 両方の断片の増幅が見られるものをノックインアリルとして相同組換えES細胞を同定した。

樹立した変異導入型 ES 細胞から蛋白質を抽出し、FLAG 抗体を用いてウェスタンブロッティングを行ったところ、野生型 ES 細胞には見られないバンドが検出され、導入したβ５が発現していることが確認できた。さらに、これらのサンプルを沈降速度解析にかけ、グリセロール濃度勾配によってわけられた画分をウエスタンブロット法で解析した。FLAG 抗体で見ると、野生型の ES 細胞ではバンドが現れないのに対し、単離された相同組換え ES 細胞ではバンドが検出された。β５抗体で見ると、野生型 ES 細胞ではバンドが一本であるのに対して、相同組み替えES細胞では、本来のβ５と一緒に、３×FLAG の分だけシフトしたバンドが確認できた。この結果、変異導入型サブユニットが正しくプロテアソームに組み込まれていることが判明した。現在、この変異導入型 ES 細胞をマウス 8 細胞期胚にマイクロイ

ンジェクションし、翌日仮親の子宮に移植し、ヘテロマウスの作製を進めている。ヘテロマウスがジャームラインに入っていることを確かめ、交配により導入型サブユニットを持った条件的ノックインマウスを作製する計画である。

E. 結論

「プロジェクトI」

ERAD に関与する糖鎖識別ユビキチンリガーゼとしてニューロン特異的な SCF^{Fbs1} とユビキタスに発現している普遍的 SCF^{Fbs2} を発見した。そして、キトビオース（蛋白質の Asp 残基に結合する GlcNAc-GlcNAc 糖）や RNase が結合した SCF^{Fbs1} の X 線結晶解析による立体構造解析に成功し、原子レベルで標的（糖蛋白質）の識別機構を解明した。さらにニューロン特異的な Fbs1 が SCF^{Fbs1} リガーゼとしての役割以外に糖蛋白質のための分子シャペロンとして機能していることを初めて見出した。この成果は、運動ニューロンの変性機構を解明するための有益な情報を得ることが出来ると期待できる。

「プロジェクトII」

本研究では変異導入によるプロテアソームの活性低下モデルの作製に取り組んだ。立体構造から予測した触媒活性部位近辺のアミノ酸を構造の異なる種々のアミノ酸に置換することにより、プロテアソームの Caspase 様活性および trypsin 様活性には全く影響せず、最も重要な Chymotrypsin 様活性のみを選択的に 25-50%程度に低下させた変異型プロテアソームを出芽酵母で作製することに成功した。現在、この変異を導入した条件的ノックインマウスを作製中である。すでに変異導入型 ES 細胞の取得に成功、現在、ヘテロマウスの作製に取り組んでいる。このプロジェクトは、プロテアソームがなぜ３種の異なった活性を持っているのかという基本的な命題に答えることができるのみならずプロテアソームの機能低下が神経変性疾患の発症にどうのように関係するかについての個体レベルでの解析が初めて可能になる。

F. 健康危険情報

無し。

G. 研究発表

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H. 知的財産権の出願・登録状況

1. 特許取得

無し

2. 実用新案登録

無し

3. その他

無し

Ⅲ. 研究成果の刊行に関する一覧

研究成果の刊行に関する一覧表

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発表者名	論文タイトル名	発表誌名	巻・号	ページ	出版年
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Experimental Neurology xx (2006) xxx–xxx

Experimental Neurology

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Review

Pathogenesis, animal models and therapeutics in Spinal and bulbar muscular atrophy (SBMA)

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Received 12 December 2005; revised 18 January 2006; accepted 20 January 2006

Abstract

Spinal and bulbar muscular atrophy (SBMA) is a hereditary neurodegenerative disease characterized by slowly progressive muscle weakness and atrophy of bulbar, facial, and limb muscles. The cause of SBMA is expansion of a trinucleotide CAG repeat, which encodes the polyglutamine tract, in the first exon of the androgen receptor (AR) gene. SBMA chiefly occurs in adult males, whereas neurological symptoms are rarely detected in females having mutant AR gene. The cardinal histopathological finding of SBMA is loss of lower motor neurons in the anterior horn of spinal cord as well as in brainstem motor nuclei. Animal models carrying human mutant AR gene recapitulate polyglutamine-mediated motor neuron degeneration, providing clues to the pathogenesis of SBMA. There is increasing evidence that testosterone, the ligand of AR, plays a pivotal role in the pathogenesis of neurodegeneration in SBMA. The striking success of androgen deprivation therapy in SBMA mouse models has been translated into clinical trials. In addition, elucidation of pathophysiology using animal models leads to emergence of candidate drugs to treat this devastating disease: HSP inducer, Hsp90 inhibitor, and histone deacetylase inhibitor. Utilizing biomarkers such as scrotal skin biopsy would improve efficacy of clinical trials to verify the results from animal studies. Advances in basic and clinical researches on SBMA are now paving the way for clinical application of potential therapeutics.

