News Release

Title

An RNA-binding protein MID1 is associated with vulnerability of motor neurons in spinal and bulbar muscular atrophy

Key Points

- **The mechanism for the vulnerability of motor neurons in spinal and bulbar muscular atrophy (SBMA) remains unknown.**
- **MID1, which is specifically expressed in motor neurons, is dysregulated in the spinal cord of SBMA model mice.**
- **MID1 increases the production of pathogenic androgen receptor.**
- **Experiments in mouse spinal cord culture demonstrated that MID1 exacerbates the impairments of axonal elongation in the presence of androgen.**

Summary

The research team led by Prof. Masahisa Katsuno, Department of Neurology in collaboration with Prof. Takaki Miyata, Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), has revealed that MID1, specifically expressed in motor neurons increases pathogenic androgen receptor levels, thereby causing axonal impairments.

Spinal and bulbar muscular atrophy (SBMA) is a hereditary disease that affects motor neurons in the brainstem and spinal cord. The prevalence is around 2 per 100,000 worldwide. SBMA is caused by the expanded trinucleotide CAG repeats in the first exon of *androgen receptor* (*AR*) gene. A CAG repeat is translated into polyglutamine, and AR harboring a polyglutamine expansion exerts cytotoxicity in the presence of androgen. However, the mechanism for “vulnerability”, by which motor neurons are susceptible for damage compared with other types of cells in SBMA, remains unknown. Through comprehensive gene expression analyses, we found that MID1, specifically expressed in motor neurons, promotes the production of pathogenic AR with a polyglutamine expansion. Furthermore, mechanistic analyses using cultured mouse spinal cord slices demonstrated that MID1 exacerbates the impairments of axonal elongation in the presence of androgen.

This study revealed a key mechanism for the vulnerability of motor neurons in SBMA, whereby MID1 specifically expressed in motor neurons causes axonal impairments through the increased production of pathogenic AR protein. Our work was published in the journal *Cell Death & Disease* on July 13, 2022.

Research Background

SBMA is a disease characterized by progressive atrophy of muscles for speech and swallowing, and movements of limbs and trunk. SBMA is an intractable heritable disease designated in Japan and classified as a motor neuron disease, in which motor neurons in the brainstem and spinal cord are impaired and eventually lost by cell death. Although leuprorelin acetate is approved in Japan, its efficacy on neurological symptoms is limited. SBMA is caused by a trinucleotide CAG repeat expansion in the first exon of *androgen receptor* (*AR*) gene. A CAG repeat is translated into polyglutamine and AR harboring a polyglutamine expansion exerts cytotoxicity in the presence of androgen, eventually forming insoluble nuclear aggregates and leading to motor neuron death. In a previous study, we developed transgenic mice harboring human *AR* with an expanded CAG repeat (SBMA model mice). The male mice phenocopy androgen-dependent motor deficits. While AR is expressed ubiquitously, the mechanism for the vulnerability of motor neurons in SBMA is not fully understood. Therefore, we aimed to uncover it using the SBMA model mice.

Research Results

Using microarray data from mouse spinal cords, we first searched for genes that are specifically expressed in spinal motor neurons and dysregulated in SBMA mice, and focused on MID1, which is upregulated at early stages before the manifestation of overt motor symptoms. Immunohistochemical staining confirmed that MID1 is specifically expressed in motor neurons in the spinal cord of both mice and human (Fig.1).

