Original Article

Figure 2. ' L D E H W H V L Q G X F H G $ β_{1-42} accumulation and downregulated the expression of BDNF and MAP2. (A-B) The L P P X Q R K L V W R F K H P L F D O β_{1-42} and BDNF in CA1, CA3, and PFC of the hippocampus. (C) The immunofluorescence staining of MAP2 in CA1, CA3, and PFC. CON: control, PFC: prefrontal cortex, CA1: cornu ammon1, and CA3: cornu ammon3. 6 F D O H E D U P D Q G 1

RESULTS

Diabetes resulted in cognitive decline with inferior learning and memory function

All mice were trained to learn how to locate the platform throughout six blocks. As shown in Fig. 1A, there is a significant difference in their latency to reach the platform during the six training blocks. db/db mice took longer to reach the platform from block 2 to block 6, when

$ D O P R V 1 W Q B $ immunoreactivity was observed in the hippocampal CA1 and CA3 in mice from the CON group, but the signals were markedly more intense in the hippocampus of db/db mice. BDNF contributes to the induction of synaptogenesis, spine density, and postsynaptic receptor expression in neurons, and its expression is significantly suppressed in the brain of AD patients. Thus, we detected the expression of BDNF. As shown as Fig. 2B, BDNF was normally expressed in mice from the CON group, but its expression was markedly lower in db/db mice. Moreover, we identified a significant reduction of MAP2 immunoreactivity in the hippocampus of db/db mice when compared to mice from the CON group (Fig. 2C). These results indicate that BDNF and MAP2 expression are attenuated by diabetes.

Figure 3. The morphological structure and level of apoptosis in the hippocampus in diabetes. (A) H&E staining of CA1 and DG. (B) TUNEL staining of the hippocampus of CON mice and db/db mice. CON: control. Scale bar = 50 P D Q G 1

The morphological structure and level of apoptosis in the hippocampus of diabetic mice

Animal models of AD show necrotic changes in hippocampal neurons. In the current study, we found that hippocampal CA1 and DG exhibited a clear hippocampus structure in mice from the CON group. They displayed normal cell morphology, large round or oval-shaped nuclei, evident nucleoli, and clear nuclear membranes (Fig. 3A). In the db/db group, neuronal cells were arranged sparsely, and the cell boundaries were blurred (Fig. 3A). However, there was no visible difference on pyknosis when compared to mice from the CON group (Fig. 3A). Moreover, we also detected the level of cell apoptosis using the TUNEL assay. We found no obvious neuronal apoptosis in the hippocampus of db/db mice compared to mice from the CON group (Fig. 3B).

Autophagy was activated in the hippocampus of db/db mice

The conversion of LC3 from the soluble form (LC3-I) to the autophagosome-associated form (LC3-II) and the degradation of P62 (also known as SQSTM1) imply an increase of autophagic activation [16,17]. In the current study, we invest5(y)5(ply)-117(a)4(n)] TJ ET Q q 0.0000091

This then changes synaptic plasticity, which subsequently leads to the decline of LTP and affects the learning and memory processes [33]. Thus, we focused on the effect of diabetes on CA1 and CA3. Consistent with previous studies, the present study confirmed that diabetes induces $A\beta_{1-42}$ deposition in hippocampal CA1 and CA3 and reduces the expression of BDNF and MAP2. Our results have confirmed that diabetes impaired cognitive

complications, such as retinopathy and nephropathy [42,43]. Whether there is cross-talk between ER stress and autophagy during DACD is still unclear. A recent study demonstrated that inhibition of ER stress reversed AGEs-induced autophagy in mesangial cells, but autophagy inhibition did not influence AGEs-induced ER stress [44]. Additionally, diabetes influenced the expression of ER stress-related genes in the hippocampus, and hippocampal cells adapted to prolonged ER stress induced by T2D with partial suppression of Xbp1 [45]. Thus, we speculate that inhibition of ER stress is involved in the neuroprotective role of autophagy during DACD, but this needs to be further elucidated in future studies.

