News Release

Title

Src inhibition attenuates polyglutamine-mediated neuromuscular degeneration in spinal and bulbar muscular atrophy

Key Points

○Spinal and bulbar muscular atrophy (SBMA) is an X-linked, adult-onset neuromuscular disease characterized by muscle weakness and atrophy.

○Levels of phosphorylated Src were elevated in the spinal cords and skeletal muscles of a mouse model of SBMA (AR-97Q) before the time of disease onset compared with the levels in wild-type mice.

○Phosphorylated Src was also up-regulated in autopsy specimens of spinal cord and skeletal muscle from patients with SBMA compared with specimens from control patients.

○A Src kinase inhibitor (SKI) suppresses neuromuscular degeneration in the mouse model of SBMA.

○SKIs appear to be promising candidate therapeutics for the treatment of neurodegeneration.

Summary

A group of researchers, headed by Prof. Masahisa Katsuno, Department of Neurology, Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu, M.D., Ph.D.) have revealed that Src kinase is a pivotal therapeutic target for neuromuscular degeneration in spinal and bulbar muscular atrophy (SBMA). This work was published online in *Nature Communications* on September 19, 2019.

SBMA is a neuromuscular disease caused by an expanded CAG repeat in the androgen receptor (AR) gene. The research group headed by Prof. Katsuno performed a comprehensive analysis of signaling pathways in a mouse model of SBMA (AR-97Q mice) utilizing a phosphoprotein assay. They measured the levels of 17 phosphorylated proteins in spinal cord and skeletal muscle of AR-97Q mice at three stages: pre-onset, early symptomatic and advanced. The level of phosphorylated Src (p-Src) was markedly increased in the spinal cords and skeletal muscles of AR-97Q mice prior to the onset. Intraperitoneal administration of a Src kinase inhibitor improved the behavioral and histopathological phenotypes of the transgenic mice. They also identified p130Cas as an effector molecule of Src and showed that the phosphorylated p130Cas was elevated in murine and cellular models of SBMA. This study suggested that Src kinase inhibition is a potential therapy for SBMA.

Research Background

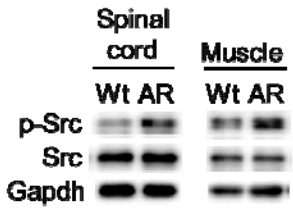
SBMA is an adult-onset neuromuscular disease caused by abnormal CAG repeat expansions in AR gene. SBMA is characterized by muscle weakness, atrophy and fasciculation of the limb and bulbar muscles. Ligand-dependent toxicity of the pathogenic polyglutamine-expanded AR protein is central to the pathogenesis of SBMA, and leuprorelin acetate, a luteinizing hormone-releasing hormone (LHRH) agonist, potentially suppresses neurological symptoms in SBMA patients. However, the benefit of this drug is limited by its side effects, such as sexual dysfunction and anti-anabolic actions on skeletal muscle, so that the development of the novel therapeutics for SBMA is required.

Research Results

The researchers investigated the expression levels of 17 phosphorylated proteins that are well known to play essential roles in cellular survival and function using Bio-rad Bio-plex phosphoprotein assay, in the spinal cord and skeletal muscle specimen of the mouse model of SBMA (AR-97Q mice) at three stages: pre-onset, early symptomatic, and advanced. The most outstanding changes were found in Src signaling pathway. The significant activation of Src sustained until the advanced stage of the disease in the spinal cord of SBMA mice. Levels of phosphorylated Src were also elevated in the skeletal muscles of AR-97Q mice before and around the time of disease onset compared with the levels in wild-type mice (Fig 1).