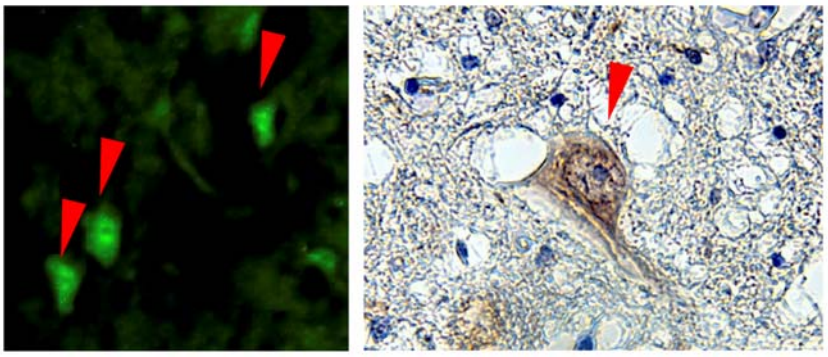


Fig.1 Immunostaining of spinal cord from SBMA model mouse (left) and SBMA patient (right)
Expression of MID1 protein in spinal motor neurons (arrowheads) is captured by green fluorescence detected by fluorescence antibody method in the left panel and as brown staining by enzyme antibody method in the right panel. Scale bars are 50 μm.

MID1 is known to bind to CAG repeats within mRNAs, such as *AR* mRNA, and promotes their translation. In our experiments using cultured motor neuron model cells, MID1 markedly increased the protein levels of pathogenic AR in the presence of androgen (Fig. 2). On the other hand, no increase in the protein levels was observed in the cells expressing *AR* with unexpanded CAG repeats.

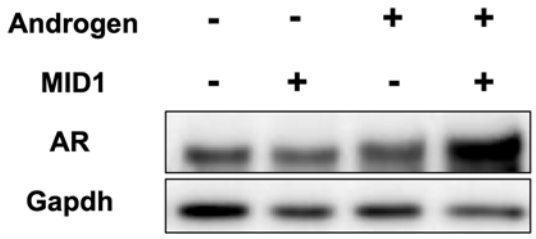


Fig.2 Overexpression of MID1 Increases pathogenic AR protein in the presence of androgen
Detection of AR protein by immunoblotting in cultured motor neuron-like cells (NSC-34). The highest AR protein level was obtained when MID1 was expressed in the presence of androgen (far right lane). Gapdh was used as control.

It is known that MID1 expression in humans and mice already begins during the fetal period, which we confirmed by mouse studies. To evaluate the involvement of MID1 in motor neuron damage in SBMA, we conducted an experiment with slice cultures of fetal mouse spinal cord to examine MID1 effect on axon outgrowth. In spinal cord slices derived from SBMA mice, many axons were found to elongate from motor neurons under no androgen exposure, but the axonal elongation was markedly suppressed in the presence of androgen (Fig. 3A). In addition, the axonal defect by androgen treatment was also found, albeit to a lesser degree, in female-derived spinal cord cultures. Cultures of female-derived spinal cord slices with androgen and lentivirus overexpressing MID1 resulted in increase of pathogenic AR protein and further suppression of axonal elongation (Fig. 3B). On the other hand, cultures of male-derived spinal cord slices with androgen and lentivirus expressing MID1-knockdown shRNA resulted in reduction of pathogenic AR protein and restoration of axonal elongation (Fig. 3C). These results showed that MID1 exacerbates the impairment of axonal elongation of SBMA motor neurons in the presence of androgen.