Keywords: Spinal and bulbar muscular atrophy; Polyglutamine; Androgen receptor; Testosterone; Luteinizing hormone-releasing hormone analog; Heat shock protein; Geranylgeranylacetone; 17-Allylamino geldanamycin; Histone deacetylase inhibitor; Axonal transport

Contents

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0014-4886/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2006.01.021

History and nomenclature

More than a hundred years have elapsed since the first description of spinal and bulbar muscular atrophy (SBMA) from Hiroshi Kawahara, who described the clinical and hereditary characteristics of two Japanese brothers with progressive bulbar palsy (Kawahara, 1897). This work was followed by several reports on similar cases with or without X-linked pattern of inheritance (Katsuno et al., 2004). SBMA is also known as Kennedy disease (KD), named after William R. Kennedy, whose study on 11 patients from 2 families depicted the clinical, genetical, and pathological features of this disorder (Kennedy et al., 1968). Other names for this disease are bulbospinal neuronopathy and bulbospinal muscular atrophy.

In 1991, the cause of SBMA was identified as the expansion of a trinucleotide CAG repeat in the androgen receptor (AR) gene (La Spada et al., 1991). This was the first discovery of polyglutamine-mediated neurodegenerative diseases, and subsequent studies using transgenic animal models opened the door to development of pathogenesis-based therapies for this devastating disease.

Clinical features

SBMA exclusively affects adult males. The prevalence of this disease is estimated to be 1–2 per 100,000, whereas a considerable number of patients may have been misdiagnosed as other neuromuscular diseases including amyotrophic lateral sclerosis (Fischbeck, 1997). Patients of various ethnic backgrounds have been reported around the world.

Major symptoms of SBMA are weakness, atrophy, and fasciculations of bulbar, facial and limb muscles (Sperfeld et al., 2002; Katsuno et al., 2004). In extremities, involvement is usually predominant in proximal musculature. The onset of weakness is usually between 30 and 60 years but is often preceded by nonspecific symptoms such as postural tremor and muscle cramps. Although fasciculations in the extremities are rarely present at rest, they are easily induced when patients hold their arms horizontally or bend their legs while lying on their backs. These contraction fasciculations are especially noticeable in the face, neck, and tongue and are usually present in the early stage. Fatigability after exercise might also be accompanied. Bilateral facial and masseter muscle weakness, poor uvula and soft palatal movements, and atrophy of the tongue with fasciculations are often encountered. Speech has a nasal quality in most cases due to reduced velopharyngeal closure. Advanced cases often develop dysphagia, eventually resulting in aspiration or choking. Muscle tone is usually hypotonic, and no pyramidal signs are detected. Deep tendon reflex is diminished or absent with no pathological reflex. Sensory involvement is largely restricted to vibration sense which is affected distally in the legs. Cerebellar symptoms, dysautonomia, and cognitive impairment are absent. Patients occasionally demonstrate signs of androgen insensitivity such as gynecomastia, testicular atrophy, dyserection, and decreased fertility, some of which are detected before the onset of motor symptoms. Abdominal obesity is common, whereas male pattern baldness is rare in patients with SBMA.

Electromyogram shows neurogenic abnormalities, and distal motor latencies are often prolonged in nerve conduction study. Both sensory nerve action potential and sensory evoked potential are reduced or absent. Endocrinological examinations frequently reveal partial androgen resistance with elevated serum testosterone level. Serum creatine kinase level is elevated in the majority of patients. Hyperlipidemia, liver dysfunction, and glucose intolerance are also detected in some patients. Profound facial fasciculations, bulbar signs, gynecomastia, and sensory disturbance are the main clinical features distinguishing SBMA from other motor neuron diseases, although gene analysis is indispensable for diagnosis. Female patients are usually asymptomatic, but some express subclinical phenotypes including high amplitude motor unit potentials on electromyography (Sobue et al., 1993).

The progression of SBMA is usually slow, but a considerable number of patients need assistance to walk in their fifties or sixties. Life-threatening respiratory tract infection often occurs in the advanced stage of the disease, resulting in early death in some patients. No specific therapy for SBMA has been established. Testosterone has been used in some patients, although it has no effects on the progression of SBMA.

Etiology

The cause of SBMA is expansion of a trinucleotide CAG repeat, which encodes the polyglutamine tract, in the first exon of the androgen receptor (AR) gene (La Spada et al., 1991). The CAG repeat within AR ranges in size from 9 to 36 in normal subjects but from 38 to 62 in SBMA patients. Expanded polyglutamine tracts have been found to cause several neurodegenerative diseases including SBMA, Huntington's disease, several forms of spinocerebellar ataxia, and dentatorubral-pallidoluysian atrophy (Gatchel and Zoghbi, 2005). These disorders, known as polyglutamine diseases, share salient clinical features including anticipation and somatic mosaicism, as well as selective neuronal and nonneuronal involvement despite widespread expression of the mutant gene. 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 in SBMA as documented in other polyglutamine diseases (Doyu et al., 1992). These observations explicitly suggest that common mechanisms underlie the pathogenesis of polyglutamine diseases.

