Dysregulated O-GlcNAcylation is a molecular link to Alzheimer's disease

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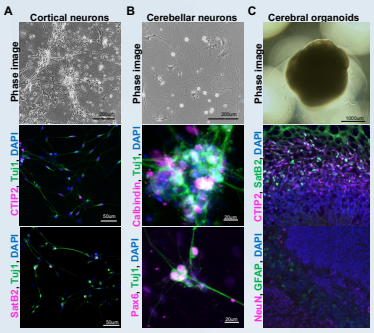
Background

Sporadic Alzheimer's disease (sAD) is the most common type of neurodegenerative disease. Recent studies show that detectable impairment of brain glucose metabolism occurs years before onset of AD symptoms, and the dysregulated O-GlcNAc levels likely arises from impaired glucose metabolism and correlates with AD pathogenesis. So far, the mechanism of sAD and the role of O-GlcNAcylation in AD pathology remained largely unknown due to a lack of a human sAD model.

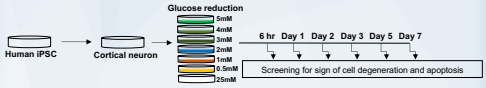
Methods

Generating neurons and cerebral organoids from human pluripotent stem cells



We generated human cortical neurons (A), cerebellar neurons (B) and cerebral organoids (C) from healthy human pluripotent stem cells based on established protocols.

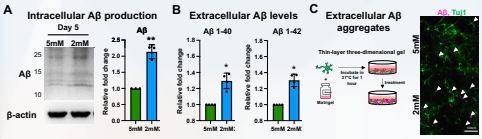
Screening for optimal glucose concentration for inducing degeneration



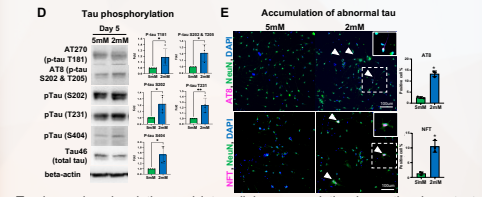
After maturation, the neurons were treated with glucose reduction media ranging from 0.5 to 5 mM, in the course of 6hr to 7 days, to study the effect of low glucose on the degenerative status of neuron.

Results

Low glucose induces AD-like phenotypes in cortical neuron

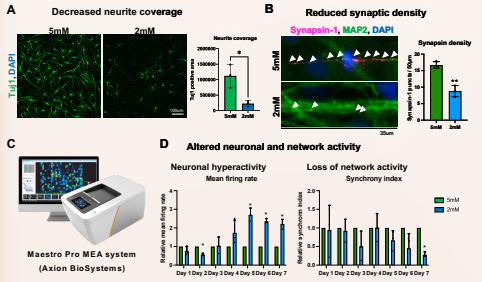


Increased production of amyloid beta is one of the features of AD. Therefore, we detected intracellular and extracellular amyloid beta level using western blotting (A) and ELISA (B), respectively. Extracellular amyloid beta aggregations were observed in three-dimensional cultured system (C). Our results demonstrate that lowering glucose levels to 2mM significantly induces amyloid beta production and secretion.



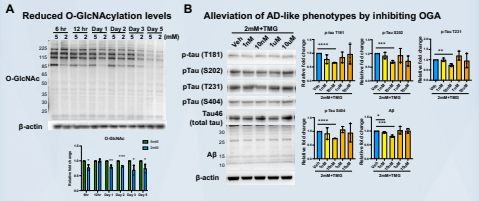
Tau hyperphosphorylation and intracellular accumulation is another important feature in AD brains. We found increasing phosphorylation on tau at several sites in low glucose treated neurons (C). The accumulation of hyperphosphorylated tau (stained by AT8) and neurofibrillary tangles (stained by NFT) in low glucose treated neurons were revealed by immunofluorescence staining.

Low glucose induces degenerative phonotypes in cortical neurons



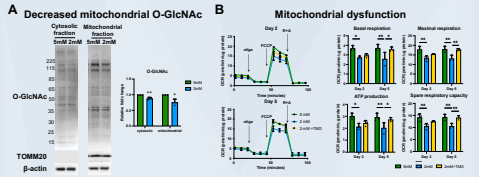
The early stages of many neurodegenerative diseases and age-related degeneration are characterized by neurite damage and compromised synaptic function that precede neuronal cell death. To reveal the effects of low glucose on neuron structure and function, we performed immunofluorescence staining of Tuj1 for observing neurites (A), and synapsin-1 for observing synapses (B). We found that low glucose damaged both neurite and synaptic structure. Therefore, we further utilized microelectrode array (MEA) to analyze neuronal network activity and found that neurons are hyperactive while have decreased connectivity under low glucose treatment (C&D).