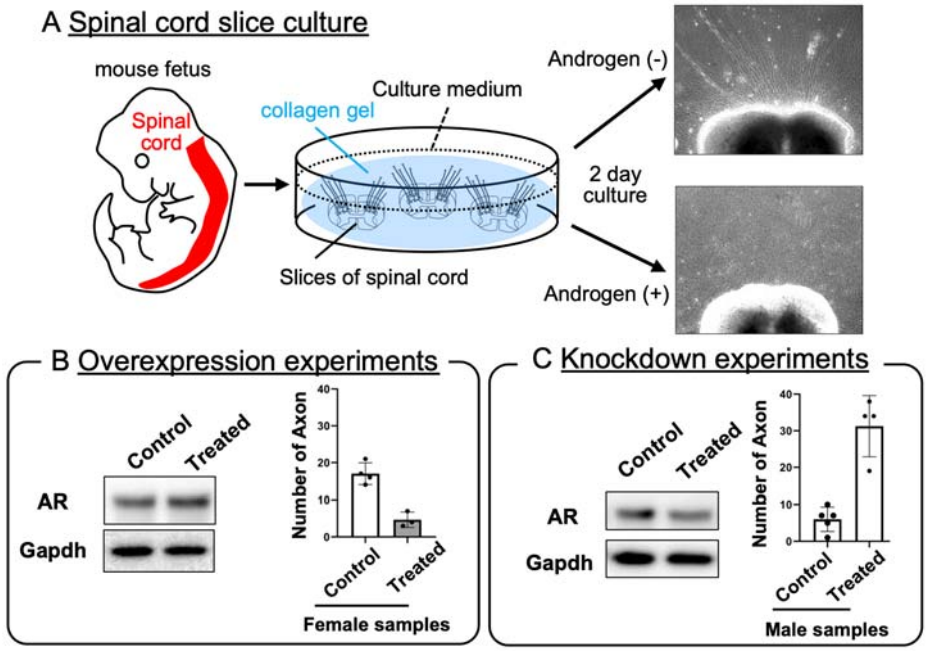
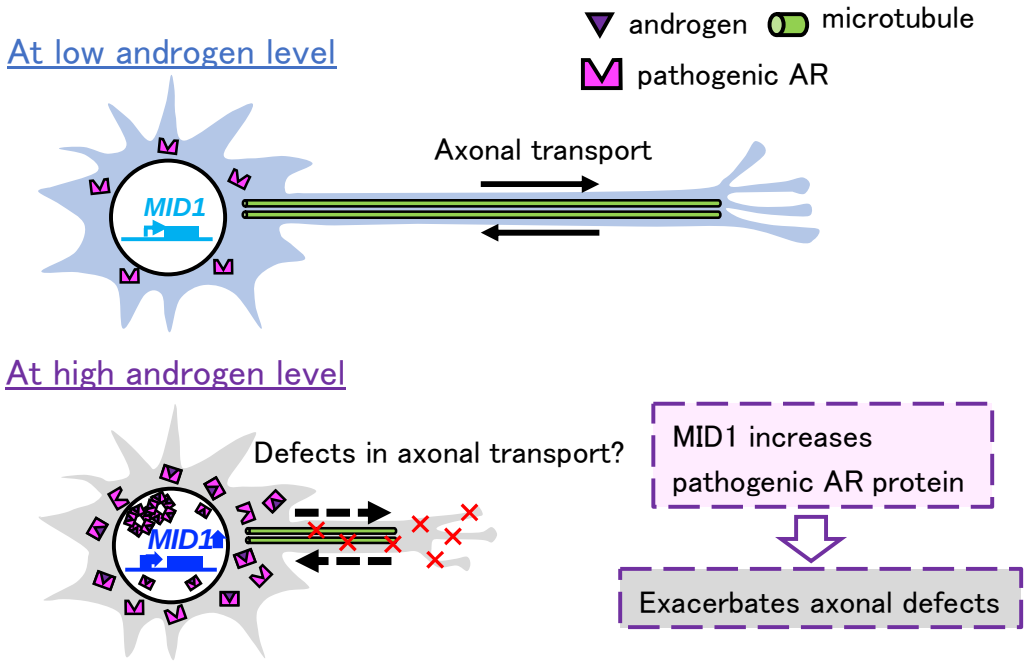


Fig. 3 MID1 is involved in increased protein levels of pathogenic AR and inhibition of axonal elongation
A) Cultures of spinal cord slices derived from SBMA model mice under androgen treatment. B) Lentiviral overexpression of MID1 increased the protein level of pathogenic AR and decreased the number of axons. C) Lentiviral knockdown of MID1 decreased the protein level of pathogenic AR and increased (restored) the number of axons. Control spinal cord slices were treated with control lentivirus.

Future Perspective

Our study suggests that elevated expression of MID1, a protein inherently expressed in motor neurons, contributes to the selective vulnerability of motor neurons in SBMA. Previous studies using mouse models of SBMA reported that pathogenic AR protein impairs axonal transport. Since MID1 also has binding ability to microtubule present in axon, pathogenic AR protein may impair the formation and maintenance of axon. In the future, we hope to elucidate the mechanism by which MID1 causes axonal impairments in a manner dependent on pathogenic AR, and develop therapeutic agents that target MID1 or proteins interacting with MID1.



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