AR, the causative protein of SBMA, is an 110-kDa nuclear receptor which belongs to the steroid/thyroid hormone receptor family (Poletti, 2004). AR mediates the effects of androgens, testosterone, and dihydrotestosterone, through binding to an androgen response element in the target gene to regulate its expression. AR is essential for major androgen effects including normal male sexual differentiation and pubertal sexual development, although AR-independent nongenomic function of androgen has been reported. AR is expressed not only in primary and secondary sexual organs but also in nonreproductive organs including the kidney, skeletal muscle, adrenal gland, skin, and nervous system, suggesting its far-reaching influence on a variety of mammalian tissues. In the central nervous system, the expression level of AR is relatively high in spinal and brainstem

motor neurons, the same cells which are vulnerable in SBMA. The AR gene is located on chromosome Xq11–12. This 90-kb DNA contains eight exons coding for the functional domains specific to the nuclear receptor family. The first exon codes for the N-terminal transactivating domain. Exons 2 and 3 code for the DNA-binding domain, whereas exons 4 through 8 code for the ligand-binding domain. The N-terminal transactivating domain, in which a CAG trinucleotide repeat locates, possesses a major transactivation function maintained by interaction with general transcriptional coactivators such as c-AMP response element binding protein-binding protein (CBP), TAFII130, and steroid receptor coactivator-1 (SRC-1). The CAG repeat beginning at codon 58 in the first exon of AR encodes polyglutamine tract. The length of this repeat is highly variable because of the slippage of DNA polymerase upon DNA replication. Whereas its abnormal elongation causes SBMA, the shorter CAG repeat is likely to increase the risk of prostate cancer (Clark et al., 2003). Transcriptional coactivators also possess glutamine-rich regions modulating protein–protein interaction with the N-terminal transactivating domain of AR.

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 possesses 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, probably because it disrupts interaction between the N-terminal transactivating domain of AR and transcriptional coactivators (Poletti, 2004). Although this loss of function of AR may contribute to the androgen insensitivity in 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 (Tfm) patients lacking AR function or in AR knockout mice. 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 (Adachi et al., 2001).

Aggregation of abnormal protein has been considered to be central to the pathogenesis of neurodegenerative diseases such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and prion disease. An expanded polyglutamine stretch alters conformation of causative proteins, resulting in aggregation of the proteins. It is now widely accepted that aggregation of these abnormal proteins in neurons is the primary 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 form after a repeat length-dependent conformational change of polyglutamine monomer from a random coil to a parallel, helical β-sheet (Wyttenbach, 2004). 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 (Muchowski and Wacker, 2005). This hypothesis is likely to be the case in SBMA.

Pathology

Histopathological studies provide important information on the pathogenesis of polyglutamine-mediated neurodegeneration. The fundamental histopathological finding of SBMA is loss of lower motor neurons in the anterior horn of spinal cord as well as in brainstem motor nuclei except for the third, fourth and sixth cranial nerves (Fig. 1A) (Sobue et al., 1989). The

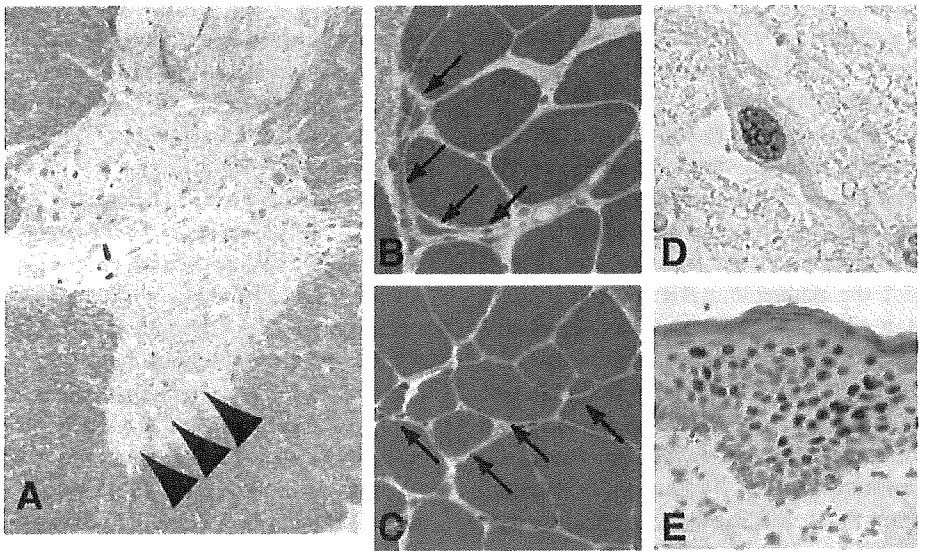

Fig. 1. Histopathology of SBMA. (A) A transverse section of spinal cord demonstrates marked depletion of motor neurons in the anterior horn. (B and C) HE staining of skeletal muscle shows both neurogenic (B, arrows) and myogenic changes (C, arrows). (D) A residual motor neuron in the lumbar anterior horn shows a diffuse nuclear accumulation of pathogenic androgen receptor detected by anti-polyglutamine antibody. (E) Nuclear accumulation of pathogenic AR is also detected in nonneuronal tissues such as scrotal skin (E).

number of nerve fibers is reduced in the ventral spinal nerve root, reflecting motor neuronopathy. Sensory neurons in the dorsal root ganglia were less severely affected, and large myelinated fibers demonstrate a distally accentuated sensory axonopathy in the peripheral nervous system. Neurons in the Onufrowicz nuclei, intermediolateral columns, and Clarke's columns of the spinal cord are generally well preserved. 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 (Figs. 1B and C).

