News Release

Title

Elucidation of novel mechanism for insulin secretion by TDP-43: unexpected link between ALS and diabetes.

Key Points

- Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by selective motor neuron death. TAR DNA-binding protein of 43kDa (TDP-43) is a neuropathological marker of ALS, which is lost in the nucleus of motor neurons of ALS.
- We here found that ALS patients have impaired insulin secretion and loss of TDP-43 in the nucleus of pancreas in autopsied ALS cases.
- Furthermore, experiments with cell and mouse showed that TDP-43 regulates the transcriptional activity of *Cacna1c*, the gene encodes CaV1.2 protein.
- The results reveal that TDP-43 regulates the early-phase insulin secretion via CaV1.2 protein.
- The insulin secretion capacity appears to be a biomarker of disease state or prognostic factor of ALS.
- The role of TDP-43 in pancreas may explore novel pathomechanism of diabetes.

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 TDP-43 regulates cellular exocytosis mediated by L-type voltage dependent calcium channel subunit (CaV1.2) and thus plays an important role in the early-phase of insulin secretion by pancreatic islets. This work was published online in *Journal of Clinical Investigation* on June 29, 2019.

Amyotrophic lateral sclerosis (ALS) is a selective motor neuron disorder, leading to death in 3-5 years after the onset due to rapid progression of muscle atrophy, including respiratory and bulbar muscles. TDP-43 pathology is seen in 90-95% of sporadic ALS subjects. The nuclear loss of TDP-43 results in motor neuron degeneration, while the cytoplasmic aggregation of TDP-43 is also cytotoxic. The research group headed by Prof. Katsuno has been focusing on TDP-43 dysfunction (loss of function), but the molecular mechanism from nuclear loss of TDP-43 to neuronal death is elusive.

Normal TDP-43 protein is abundant and ubiquitous in both neuronal and non-neuronal tissues, with being highly expressed in the pancreas, suggesting the possibility that TDP-43-related pathology underlies the non-neuronal manifestation of ALS. The glucose tolerance test in ALS patients showed impaired insulin secretion, and immunostaining of autopsy pancreatic tissue showed nuclear loss of TDP-43 staining. Based on the hypothesis that nuclear loss of TDP-43 causes impaired insulin secretion, the research group used a beta

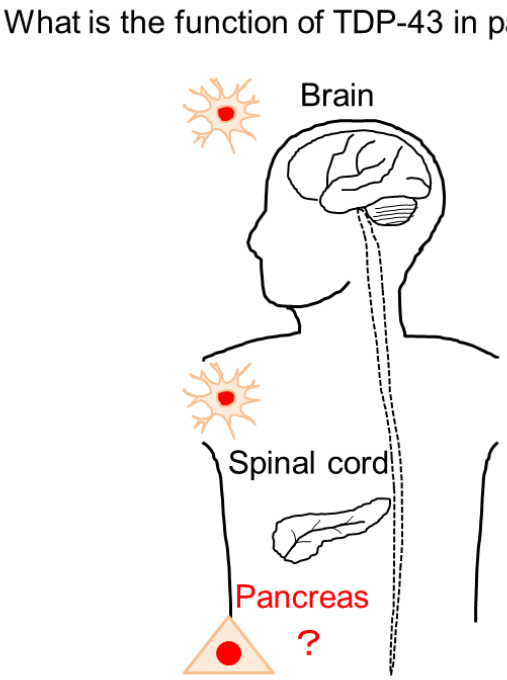
cell line (MIN6 cells) in vitro, and made the pancreas specific TDP-43 knockout mice in vivo. The TDP-43 knocked-down MIN6 cells had decreased insulin secretion. Next, comprehensive gene analysis using MIN6 cells revealed that TDP-43 knock-down down-regulates voltage-dependent Ca channel subunit (CaV1.2), and that TDP-43 binds to the promoter region of *Cacna1c*, which encodes CaV1.2. The pancreas-specific TDP-43 knockout mice also had impaired insulin secretion and down-regulation of CaV1.2 in beta cells.

This study provides the novel insight that loss of TDP-43 inhibits exocytosis via the CaV1.2 calcium channel and reduces the early-phase of the glucose-induced insulin secretion. In the future, insulin secretion capacity can be a biomarker of a disease state for ALS patients. In addition, the elucidation of a novel molecular pathogenesis of diabetes is also expected.