The ratio of phosphorylated proteins
0.50-0.85
1.15-2.00
≧2.00
*FDR<0.1

	AR-97Q / Wild-type					
	spinal cord			muscle		
	6w	9w	13w	6w	9w	13w
Src	1.15	*1.14	*1.32	1.81	3.64	0.51
Stat3	*1.34	1.05	0.88	*1.90	1.16	1.26
p38MAPK	*1.41	0.85	1.16	9.99	1.65	1.46
c-Jun	1.17	0.90	*1.24	1.71	1.64	*3.52
IGF-1R	1.17	*1.19	*0.89	1.29	*2.65	1.28
IRS-1	1.21	0.65	1.26	*3.88	5.24	3.79
Akt	*1.57	*0.61	0.94	2.37	*4.16	1.47
IkBa	*1.60	1.35	1.06	4.57	*1.21	1.66
p70s6k	1.2	0.91	1.11	1.75	1.70	*2.61
Erk1/2	1.12	1.22	*0.81	2.33	0.80	0.55
Smad2	*1.13	*0.84	1.05	1.95	1.42	1.84
GSK	1.05	0.97	1.03	*1.20	*1.37	*1.45
c-Abl	1.14	0.97	1.01	1.17	0.96	1.59
JNK	1.09	1.19	1.11	*1.83	*4.26	*2.85
VEGFR	1.08	0.96	*1.30	1.46	1.84	1.42
mTOR	0.97	0.81	1.25	1.24	1.89	*2.98
HSP27	1.12	1.03	1.21	0.64	*1.47	2.47



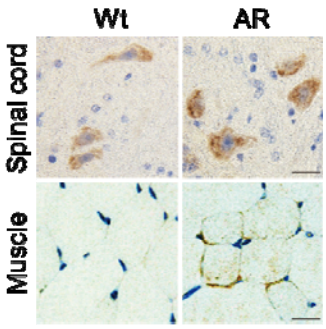


Figure 1. The results of Bio-Plex phosphoprotein assay, immunoblots and immunohistochemical analysis for p-Src in the spinal cord and skeletal muscle of 6-week-old mice.

Moreover, induced pluripotent stem cell (iPSC)-derived motor neurons from patients with

SBMA displayed an increased level of phosphorylated Src compared with the level in motor neurons derived from the iPSCs from healthy controls. Phosphorylated Src was also up-regulated in the biopsied skeletal muscles of patients with SBMA compared with those of control subjects, indicating that Src phosphorylation is elevated in the skeletal muscle of SBMA patients at an early stage. Furthermore, the levels of phosphorylated Src were increased in autopsy specimens of spinal cord and skeletal muscle from patients with SBMA compared with specimens from control patients (Fig 2).

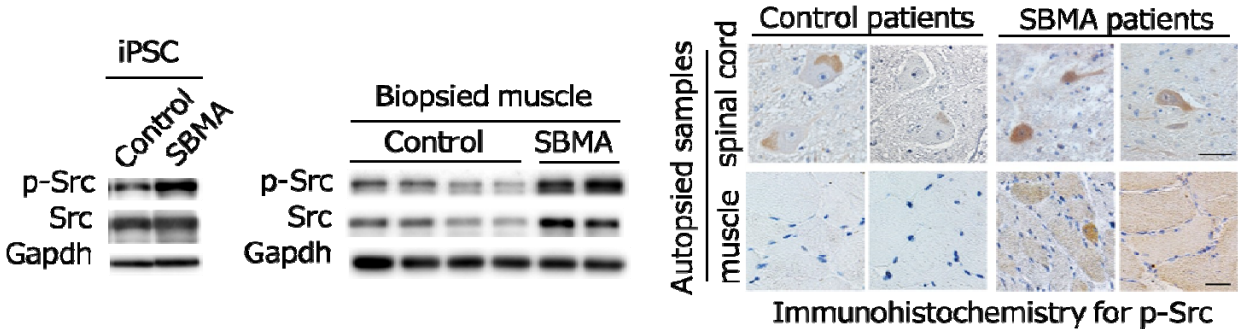


Figure 2. The levels of phosphorylated Src were elevated in patients with SBMA.

Based on the findings in these assays, the researchers analyzed the activation levels of Src pathway and evaluated the efficacy of Src kinase inhibitor (SKI) in NSC34 and C2C12 cells stably expressing AR-97Q. Src pathway was up-regulated in neuronal and muscular cellular models of SBMA and the treatment with A419259 trihydrochloride (A419259), an SKI, improved cellular viability of 97Q cells (Fig 3).