A pathologic hallmark of polyglutamine diseases is the presence of nuclear inclusions (NIs). In SBMA, NIs containing the pathogenic AR are found in the residual motor neurons in the brainstem and spinal cord as well as in nonneuronal tissues including prostate, testis, and skin (Li et al., 1998). 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 AR is truncated or masked upon formation of NI. A full-length AR protein with expanded polyglutamine tract is cleaved by caspase-3, liberating a polyglutamine-containing toxic fragment, and the susceptibility to cleavage is polyglutamine repeat length-dependent (Kobayashi et al., 1998). 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 nonneural inclusions, in contrast to the other polyglutamine diseases in which NIs take the form of filamentous structures. Although NI is a disease-specific histopathological finding, its role in pathogenesis has been heavily debated. Several studies have suggested that NI may indicate cellular response coping with the toxicity of abnormal polyglutamine protein (Arrasate et al., 2004). Instead, the diffuse nuclear accumulation of the mutant protein has been considered essential for inducing neurodegeneration in polyglutamine diseases including SBMA.

An immunohistochemical study on autopsied SBMA patients using an anti-polyglutamine antibody demonstrates that diffuse nuclear accumulation of the pathogenic AR is more frequently observed than NIs in the anterior horn of spinal cord (Adachi et al., 2005). 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. Similar findings have also been reported on other polyglutamine diseases. 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 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 (Gatchel and Zoghbi, 2005).

Since 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 nonneuronal tissues such as scrotal skin (Figs. 1D and E). 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 (Banno et al., in press). These findings indicate that scrotal skin biopsy with anti-polyglutamine 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.

Molecular pathogenesis and therapeutic strategies

Ligand-dependent pathogenesis in animal models of SBMA

SBMA is unique 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. AR is normally confined to a multiheteromeric 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 important roles 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 enhancer and a chicken β-actin promoter (Katsuno et al., 2002). This model recapitulated not only the neurologic disorder but also the phenotypic difference with gender which is a specific feature of SBMA. The mice with 97CAGs (AR-97Q) exhibited progressive motor impairment, although those with 24 CAGs did not show any manifested phenotypes. Affected AR-97Q mice demonstrated small body size, short life span, progressive muscle atrophy, and weakness as well as reduced cage activity, all of which were 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 onset of motor impairment detected by the rotarod task was at 8 to 9 weeks of age in the male AR-97Q mice while 16 weeks or more in the females. 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 to 30% at more than 210 days. 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. The nuclear fraction contained the most of smearing pathogenic AR protein. Diffuse nuclear staining and less frequent NIs detected by 1C2, an antibody specifically recognizing the expanded polyglutamine tract, were demonstrated in the neurons of spinal cord, cerebrum, cerebellum, brainstem, and dorsal root ganglia as well as in nonneuronal tissues such as heart, muscle, and

pancreas. Male AR-97Q mice showed markedly more abundant diffuse nuclear staining and NIs than females, in agreement with the symptomatic and Western blot profile differences with gender. Despite the profound sexual difference of the pathogenic AR protein expression, there was no significant difference in the expression of the transgene mRNA between the male and female AR-97Q mice. 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.

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 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. The nuclear accumulation of the pathogenic AR protein with an expanded polyglutamine tract is likely essential in inducing neuronal cell dysfunction and degeneration in the majority of polyglutamine diseases. It thus appears logical that reduction in testosterone level improves phenotypic expression by preventing nuclear localization of the pathogenic AR. In support of this hypothesis, the ligand-dependent neurodegeneration has also been revealed in a fruit fly model of SBMA (Takeyama et al., 2002). Alternatively, castration may enhance protective effects of molecular chaperones, which are normally associated with AR and dissociate upon ligand binding.

Testosterone blockade therapy for SBMA

Successful treatment of AR-97Q mice with castration inspired us testosterone blockade therapies using leuprorelin and flutamide (Katsuno et al., 2003). Leuprorelin is a potent luteinizing hormone-releasing hormone (LHRH) analog suppressing the releases of gonadotrophins, luteinizing hormone and follicle-stimulating hormone. This drug has been used for a variety of sex hormone-dependent diseases including prostate cancer, endometriosis, and prepuberty. The primary pharmacological target of leuprorelin is the anterior pituitary. Through its agonizing effect on LHRH-releasing cells, it initially promotes the releases of gonadotrophins, resulting in transient increase in the serum level of testosterone or estrogens. After this surge, the continued use of this drug induces desensitization of the pituitary by reducing LHRH receptor binding sites and/or uncoupling of receptors from intracellular processes. Within about 2 to 4 weeks of leuprorelin administration, human serum testosterone level decreases to the extent achieved by surgical castration. The effects are maintained during the treatment, suggesting that continuous administration of leuprorelin is required for its clinical use. This drug thus has been provided as sustained release depot taking the form of polymer microspheres. On the other hand, 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 analog, 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.