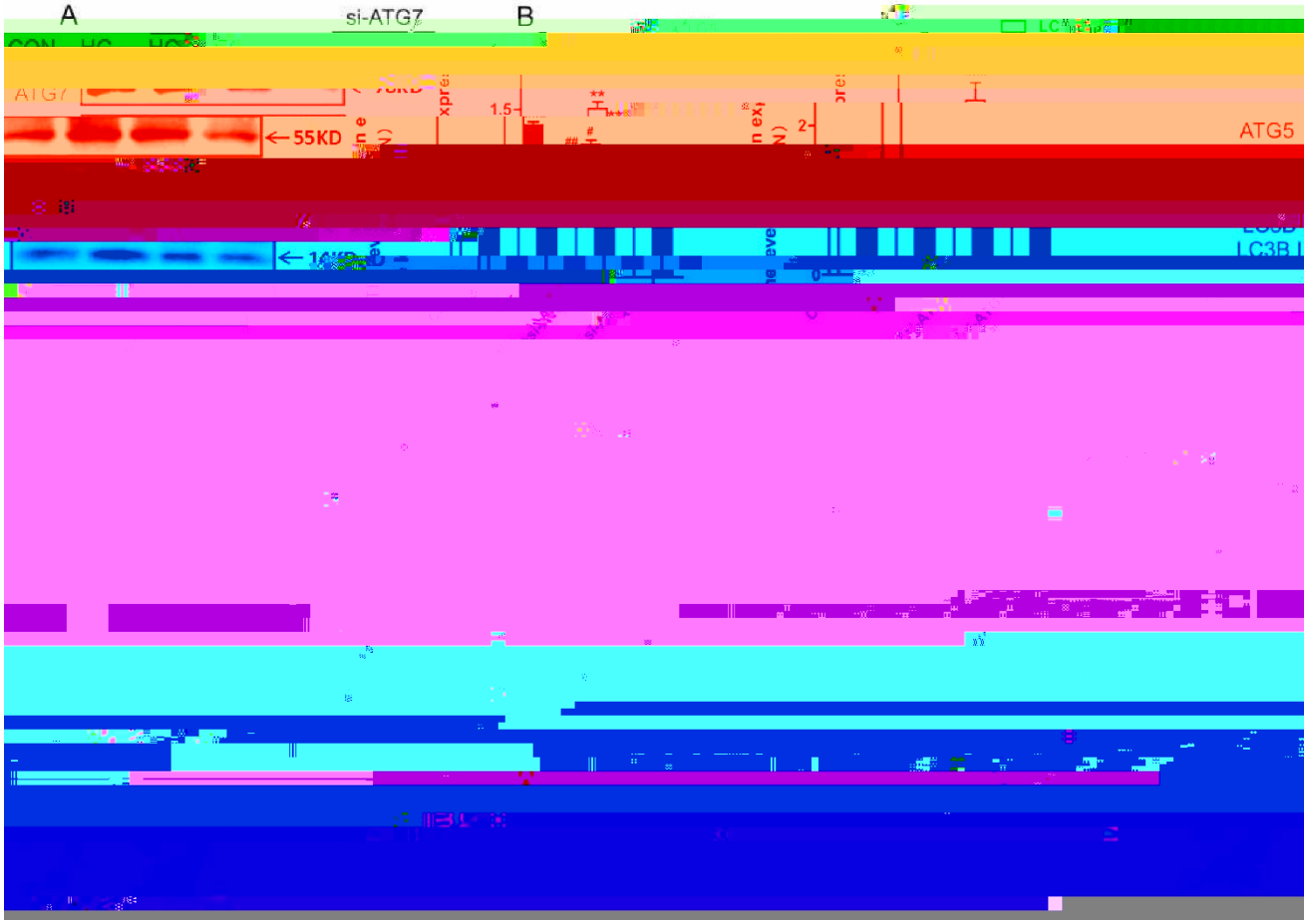

Figure 8. ATG7 siRNA treatment abolished the neuroprotective role of autophagy under HG conditions. (A) Western blot analysis of ATG7, ATG5, P62, and LC3B expression. (B) Quantitative analysis of ATG5 and ATG7. (C) Quantitative analysis of LC3B and P62. (D) Immunofluorescence staining of LC3B in PC12 cells. (E) The results of flow cytometry under different conditions. (F) The level of apoptosis in PC12 cells. CON: control, HG: high glucose. Scale bar = 50 μm. *P < 0.05 vs. CON, **P < 0.01 vs. CON, ***P < 0.001 vs. CON, #P < 0.05 vs. HG group, ##P < 0.01 vs. HG group, ###P < 0.001 vs. HG group, and N = 3.

Generally, astrocytes are considered to support and nourish neurons in the central nervous system. Recently, increasing evidence has demonstrated that astrocytes are also involved in neurotransmitter release in the presynaptic neuron, which receives and integrates the neural signals from multiple adjacent neurons [46,47]. It has also been reported that astrogliosis contributes to diabetes-associated memory impairment [48]. Using the metabonomics analytical method, it was also revealed that diabetes induces astrocyte proliferation and neuronal apoptosis in the hippocampus, and it disturbs energy metabolism and the glutamate-glutamine cycle between astrocytes and neurons [49]. The current study indicates that autophagy plays a neuroprotective role during DACD. It is well known that autophagy could be induced by a limited availability of ATP or a lack of essential nutrients, such as glucose and amino acids, and the accumulation of specific metabolites or metabolic

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