Decreased O-GlcNAcylation levels are involved in early stage of AD-like alternations



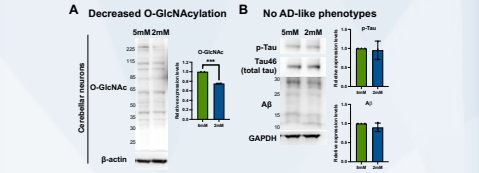
To elucidate the role of O-GlcNAcylation in low glucose-induced AD-like alternations, we detected O-GlcNAcylation levels at different time points after reducing glucose level and found that O-GlcNAcylation levels are significantly reduced soon after low glucose treatment and last till the end of experiment (A). Furthermore, specifically raising O-GlcNAcylation levels by inhibiting O-GlcNAcase, an enzyme that removes O-GlcNAc, even in low glucose treated neurons, rescues low glucose-induced AD-like changes (B). TMG: thiamet-G

Mitochondrial dysfunction induced by lower O-GlcNAcylation level prior to AD phenotypes

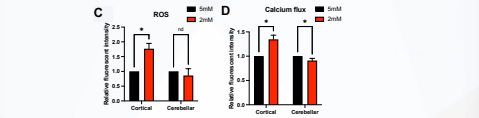


O-GlcNAcylation is abundant and important in mitochondria. To explore the effects of low glucose on O-GlcNAcylation levels in mitochondria and its functions, we used western blotting to detect O-GlcNAcylation levels and Seahorse assay to examine mitochondrial functions. Our results show that O-GlcNAcylation levels are decreased in mitochondria (A) along with mitochondrial dysfunction (B) by low glucose treatment, which occurs before any other degenerative phenotype appeared. Moreover, the mitochondrial abnormality can be reversed by TMG treatment (B).

Cerebellar neurons are relatively resistant to low glucose-induced AD-like alternations

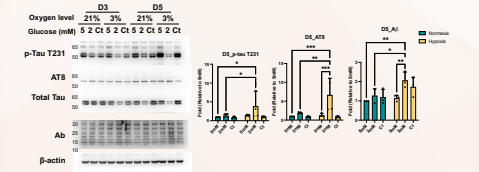


AD affects brain regions and neuronal populations in a different and specific manner. Cerebellum is well known to be relatively resistant to AD pathology, therefore, we tested our model on cerebellar neurons to investigate whether it recapitulates the predictable region-specific manner of AD. In low glucose-treated cerebellar neurons, O-GlcNAcylation levels are reduced (A); however, there is no change in AD-like phenotypes, including tau phosphorylation and amyloid beta levels (B).

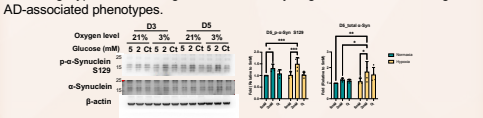


To investigate the mechanism underlying selective vulnerability, we detected oxidative stress by CM-H2DCFDA (C) and calcium flux by Fluo4 (D) in both low glucose-treated cortical and cerebellar neurons. While glucose insufficiency affected redox and calcium homeostasis in cortical neurons, it did not have the same effects on cerebellar neurons, suggesting that a different regulation of O-GlcNAcylation profile in mitochondria could be involved in selective vulnerability

Low oxygen levels exert a synergistic effect with low glucose in inducing AD-like phenotypes in cortical neurons

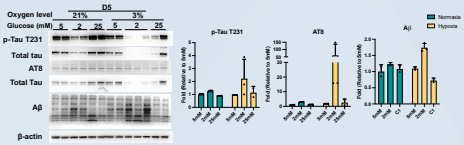


Hypoxia, a result of hypoperfusion, is one of the major factors contributing to the pathogenesis of AD. We investigated the effect of hypoxia on inducing AD-like phenotypes. Our western blot results suggest that tau phosphorylation and amyloid beta levels resulting from hypoxia alone are not significant. However, combining hypoxia and low glucose exerts a synergistic effect on inducing both AD-associated phenotypes.