Leuprorelin successfully inhibited nuclear accumulation of the pathogenic AR, resulting in marked amelioration of neuromuscular phenotypes seen in the male AR-97Q mice (Fig. 2). Leuprorelin initially increased the serum testosterone level by agonizing the LHRH receptor but subsequently reduced it to undetectable levels. Androgen blockade effects were also confirmed by reduced weights of the prostate and seminal vesicle. The leuprorelin-treated AR-97Q mice showed longer lifespan, larger body size, and better motor performance compared with vehicle-treated mice. Although leuprorelin-induced infertility was prevented by dose reduction, the therapeutic effects on neuromuscular phenotypes were insufficient at a lower dose of leuprorelin. In the Western blot analysis and anti-polyglutamine immunohistochemistry, the leuprorelin-treated male AR-97Q mice demonstrated a markedly diminished amount of the pathogenic AR in the nucleus, suggesting that leuprorelin successfully reduced nuclear AR accumulation. Testosterone, which was given from 13 weeks of age, markedly aggravated neurological symptoms and pathologic findings of leuprorelin-treated male AR-97Q mice. Leuprorelin appears to improve neuronal dysfunction by preventing ligand-dependent nuclear translocation of the pathogenic AR in the same way as castration. Given its minimal invasiveness and established safety, leuprorelin appears to be a promising therapeutic agent for SBMA. In a preliminary open trial, 6-month treatment with leuprorelin significantly diminished nuclear accumulation of pathogenic AR in the scrotal skin of patients, suggesting that androgen deprivation intervenes in the pathogenic process of human SBMA, as demonstrated in animal studies (Banno et al., in press). Another trial on a larger scale is currently underway to verify clinical benefits of leuprorelin for SBMA patients.

Leuprorelin-treated AR-97Q mice showed deterioration of body weight and rotarod task at the age of 8–9 weeks, when serum testosterone initially increased through the agonistic effect of leuprorelin. This change was transient and followed by sustained amelioration along with consequent suppression of testosterone production. The foot print analysis also revealed temporary exacerbation of motor impairment. Immunostaining of tail specimen, sampled from the same individual mouse, demonstrated an increase in the number of the muscle fibers with nuclear 1C2 staining at 4 weeks of leuprorelin administration,

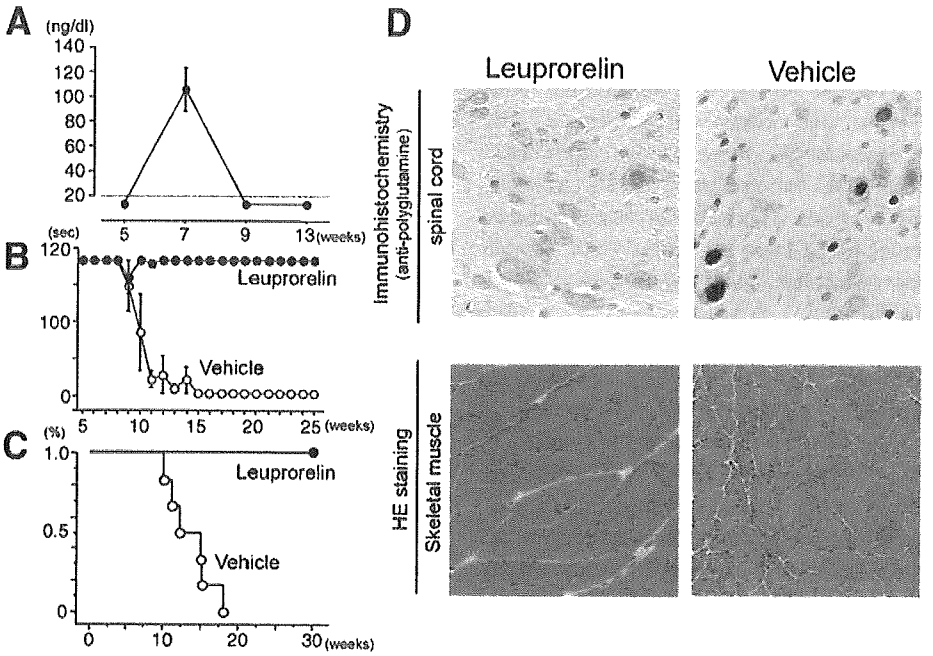

Fig. 2. Effects of leuprorelin on mutant androgen receptor (AR) expression and neuropathology of male AR-97Q mice. (A) Serum testosterone level in AR-97Q mice. Leuprorelin initially increased serum testosterone level but subsequently reduced it to undetectable levels. (B and C) Rotarod task (B) and survival rate (C) of the AR-97Q mice. Leuprorelin markedly improved motor function of the mice at the dose. (D) Immunohistochemistry using 1C2 showed marked differences in diffuse nuclear staining and nuclear inclusions between the leuprorelin-treated and vehicle-treated AR-97Q male mice in the spinal anterior horn. HE staining of the muscle in the vehicle-treated male mouse revealed apparent grouped atrophy and small angulated fibers, which were not seen in the leuprorelin-treated mice.

