<Background of research>

Neurodegenerative diseases are a group of disorders that affect a certain population of neurons, and are characterized by adult-onset and progressive impairment of cognitive and/or motor function. Since the molecular pathogenesis of neurodegenerative diseases is still largely unknown, there is no established cure for most of these devastating disorders. Intra- and extracellular accumulation of abnormal proteins is a common histopathological feature of neurodegenerative diseases, although it is unclear how these deposits cause neuronal dysfunction and eventual cell death.

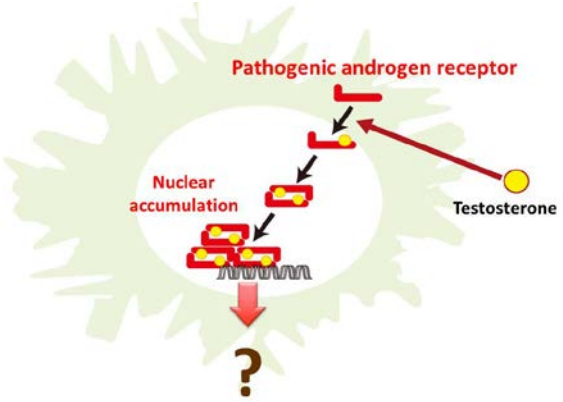

Fig. 1. Putative pathogenesis of SBMA

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a late-onset motor neuron disease characterized by progressive weakness and the atrophy of bulbar, facial, and limb muscles, which is attributable to the degeneration of the lower motor neurons in the spinal cord and brainstem. This disease is caused by an abnormal expansion of the CAG repeat within the gene encoding the androgen receptor (AR), and it exclusively affects males carrying this type of mutation. The pathogenic AR protein accumulates in the nucleus of the motor neurons in a testosterone-dependent manner, and causes several molecular changes, such as transcriptional dysregulation. However, it is unknown which genes are predominantly involved in the pathogenesis of neurodegeneration [Fig. 1].

<Results of research>

We performed a microarray analysis of the spinal cord of a mouse model of SBMA (AR-97Q) to identify the changes in gene expression that play a crucial role in polyglutamine-mediated neurodegeneration, and found that the expression of 124 genes increased (> 1.5-fold) or decreased (> 33%) in the male AR-97Q mice compared with controls at the before-onset stage [Fig. 2]. Among these candidates, we focused on the gene encoding calcitonin gene-related peptide 1 (Cgrp1) for the

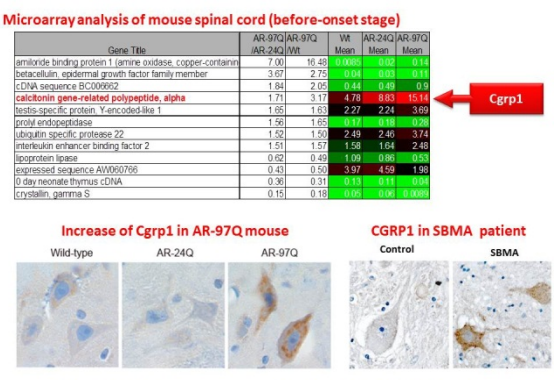

Fig. 2. Microarray analysis of AR-97Q mice

following reasons: i) this gene, *Calca*, was significantly up-regulated in the AR-97Q mice compared with the other types of mice; ii) the expression of this gene showed a further increase in the AR-97Q mice in a stage-dependent manner; and iii) this gene is chiefly expressed in lower motor neurons and DRG sensory neurons, not in glial or vascular endothelial cells. We confirmed the results of the microarray using RT-PCR and immunohistochemistry. Moreover, the immunohistochemical analysis of the autopsied human specimens showed an increased expression of CGRP1 in the spinal motor neurons of SBMA patients. We further investigated the role of CGRP1 in polyglutamine-mediated neurotoxicity in differentiated human neuroblastoma SH-SY5Y cells stably expressing the human AR containing 97 glutamines (AR97Q). The transient over-expression of CGRP1 reduced the viability and increased the cellular damage, while the knock-down of *CALCA* expression using siRNA restored the viability and diminished the damage of the cells stably expressing AR97Q. As for the molecular mechanism by which CGRP1 induces neuronal damage, we clarified that CGRP1 activates the JNK pathway and that pharmacological inhibition of JNK attenuates CGRP1-mediated neurotoxicity.

To clarify the role of CGRP1 in the pathogenic processes of SBMA, we next investigated the biological effects of the depletion of *Calca* in the male AR-97Q mice. The homozygous deletion of the *Calca* gene improved motor function and histopathological signs of motor neuron degeneration via suppression of JNK in the male AR-97Q mice. Given that CGRP1 has been implicated in the molecular pathogenesis of migraine, and 5-HT1B/1D receptor agonists are shown to suppress the expression and secretion of CGRP1 in neurons, we tested whether these anti-migraine drugs could alleviate the polyglutamine-mediated neurotoxicity by decreasing the expression of CGRP1 in neuronal cells. The 5-HT1B/1D receptor agonist naratriptan reduced the cellular damage and restored the viability of SH-SY5Y cells that stably expressed AR97Q. Naratriptan restored the cell viability and suppressed cell death in the SH-SY5Y cells stably expressing AR97Q. The oral administration of naratriptan improved motor function of the male AR97Q mice in a dose-dependent manner. This pharmacological intervention also resulted in an increase in the body weight and lifespan, although it showed no detectable effects on phenotypes of wild-type mice. The histopathological analyses indicated that oral naratriptan ameliorated the neurodegeneration in the AR-97Q mice [Fig. 3].

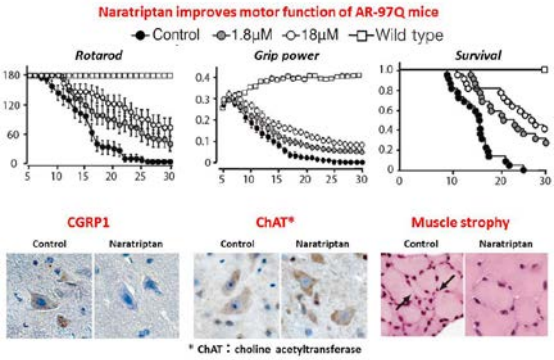

Fig. 3. Effects of naratriptan in AR-97Q mouse

These observations suggest that the pharmacological inhibition of CGRP1-JNK pathway by naratriptan is a novel therapeutic strategy for SBMA and other polyglutamine-related neurodegenerative diseases.

<Future perspectives>

Our approach to identify the critical molecule that mediates polyglutamine-induced neurotoxicity appears to be applicable to other polyglutamine diseases including Huntington's disease and spinocerebellar ataxias. We are now conducting a clinical trial of leuprorelin for SBMA, and once this trial is finished, we are going to plan a phase II clinical trial of naratriptan.

<Acknowledgement>

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<Publication>

Minamiyama M, Katsuno M, Adachi H, Doi H, Kondo N, Iida M, Ishigaki S, Fujioka Y, Matsumoto S, Miyazaki Y, Tanaka F, Kurihara H, Sobue G. Naratriptan mitigates CGRP1-associated motor neuron degeneration caused by expanded polyglutamine. ***Nat Med.*** 18: 1531-1538, 2012. doi: 10.1038/nm.2932.