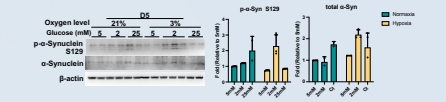


Emerging evidence suggests that, in addition to tau and amyloid beta, alpha-synuclein could be involved in AD pathology. With our model, we detected phosphorylated and total alpha-synuclein levels under different conditions. We found that both phosphorylated and total alpha-synuclein levels are induced by low glucose and further elevated by the combination of low glucose and low oxygen.

Synergistic effect of low glucose and low oxygen on inducing AD-like phenotypes was reproduced in cerebral organoids

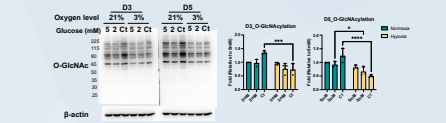


Brain organoids are three-dimensional cellular aggregates that recreate different neural cell interactions and tissue characteristics in culture. Therefore, we used organoids to verify the synergistic effect of low glucose and low oxygen observed in two-dimensional neuron culture.



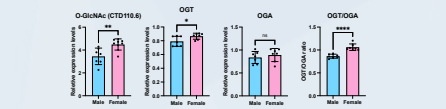
Similarly, phosphorylated and total alpha-synuclein levels are up-regulated by combining low glucose and hypoxia in cerebral organoids.

Hypoxia further reduced O-GlcNAcylation levels in low glucose-treated neurons



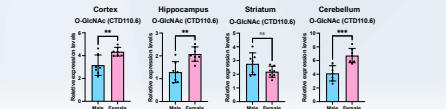
Hypoxia is an important regulator of O-GlcNAcylation. Thus, we were interested in the involvement of O-GlcNAcylation in the synergistic effect of combing hypoxia and low glucose. The result demonstrated that the O-GlcNAcylation levels decreased by low glucose treatment are further reduced by hypoxia on day 5.

Females have higher O-GlcNAcylation levels than males in mice brains



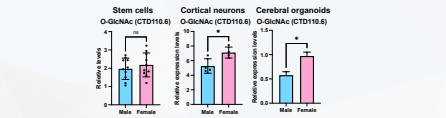
Mice brains were harvested at 6-week-old. O-GlcNAcylation levels, OGT, OGA and OGT/OGA ratio were detected by western blot. The results showed that O-GlcNAcylation, OGT, and OGT/OGA ratio are higher in females than males in mice brains.

Sex- and regional specific regulation of O-GlcNAcylation levels



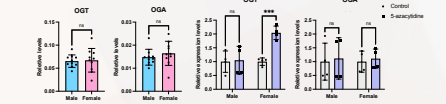
Upon more detailed regional dissection and analysis, we discovered that O-GlcNAcylation levels are higher in females within the cortex, hippocampus, and cerebellum, while males appear to have slightly higher levels of O-GlcNAcylation in the striatum.

Sex differences in O-GlcNAcylation levels in human cells



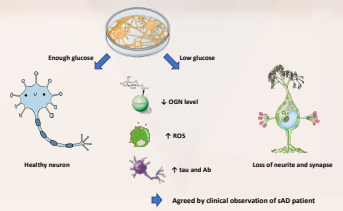
Sex differences in O-GlcNAcylation levels could also be observed in cortical neurons and cerebral organoid derived from human pluripotent stem cells but not in the stem cells themselves.

Sex differences in O-GlcNAcylation and OGT levels could be due to post-transcriptional regulation



RT-qPCR data showed that there is no significant difference of OGT and OGA expression levels between male and female neurons (A). To further confirm that OGT gene, an X-link gene, does not reactivate after neuronal differentiation, the neurons were treated with 5-Azacytidine, a DNA methylation inhibitor, and the gene expression levels of OGT and OGA were determined by RT-qPCR (B). The results suggested that OGT gene remines inactivated after neuronal differentiation, and sex-difference in OGT protein level could be due to post-transcriptional regulation.

Summary



we established a human model that mimics the main features of AD-like changes, which agrees with clinical observations of sAD patients. Our results also suggest that dysregulated O-GlcNAc might be a direct molecular link between hypometabolism and sAD-like alternations, and that O-GlcNAc could play a mechanistic role in sex and region-specific vulnerabilities to AD. Therefore, this platform can serve as a tool to better understand molecular processes involved in sAD and for drug development.