although this 1C2 staining was diminished by another 4 weeks of treatment. Our results indicate that preventing nuclear translocation of the pathogenic AR is enough to reverse both symptomatic and pathologic phenotypes in our AR-97Q mice. In support with these observations, testosterone deprivation by means of castration reverses motor dysfunction in another transgenic mouse model of SBMA showing fairly slow progression (Chevalier-Larsen et al., 2004). Since the pathophysiology of AR-97Q mice is neuronal dysfunction without neuronal cell loss, our results indicate that the pathogenesis of polyglutamine diseases is reversible at least in its initial stage. From therapeutic point of view, it is of importance to determine the early dysfunctional period in the natural history of human SBMA.

By contrast, flutamide, AR antagonist, did not ameliorate symptoms, pathologic features, or nuclear localization of the pathogenic AR in the male AR-97Q mice, although there was no significant difference in the androgen blockade effects between flutamide and leuprorelin. Flutamide does not inhibit, but may even facilitate, the nuclear translocation of AR. In *consistency* with mouse study, this AR antagonist also promotes nuclear translocation of the pathogenic AR containing an expanded polyglutamine in cellular and fly models of SBMA (Takeyama et al., 2002; Walcott and Merry, 2002). This appears to be the reason why flutamide demonstrated no therapeutic effect in our transgenic mouse model of SBMA. Flutamide is not likely to be a proper therapeutic agent for SBMA.

The castrated or leuprorelin-treated AR-97Q mice showed phenotypes similar to those seen in the female AR-97Q mice, implying that motor impairment of SBMA patients can be reduced to the level in females. SBMA has been considered an X-linked disease, whereas other polyglutamine diseases show autosomal dominant inheritance. In fact, SBMA female patients hardly manifest clinical phenotypes, although they *possess* similar number of a CAG repeat in the disease allele of AR gene as their siblings with SBMA (Sobue et al., 1993; Mariotti et al., 2000). Reduction in the mutant AR expression due to X-inactivation may prevent females from disease manifestation, but hormonal intervention studies using mouse and fly models clearly suggest that low level of testosterone prevents nuclear accumulation of the pathogenic AR protein, resulting in a lack of neurological phenotypes in the females. This view is strongly supported by the observation that manifestation of symptoms is minimal even in homozygous females of SBMA (Schmidt et al., 2002). Therefore, it seems inappropriate to regard SBMA as an X-recessive inherited disease, but rather its neurological phenotype is likely to depend on testosterone concentration.

Role of heat shock proteins in pathogenesis of SBMA

Many components of ubiquitine-proteasome and molecular chaperones are known to colocalize with polyglutamine-containing NIs, implying that failure of cellular defense mechanism underlies neurodegeneration in polyglutamine diseases. Heat shock protein (HSP), a stress-inducible molecular chaperone, is another key to elucidation of the pathogenesis of SBMA. HSPs are classified into different families according to molecular size: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small HSPs (Macario and Conway de Macario, 2005). These HSPs are either constitutively expressed or inducibly synthesized after cellular stress. HSPs play a crucial role in maintaining correct folding, assembly, and intracellular transport of proteins.

For example, Hsp70 and Hsp90, essential components of AR-chaperone complex in the cell cytoplasm, regulate function, nuclear translocation, and degradation of AR (Heinlein and Chang, 2001). Under toxic conditions, HSP synthesis is rapidly upregulated, and nonnative proteins are refolded as a consequence. Therefore, HSPs have attracted a great deal attention as cytoprotective agents against detrimental conditions including ischemia and malignancy.