Research Background

Since TDP-43 was identified as a neuropathological maker of ALS in 2006, the molecular function of TDP-43 has been heavily studied. TDP-43 is a nuclear protein which possesses a variety of functions in RNA metabolism (transcription, splicing and transport), synapse (synaptic vesicle transport, neurotransmitter secretion and synaptic transmission), development and cell morphology. TDP-43 is normally localized in the nucleus and histopathology of ALS is characterized by the nuclear loss of TDP-43. Neuron-specific depletion of TDP-43 leads to progressive neurodegenerative phenotype similar to human ALS, suggesting that TDP-43 is required for the maintenance of neuronal integrity. However, the precise pathomechanism from nuclear loss of TDP-43 to neuronal death is elusive.

The research team headed by Prof. Katsuno is developing biomarkers of motor neuron disease, and detected the decreased insulin secretion in ALS patients. Moreover, nuclear TDP-43 in the beta cell disappeared in autopsied pancreas. The purpose of this study was to elucidate the mechanism of impaired insulin dynamics in ALS patients focusing on nuclear loss of TDP-43 in the beta cells of pancreatic islets.



Research Results

ALS patients reduced early-phase insulin secretion, and had nuclear loss of TDP-43 in the islets (Fig1).

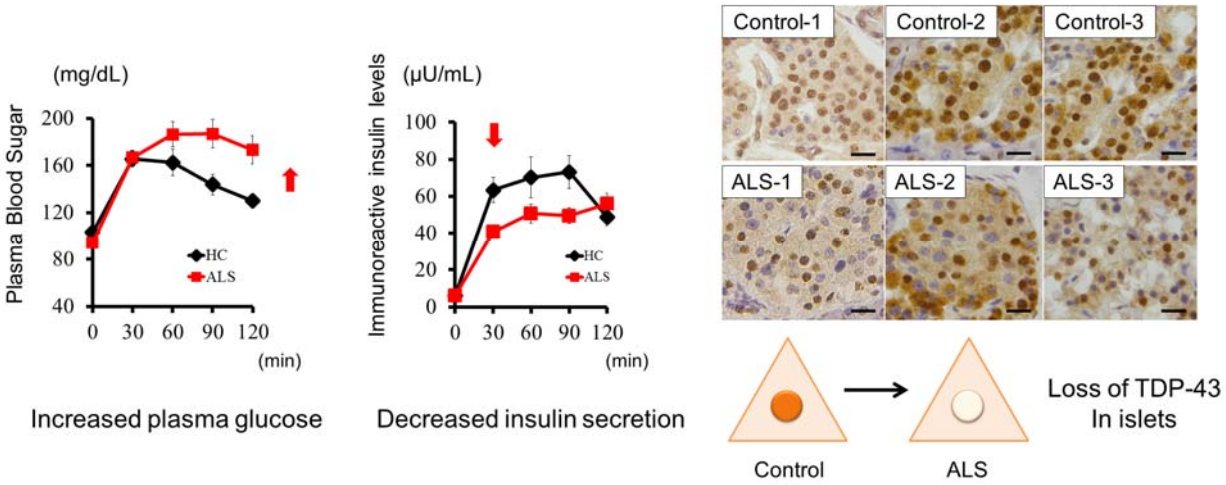


Figure1. Oral glucose tolerant test (OGTT) and immunohistochemistry of TDP-43

Insulin secretion consists of two phases, early and late, which are regulated differently. Early-phase insulin secretion is instigated by glucose uptake via glucose transporters and facilitated by the activation of potassium channels followed by Ca^{2+} influx via calcium channels. In cultured beta cell (MIN6 cell), TDP-43 knock-down inhibited the early-phase insulin secretion. Quantitative analysis of total internal fluorescence (TIRF) imaging revealed that the

glucose-stimulated 1st-phase insulin secretion was inhibited in TDP-43 knocked-down MIN6 cells.

To identify the gene expression changes, we performed microarray analysis on the TDP-43 knocked-down MIN6 cells, and found the down-regulation of voltage-dependent Ca channel (CaV1.2). The supplementation of CaV1.2 recovered glucose-induced insulin secretion in TDP-43 knocked-down MIN6 cells. In addition, in Fura2 imaging, the intracellular intracellular influx of Ca was decreased in TDP-43 knocked-down MIN6 cells. Furthermore, in whole-cell patch-clamp recordings of the Ca^{2+} current measurements, the voltage dependent calcium channel inward currents were significantly smaller in TDP-43 knocked-down MIN6 cells. Collectively, these findings demonstrate that the dysfunction in the 1st-phase of insulin secretion in TDP-43 knocked-down MIN6 cells was due to the inhibition of CaV1.2 and eventual impairment of calcium influx.

