Behavioural Brain Research 305 (2016) 265–277

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Autophagy ameliorates cognitive impairment through activation of PVT1 and apoptosis in diabetes mice

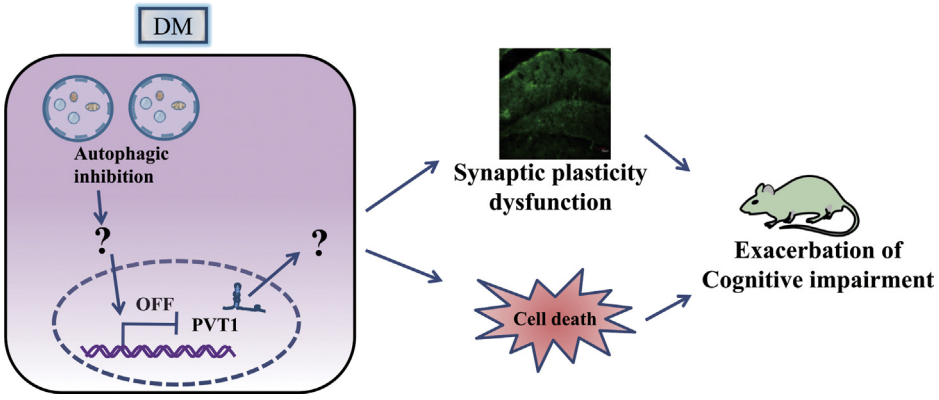
Li Zhigui, Hao Shuang, Yin Hongqiang, Gao Jing, Yang Zhuo*

College of Medicine, State Key Laboratory of Medicinal Chemical Biology, Tianjin Key Laboratory of Animal Models and Degenerative Neurological Diseases, Nankai University, Tianjin 300071, China

HIGHLIGHTS

- Autophagic inhibition exacerbated cognitive impairment in diabetic mice.
- Autophagic inhibition exacerbated dysfunction of synaptic plasticity in diabetic mice.
- PVT1 mediated autophagy induced apoptosis but not necrosis in diabetic mice.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form 24 February 2016
Accepted 9 March 2016
Available online 10 March 2016

Keywords:
Autophagy
Hippocampus
Diabetes
Cognitive impairment
Synaptic plasticity
PVT1
Long noncoding RNA
Apoptosis
Regulated necrosis

ABSTRACT

The underlying mechanisms of cognitive impairment in diabetes remain incompletely characterized. Here we show that the autophagic inhibition by 3-methyladenine (3-MA) aggravates cognitive impairment in streptozotocin-induced diabetic mice, including exacerbation of anxiety-like behaviors and aggravation in spatial learning and memory, especially the spatial reversal memory. Further neuronal function identification confirmed that both long term potentiation (LTP) and depotentiation (DPT) were exacerbated by autophagic inhibition in diabetic mice, which indicating impairment of synaptic plasticity. However, no significant change of pair-pulse facilitation (PPF) was recorded in diabetic mice with autophagic suppression compared with the diabetic mice, which indicated that presynaptic function was not affected by autophagic inhibition in diabetes. Subsequent hippocampal neuronal cell death analysis showed that the apoptotic cell death, but not the regulated necrosis, significantly increased in autophagic suppression of diabetic mice. Finally, molecular mechanism that may lead to cell death was identified. The long noncoding RNA PVT1 (plasmacytoma variant translocation 1) expression was analyzed, and data revealed that PVT1 was decreased significantly by 3-MA in diabetes. These findings show that PVT1-mediated autophagy may protect hippocampal neurons from impairment of synaptic plasticity and apoptosis, and

Abbreviation: PVT1, plasmacytoma variant translocation 1; lncRNA, long noncoding RNA; STZ, streptozotocin; 3-MA, 3-methyladenine; LTP, long term potentiation; DPT, depotentiation; PPF, pair-pulse facilitation; miRNA, microRNA; mRNA, messenger RNA; qPCR, real-time quantitative PCR; cDNA, complementary DNA; PI, propidium iodide; PBS, phosphate-buffered saline.

* Corresponding author.
E-mail address: zhuoyang@nankai.edu.cn (Z. Yang).

http://dx.doi.org/10.1016/j.bbr.2016.03.023

then ameliorates cognitive impairment in diabetes. These intriguing findings will help pave the way for exciting functional studies of autophagy in cognitive impairment and diabetes that may alter the existing paradigms.

1. Introduction

Diabetes mellitus is one of the most common serious metabolic disorders in humans. It is estimated that 382 million adults had diabetes in 2013, and that this number will reach 592 million around the world by 2035 [20]. The report estimates that health expenditure on diabetes is expected to at least USD 245 billion in US in 2012 [4]. Another report from China also reveals that 11.6% (~114 million) adults with diabetes in China in 2012 [63]. Series of studies have revealed the tight association of diabetes with central nervous system (CNS), including degeneration of learning and memory [16,54] and depression [25]. Further magnetic resonance imaging (MRI) [3,14], and computerized tomography [52] showed brain atrophy in diabetes mellitus. An even earlier study has reported structural lesions at autopsy, including axonal loss and degeneration of ganglion and cortical neurons [49].