Several studies suggest that polyglutamine elongation interferes with the protective cellular responses against cytotoxic stress (Wyttenbach, 2004). Truncated AR with an expanded polyglutamine tract delays the induction of Hsp70 after heat shock (Cowan et al., 2003). The threshold of HSP induction is known to be relatively high in spinal motor neurons (Batulan et al., 2003). Expression levels of HSPs are decreased in the brain lesion of an animal model of HD and in that of the SBMA mouse (Hay et al., 2004; Katsuno et al., 2005). Taken together, impairment of HSP induction capability is implicated in the pathogenesis of motor neuron degeneration in SBMA. Not only are HSPs implicated in the pathogenesis of neurodegeneration, but they are also potent suppressors of polyglutamine toxicity. There is increasing evidence that HSPs abrogate polyglutamine-mediated cytotoxicity by refolding and solubilizing the pathogenic proteins (Wyttenbach, 2004; Muchowski and Wacker, 2005). Hsp70 cooperates with Hsp40 in functioning as a molecular chaperone. These HSPs are proposed to prevent the initial conformation conversion of abnormal polyglutamine-containing protein from a random coil to a β-sheet, leading to attenuation of toxic oligomer formation (Wyttenbach, 2004). 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 (Kobayashi et al., 2000). Hsp70 has also been shown to facilitate proteasomal degradation of abnormal AR protein in a cell culture model of SBMA (Bailey et al., 2002). The favorable effects of Hsp70 have been verified in studies using mouse models of polyglutamine diseases. Overexpression of the inducible form of human Hsp70 markedly ameliorated symptomatic and histopathological phenotypes of our transgenic mouse model of SBMA (Adachi et al., 2003). These beneficial effects are dependent on Hsp70 gene dosage and correlate with the reduction in the amount of nuclear-localized AR protein. It should be noted that the soluble form of the pathogenic AR was also significantly decreased in amount by Hsp70 overexpression, suggesting the degradation of the pathogenic AR may have been accelerated by overexpression of this molecular chaperone.

Favorable effects obtained by genetic modulation of HSP suggest that pharmacological induction of molecular chaperones might be a promising approach to SBMA and other polyglutamine diseases. Geranylgeranylacetone (GGA), an acyclic isoprenoid compound with a retinoid skeleton, has been shown to strongly induce HSP expression in various tissues (Hirakawa et al., 1996). Oral administration of GGA upregulates the levels of Hsp70, Hsp90, and Hsp105 via activation of heat shock factor-1 in the central nervous system and inhibits nuclear accumulation of the pathogenic AR protein, resulting in amelioration of polyglutamine-dependent neuromuscular phenotypes of SBMA transgenic mice (Katsuno et al., 2005). Given its extremely low toxicity, this compound has been used as an oral anti-ulcer drug. Although a high dose appears to be needed for clinical effects, GGA appears to be a safe and promising therapeutic candidate for polyglutamine-mediated neurodegenerative diseases including SBMA.

Inhibition of Hsp90 is also demonstrated to arrest the neurodegeneration in SBMA mouse (Waza et al., 2005). Hsp90 functions in a multi-chaperone complex, assisting proper folding, stabilization, and assembly of so-called client proteins including various oncoproteins and AR (Pratt and Toft, 2003). The Hsp90-client protein complex is stabilized when it is associated with p23, a cochaperone interacting with Hsp90. 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. 17-AAG thereby inhibits nuclear accumulation of this protein, leading to marked amelioration of motor phenotypes of the SBMA mouse model without detectable toxicity (Fig. 3). Of interest is the finding that the pathogenic AR is preferentially targeted to proteasomal degradation in the presence of 17-AAG compared with wild-type AR. Given a high association between p23 and the AR containing expanded polyglutamine, it appears logical that the pathogenic AR is more dependent on Hsp90 to maintain folding and function than wild-type AR and thus is particularly susceptive to Hsp90 inhibition. 17-AAG is also capable of inducing Hsp70 in cellular and mouse models of SBMA. Collectively, 17-AAG, which is now under clinical trials for a wide range of malignancies, would be a good candidate for treatment of SBMA.

Transcriptional dysregulation in SBMA

Disruption of transcriptional machinery has also been hypothesized to underlie the pathogenesis of polyglutamine diseases (Sugars and Rubinsztein, 2003). Gene expression analysis indicates that transcriptional disruption is an early change in the pathogenesis of mouse models of polyglutamine diseases. Transcriptional coactivators such as CBP are sequestrated into the polyglutamine-containing NIs through protein–protein interaction in mouse models and patients with polyQ diseases (Nucifora et al., 2001). Alternatively, the interaction between transcriptional coactivators and soluble pathogenic protein has also been demonstrated in animal models of polyglutamine diseases as well as in postmortem tissues of patients (Steffan et al., 2001). The expression of genes regulated through CBP-mediated transcription is decreased in mouse models of polyglutamine diseases (Sugars and Rubinsztein, 2003). CBP functions as histone acetyltransferase, regulating gene transcription and chromatin structure. It has been indicated that the histone acetyltransferase activity of CBP is suppressed in cellular models of polyglutamine diseases. Taken together, transcriptional dysregulation due to decrease in histone acetylation is likely to underlie the pathogenesis of neurodegeneration in polyglutamine diseases. This hypothesis is exemplified by our experimental

