News Release

Title

Selective suppression of polyglutamine-expanded protein by lipid nanoparticle-delivered siRNA targeting CAG expansions in the mouse CNS

Key Points

・Polyglutamine (polyQ) diseases are inherited neurodegenerative disorders caused by expansion of cytosine-adenine-guanine (CAG)-trinucleotide repeats, which encode a polyglutamine tract. These diseases include spinal and bulbar muscular atrophy (SBMA), Huntington's disease, dentatorubral-pallidoluysian atrophy, and spinocerebellar ataxias.

・Previous studies have shown that CAG repeat-targeting siRNA duplexes (CAG-siRNAs) have a potential to selectively suppress polyQ-expanded proteins in *in vitro* cell models of polyQ diseases, but *in vivo* application of these siRNAs has not yet been investigated due to their easy degradability.

・Our current study revealed that lipid nanoparticle (LNP)-mediated delivery of the CAG-siRNA selectively suppresses polyQ-expanded proteins in the central nervous system (CNS) of mouse models of polyQ diseases.

・These results support the therapeutic potential of LNP-delivered CAG-siRNAs for selective suppression of polyQ-expanded proteins.

Summary

A group of researchers, headed by Prof. Masahisa Katsuno, Department of Neurology, Nagoya University Graduate School of Medicine have collaborated with a US biotech company, Arcturus Therapeutic and revealed that CAG repeat-targeting siRNA selectively suppresses polyglutamine-expanded proteins in the central system (CNS) of mice models of polyglutamine (polyQ) diseases. This work was published online in *Molecular Therapy - Nucleic Acids* on February 15, 2021.

PolyQ diseases are inherited neurodegenerative disorders caused by expansion of cytosine-adenine-guanine (CAG)-trinucleotide repeats in causative genes. These diseases include spinal and bulbar muscular atrophy (SBMA), Huntington's disease, dentatorubral-pallidoluysian atrophy, and spinocerebellar ataxias. Targeting expanded CAG repeats is a common therapeutic approach to polyQ diseases, but concomitant silencing of genes with normal CAG repeats may lead to toxicity. Previous studies have shown that CAG repeat-targeting siRNA duplexes (CAG-siRNAs) have a potential to selectively suppress mutant proteins in in vitro cell models of polyQ diseases. However, in vivo application of these siRNAs has not yet been investigated due to their easy degradability.

In this study, we demonstrate that an unlocked nucleic acid (UNA)-modified CAG-siRNA shows high selectivity for polyQ-expanded androgen receptor (AR) inhibition in in vitro cell models, and that lipid nanoparticle (LNP)-mediated delivery of the CAG-siRNA selectively suppresses polyglutamine-expanded AR and huntingtin in the central nervous system of mice models. These results support the therapeutic

potential of LNP-delivered UNA-modified CAG-siRNAs for selective suppression of mutant proteins in SBMA and other polyQ diseases.

This research was supported by AMED under Grant Number JP20lm0203005.

Research Background

Polyglutamine (polyQ) diseases are inherited neurodegenerative disorders caused by expansion of cytosine-adenine-guanine (CAG)-trinucleotide repeats in causative genes. These diseases include spinal and bulbar muscular atrophy (SBMA), Huntington's disease, dentatorubral-pallidoluysian atrophy, and spinocerebellar ataxias. Patients with polyglutamine diseases develop progressive neurological symptoms caused by the degeneration of neurons, and currently there is no curative therapy.

Reducing expanded polyQ proteins is a promising therapeutic strategy in polyQ diseases, and clinical trials applying chemically modified antisense oligonucleotides against huntingtin, the causative protein of Huntington's disease, have been launched based on the success of preclinical studies. Targeting CAG repeats itself is another potential therapeutic option in polyQ diseases, but concomitant silencing of genes with normal CAG repeats may lead to toxicity.

Recent studies have shown that CAG repeat-targeting siRNA duplexes (CAG-siRNAs) have a potential to selectively suppress mutant proteins in *in vitro* cell models of polyQ diseases. However, *in vivo* application of these siRNAs has not yet been investigated due to their easy degradability.

The aim of this study was to explore the potential application of CAG-siRNAs for mutant-allele-selective treatment for polyQ diseases in cellular and mouse models. We investigated whether CAG-siRNAs containing unlocked nucleic acid (UNA) substitutions selectively suppress polyQ-expanded proteins in cell models of SBMA. In addition, we administered a UNA-modified CAG-siRNA into the CNS of mouse models of SBMA and Huntington's disease using a lipid-enabled and UNA modified RNA (LUNAR) lipid delivery technology platform.