TDP-43 is a DNA and RNA binding protein and is known to function in transcriptional activity and RNA metabolism etc. Luciferase assays showed that TDP-43 knocked-down reduces the promoter activity of *Cacna1c,* the gene encoding CaV1.2, while there was no difference in mRNA stability of *Cacna1c* between the control and TDP-43 knocked-down MIN6 cells. These results indicate that TDP-43 depletion down-regulates CaV1.2 in MIN6 cells by decreasing gene transcripts.

Next, we created an AAV-mediated beta cell-specific TDP-43 knockout (AAV-KO) mice. The glucose tolerance test (IPGTT) and the insulin test showed a decrease in the early-phase of insulin secretion of AAV-KO mice. The isolated islet primary culture and pancreatic perfusion experiments also demonstrated impaired insulin secretion in TDP-43 knockout islets and mice.

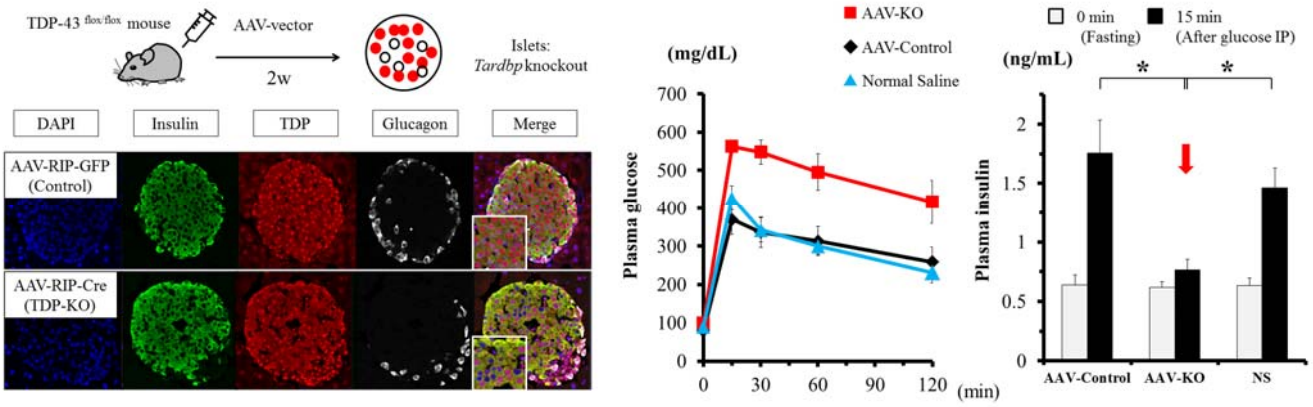


Figure2. Pancreas specific TDP-43 knockout mice, glucose tolerant test, and insulin test

Research Summary and Future Perspective

The results reveal that the loss of TDP-43 induces impaired insulin secretion in ALS, a neurodegenerative disease due to selective motor neuron death. This study provides the novel insight that TDP-43 regulates early-phase of the glucose-induced insulin secretion via the CaV1.2 calcium channel. Insulin secretion capacity can be a biomarker of a disease state for ALS patients. In addition, the elucidation of the molecular role of TDP-43 in diabetes is also

expected.

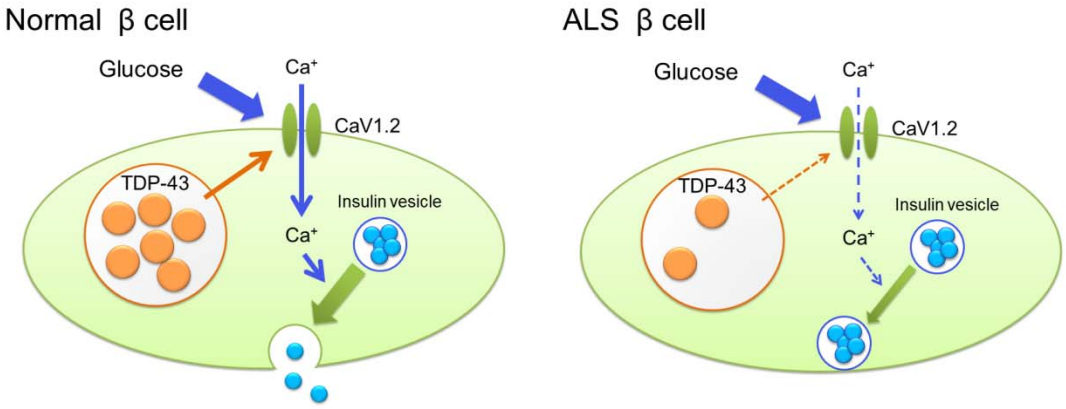


Figure3. Normal and ALS beta cell.

Publication

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