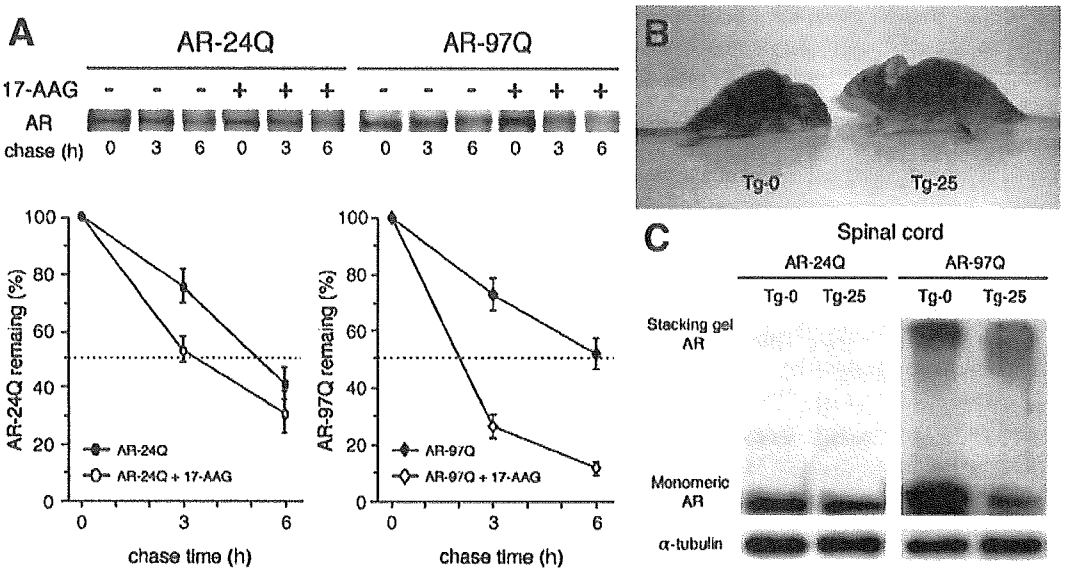

Fig. 3. Effects of 17-AAG on mutant androgen receptor (AR) turnover. (A) Pulse-chase analysis of two forms of AR. The pathogenic AR containing prolonged polyglutamine is degraded more rapidly than the wild-type AR in the presence of 17-AAG. (B) Muscle wasting is obvious in a vehicle-treated SBMA mouse (Tg-0), whereas it is hardly detected in an age-matched SBMA mouse treated with 25 mg/kg of 17-AAG (Tg-25). (C) Western blot analysis of total homogenates from the spinal cord of transgenic mice probed with an anti-AR antibody. 17-AAG decreases the amount of AR in mice bearing the pathogenic AR (AR-97Q), but this effect is only slightly observed in those expressing wild-type AR (AR-24Q).

observation that acetylation of nuclear histone H3 is significantly diminished in SBMA mice (Minamiyama et al., 2004). Additionally, dysfunction of CBP results in a decreased expression of vascular endothelial growth factor in another mouse model of SBMA, indicating the transcriptional alteration is a trigger of neurodegeneration in this disease (Sopher et al., 2004).

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

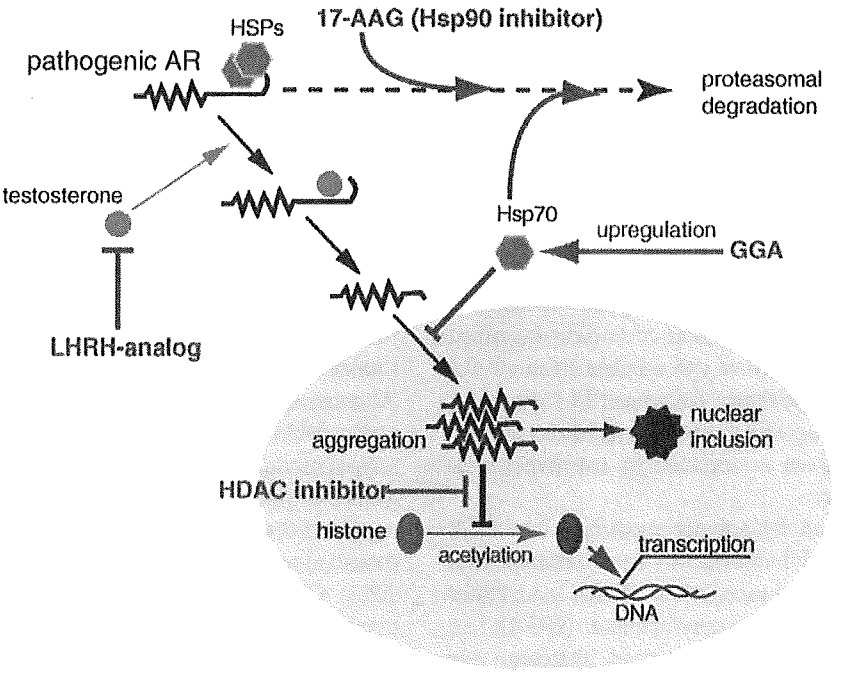

Fig. 4. Pathogenesis-targeting therapeutic approaches to SBMA. In the absence of ligand, the pathogenic AR is confined to a multi-heteromeric inactive complex with heat shock proteins (HSPs) in the cell cytoplasm. Ligand binding facilitates its dissociation from this complex and translocation into the nucleus. In the nucleus, the pathogenic AR forms aggregate and impairs histone acetylation, resulting in transcriptional dysregulation. Several candidates of therapies have been identified on the basis of insights into the molecular mechanisms of the neurodegeneration in SBMA.