Preclinical proof-of-concept study: antisense-mediated knockdown of CALM as a therapeutic strategy for calmodulinopathy

Y. Yamamoto[1], T. Makiyama[1], Y. Wuriyanghai[1], H. Kohjitani[1], J. Gao[1], A. Kashiwa[1], H. Hai[1], T. Aizawa[1], T. Imamura[1], T. Ishikawa[2], Y. Yoshida[3], S. Ohno[4], M. Horie[5], N. Makita[2], T. Kimura[1]

[1]Kyoto University, Cardiovascular Medicine, Kyoto, Japan; [2]National Cerebral & Cardiovascular Center, Omics Research Center, Suita, Japan; [3]Kyoto University, Center for iPS Cell Research and Application, Kyoto, Japan; [4]National Cerebral & Cardiovascular Center, Department of Bioscience and Genetics, Suita, Japan; [5]Shiga University of Medical Science, Center for Epidemiologic Research in Asia, Otsu, Japan

Funding Acknowledgement: Type of funding source: Private company. Main funding source(s): Nissan Chemical Corporation

Background: Calmodulin (CaM) is a ubiquitous Ca2+ sensor molecule encoded by three distinct calmodulin genes, CALM1–3, and has an important role for cardiac ion channel function. Recently, heterozygous missense mutations in CALM genes were reported to cause a new category of life-threatening genetic arrhythmias such as long-QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT), which is called as "calmodulinopathy". The patients with calmodulinopathy show poor prognosis and there is no effective treatment for them.

Purpose: Considering the dominant-negative effect of mutant calmodulin proteins produced by heterozygous missense mutations in CALMs, we aimed to prove the concept of antisense-based therapy to treat calmodulinopathy using human iPS cell-derived cardiomyocyte (hiPSC-CM) model.

Methods: We designed multiple locked nucleic acid (LNA) gapmer-antisense oligonucleotides (ASOs) targeting CALM2 and analyzed the silencing efficiency and toxicity in cultured cells to select the most potent ASO. Using CMs differentiated from hiPSCs which were generated form a 12-year-old boy with LQTS carrying a heterozygous CALM2-N98S mutation, CALM2 expression and action potentials (APs) were analyzed to evaluate the efficacy of ASOs.

Results: We identified several ASOs which reduced CALM2 expression without affecting cell viability in human cultured cells (HepG2) (ASO 50 nM, n=2; Figure 1A). Considering further experiments in vivo mouse model, we investigated the CALM2 silencing activity in mouse cultured cells (3T3-L1) without transfection (free-uptake) (ASO 1 μM, n=2; †ASOs have homologous sequence between human and mouse; Figure B). After free-uptake CALM2 silencing analysis in 3T3-L1 cells, we identified that ASO #2 has the most potent CALM2 silencing activity and low cytotoxicity (Figure 1B). ASO #2 effectively reduced CALM2 expression even in hiPSC-CMs (ASO(–): n=3, lipofection: n=4, free-uptake: n=3; $P<0.05$; Figure 1C). In action potential recordings, we demonstrated that ASO #2 ameliorated prolonged AP durations (APD90) in N98S-hiPSC-CMs at 0.5 Hz pacing (ASO(–): 666±123 ms (n=7), lipofection: 329±21 ms (n=8), free-uptake: 388±34 ms (n=12); $P<0.05$; Figure 1D).

Conclusion: Our results using patient-derived hiPSC-CM model suggest that ASO-based therapy might be a promising strategy for the treatment of calmodulinopathy.

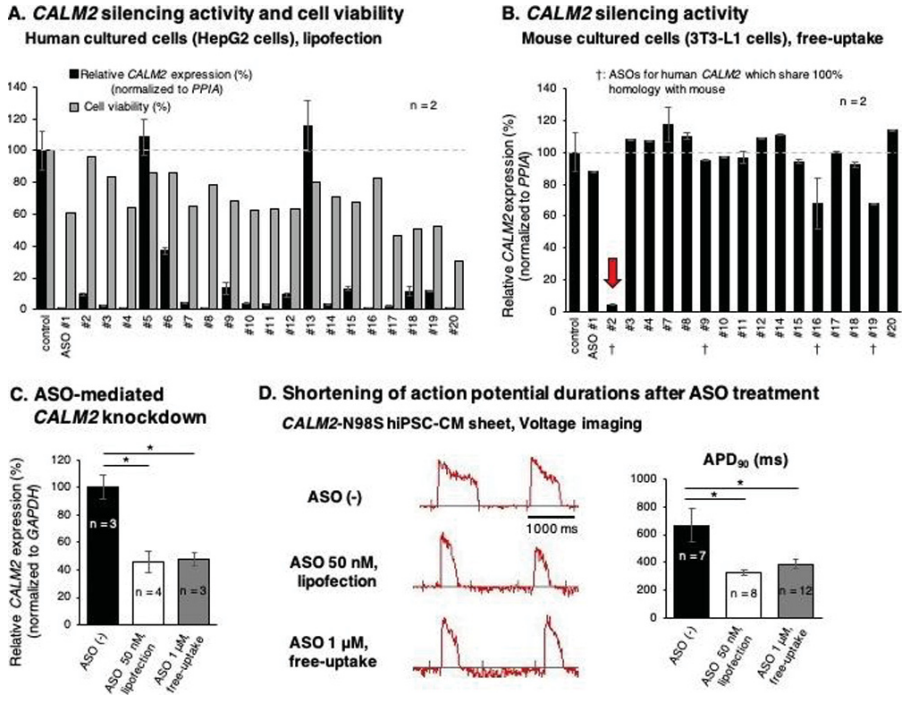

Figure 1