Research Results

We performed a luciferase reporter assay to determine the optimal position of UNA substitutions, and identified that the CAG-siRNA with UNA substitutions at position 9 and 10 (REPU910) showed high allele selectivity for polyQ-expanded protein suppression. In human fibroblasts, REPU910 had a limited effect on wild-type androgen receptor (AR) protein expression but suppressed the expression of polyQ-expanded AR by 40–65% at concentrations of 2.5 and 5 nM (Figure 1).

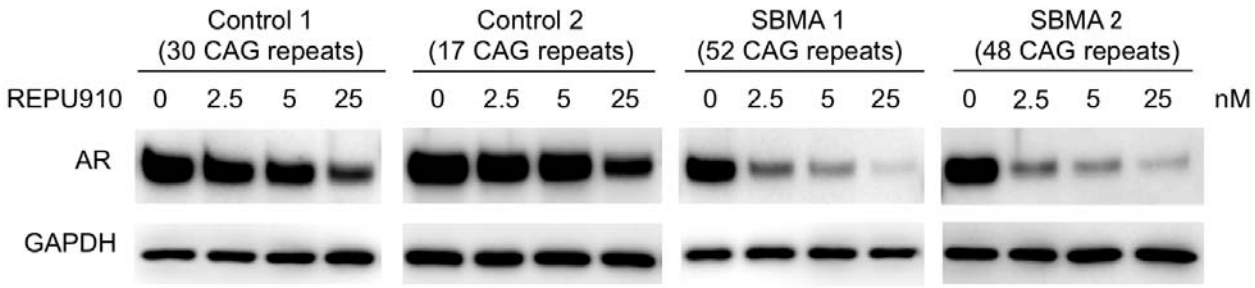

Figure 1. REPU910 siRNA selectively suppresses polyglutamine-expanded androgen receptor in human fibroblasts.

To investigated the applicability of the LUNAR platform in the CNS, we administered LUNAR nanoparticles incorporating eGFP mRNA (LUNAR-eGFP mRNA) or vehicle into the lateral ventricle of mice and assessed the expression of eGFP fluorescence three days after administration. We detected widespread eGFP expression in the brain of LUNAR-eGFP mRNA injected mouse compared to that of vehicle-injected (control) mouse (Figure 2).

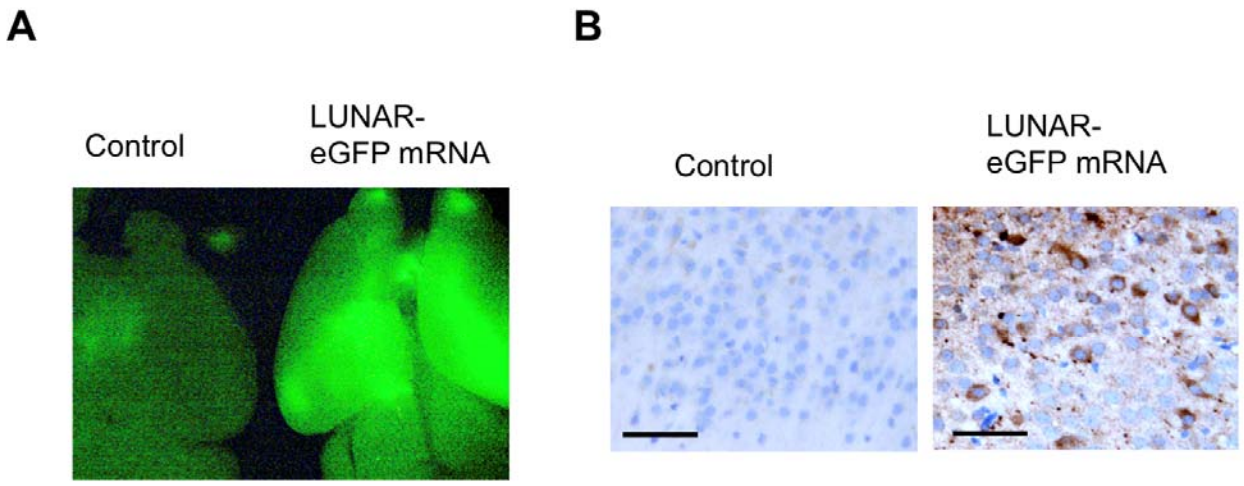

Figure 2. Distribution of LUNAR nanoparticle in the brain
A: Detection of eGFP signal by a fluorescent stereomicroscope
B: Immunohistochemistry for eGFP in the cerebral cortex (bar = 50 μm)