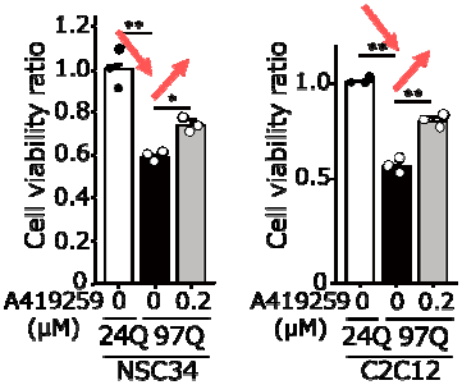


Figure 3. A419259 improved the cell viability of cellular models of SBMA.

They next intraperitoneally injected 6-week-old SBMA transgenic mice with 0.5 mg/kg/day A419259 once every three days to examine the effects of SKI *in vivo*. The intraperitoneal administration of SKI to mice beginning at 6 weeks of age improved body weight, grip strength, and performance on the rotarod task and extended the lifespan of AR-97Q mice (N=24) compared with vehicle-treated mice (N=22) (Fig 4).

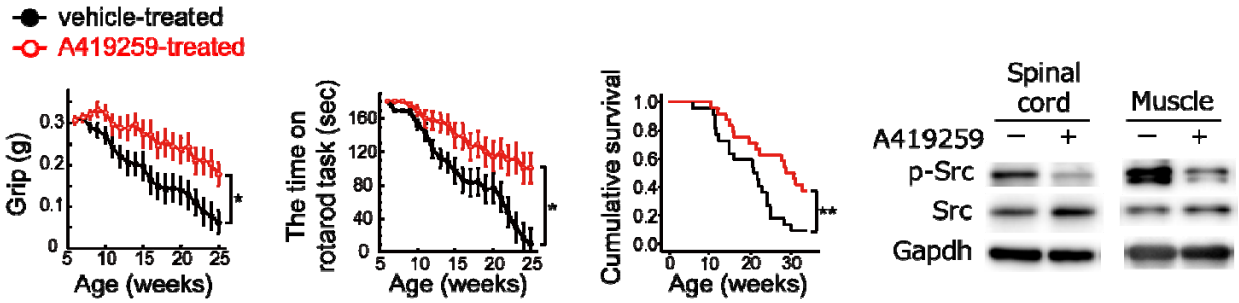


Figure 4. A419259 improves the neuromuscular phenotype of the mouse model of SBMA. A419259 suppresses Src activation in the spinal cord and skeletal muscle of AR-97Q mice.

They also investigated the alteration of phosphorylation levels of Src effector molecules using SKI-treated cellular and mouse models of SBMA and identified p130Cas as effector molecules of Src.

Finally, the researchers revealed that the interaction between Src and AR plays an essential role in the activation of Src pathway in the pathogenesis of SBMA.

Research Summary and Future Perspective

Up-regulation of Src pathway has a strong impact on the pathophysiology of SBMA and SKI is a potential therapy for SBMA (Fig 5). SKIs have been shown to be well tolerated in humans, and some of them have been used to treat leukemia. The present study provided further insight into the commonalities between neurodegeneration and cancer, and a novel promising therapy for SBMA.

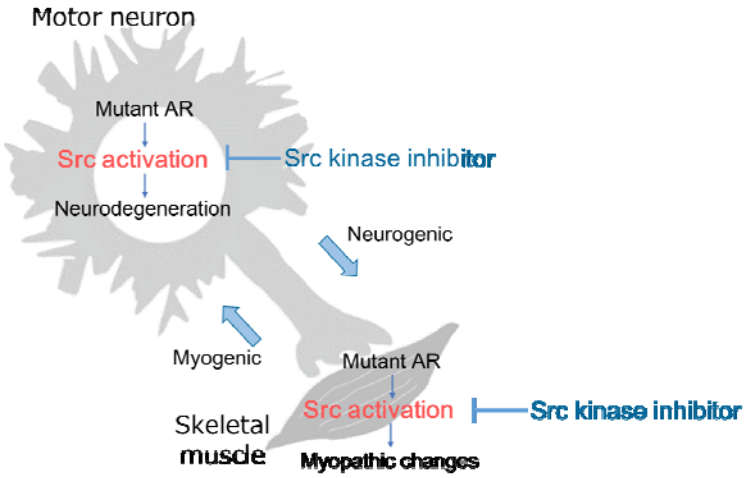


Figure 5. The abnormal Src pathway in SBMA.

Publication

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