Next, we investigated the efficacy of LUNAR-delivered REPU910 siRNA (LUNAR-REPU910) *in vivo*. To assess its mutant allele selectivity, we used our transgenic mice carrying wild-type human *AR* with 24 CAG repeats and mutant *AR* with 97 CAG repeats. We intracerebroventricularly injected LUNAR-REPU910 in the mice and assessed AR protein levels three days after administration. Western blot analysis showed that LUNAR-REPU910 suppressed polyQ-expanded AR by ~90% in the cerebral cortex, but no apparent reduction of wild-type AR was observed (Figure 3), which demonstrates the allele-selective potency of LUNAR-REPU910 against expanded CAG repeats in *in vivo* mouse brain tissue. Furthermore, the LUNAR-REPU910 also suppressed polyQ-expanded huntingtin in the CNS of a mouse model of Huntington's disease.

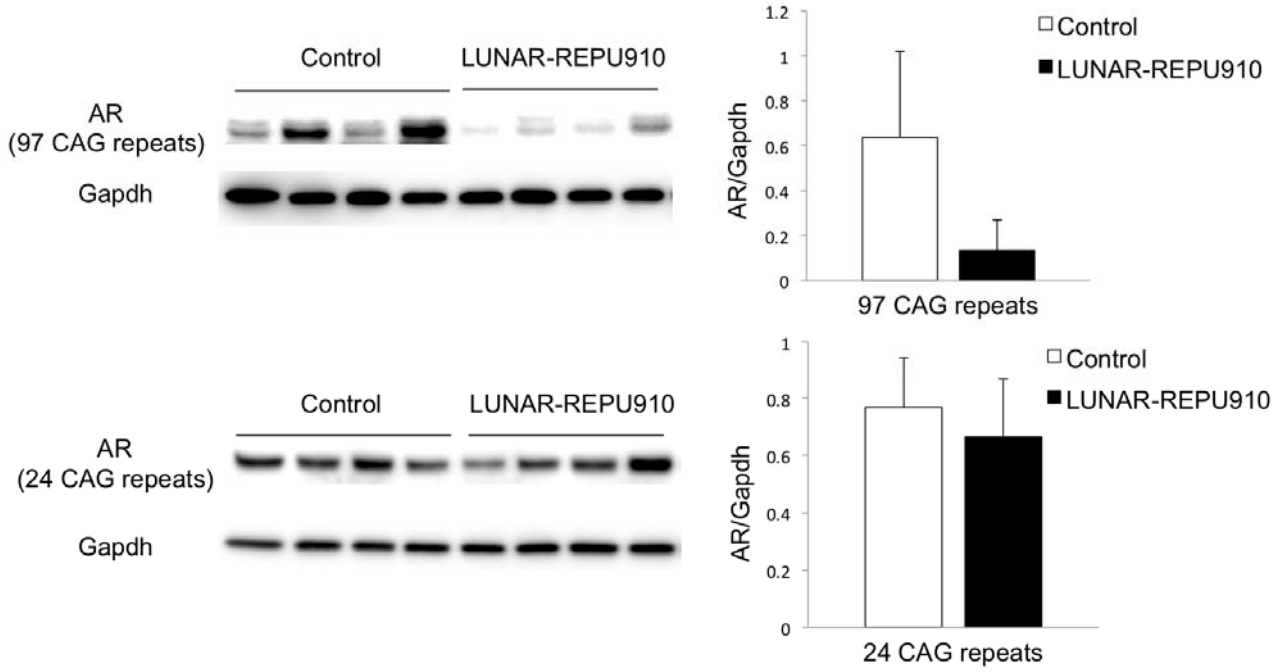

Figure 3. LUNAR-REPU910 siRNA selectively suppresses polyglutamine-expanded androgen receptor in the cerebral cortex of transgenic mice.

Research Summary and Future Perspective

The results of this study provide a proof of concept of LNP-delivered CAG-siRNA for selective suppression of polyQ-expanded protein (Figure 4). Now we are exploring the modification of nanoparticles and siRNAs to obtain the optimal distribution and duration of action to disease tissues. Our goal is to develop siRNA pharmaceuticals commonly applicable to polyQ diseases.

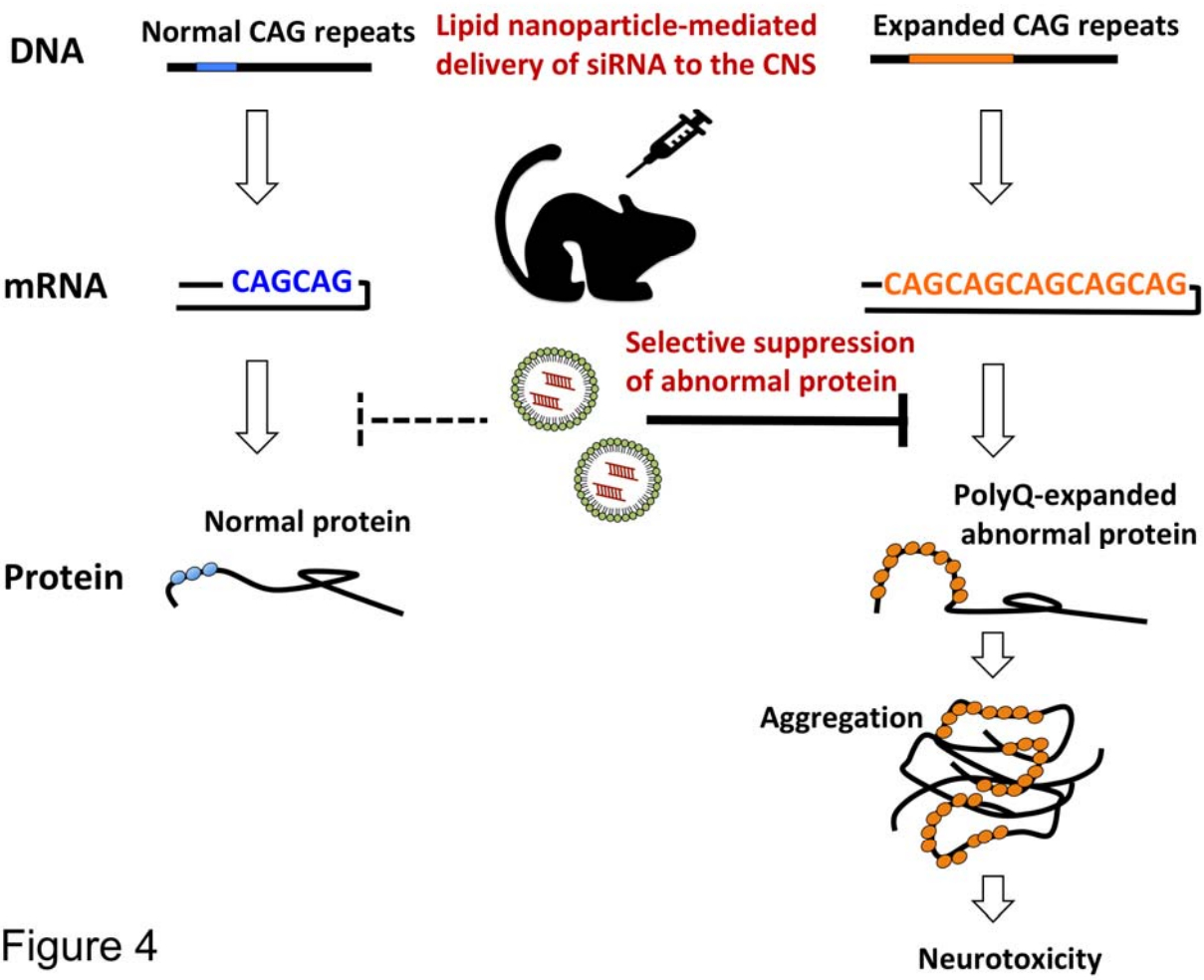

Figure 4

Publication

Tomoki Hirunagi, Kentaro Sahashi, Kiyoshi Tachikawa, Angel I Leu, Michelle Nguyen, Rajesh Mukthavaram, Priya P. Karmali, Padmanabh Chivukula, Genki Tohnai, Madoka Iida, Kazunari Onodera, Manabu Ohyama, Yohei Okada, Hideyuki Okano and Masahisa Katsuno

"Selective suppression of polyglutamine-expanded protein by lipid nanoparticle-delivered siRNA targeting CAG expansions in the mouse CNS"

Molecular Therapy - Nucleic Acids, February 15, 2021.

DOI：https://doi.org/10.1016/j.omtn.2021.02.007

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Mole_The_N_A_210216.pdf