Autophagy is a major protein degradation system that involves the degradation of cellular components through the lysosomal machinery. It includes three main forms: chaperone-mediated autophagy, microautophagy, and macroautophagy. It plays a pivotal role in cell growth, development and homeostasis, where it helps to maintain a balance between the synthesis, degradation and subsequent recycling of cellular components [39]. Researchers have revealed a key role of autophagy in neurodegenerative disease such as Alzheimer's disease (AD), in which autophagy functioned as a scavenger to clean up intracellular accumulated amyloid beta protein (Abeta) that form clumps in brain [47,64]. Perturbation of autophagy in different stages of Alzheimer disease (AD) leads to amelioration or exacerbation of AD [58]. In addition, other researches on diabetes mellitus (DM) have shown a contribution of autophagy, in which autophagy usually functioned as a protector [5,18]. However, Details have been lacking on the precise functional significance of autophagy in cognition in DM.

Long non-coding RNAs (lncRNAs) are transcribed RNA molecules (>200 nt in length) with no protein-coding capacity, and lack appreciable opening reading frames. As for functional paradigms, lncRNAs regulate cellular processes via a number of molecular mechanisms. These include regulation of epigenetics [51], transcription regulation [40,66], translational or posttranslational regulation [15,17]. Several recent studies suggest that lncRNA dysfunction is closely associated with neurodevelopmental and cognitive disorders, including Rett syndrome [46], autism [67], schizophrenia [6,37] and Fragile X syndrome [43]. In addition, a number of studies have also shown links between lncRNA expression and anxiety-like behavior [12,34]; [53], drug abuse [10,36] and suicidal behavior [48].

Despite these correlative links, little is known about the cognitive function of autophagy in DM, nor the lncRNA-mediated mechanisms, by which these transcripts influence cell fate within the context of DM. Here we determined the effect of autophagic inhibition on anxiety-like behaviors and spatial learning and memory, especially the spatial reversal memory in streptozotocin-induced diabetic mice. Further PPF, LTP and DPT were recorded to evaluate the synaptic plasticity by autophagic inhibition in DM. Subsequent hippocampal neuronal cell death, including apoptosis and regulated necrosis, was identified. Finally, we determined the long non-coding RNA PVT1 expression, which may contribute to the neuronal dysfunction and cognitive impairment in DM. Our aims were to elucidate a possible mechanism in the autophagic process in DM and to manipulate the autophagic related pathway in an effort to develop a novel therapeutic strategy for utilizing autophagy against diabetic cognitive impairment.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the local ethical committee at Nankai University, and met the guidelines of the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Documentation 55, 2001). All efforts were made to minimize the number and suffering of animals used for the experiments.

Experiments were conducted with male C57BL/6J 6-week-old mice (Experimental Animal Center of the Chinese Academy Medical Sciences, Beijing, China), weighing 20–25 g. Mice were maintained in a temperature-controlled (24 ± 1 °C) facility room with a 12-h light-dark cycle and were given free access to food and water, unless stated. Animals were housed in specific pathogen-free environment.

2.2. Animal models and treatment

Animals were kept in the animal house for one week to acclimate well before starting the experiment. Subsequently, animals were divided into two groups randomly: the control group (Con, n = 22) and the STZ-injected group (DM, n = 22). A low-dose mouse model of STZ-induced type 1 diabetes mellitus was carried out as previously described in the literature [55].

Before each experiment, aliquots of STZ were preweighed into plastic microfuge tubes, which were then wrapped in aluminum foil (to protect from light) and stored at −20 °C until use. Sodium citrate buffer (10 mmol/L, pH 4.5) was prepared by dissolving 147 mg of trisodium citrate in 49.5 mL of normal saline and adjusting the pH to 4.5 with 1 mol/L citric acid. The citrate buffer should be used fresh or frozen in aliquots and stored at −20 °C. After thawing, each vial of frozen citrate buffer should be used immediately and the remaining contents discarded.

Animals were fasted for 6 h prior to injection. To induce diabetes, a microfuge tube containing preweighed STZ was mixed immediately before being used with a predetermined volume of sodium citrate buffer to produce a final concentration of 7 mg/mL before use. The microfuge tube containing solution was wrapped in aluminum foil and kept on ice immediately. This solution was then injected intraperitoneally into each prestarved mouse at 55 mg/kg using a 29G insulin needle. STZ degraded quickly in aqueous solutions and should be administered rapidly to obtain the best experimental results. Any remaining contents should be discarded according to the safety protocols of the researcher's institute. This procedure was repeated so that each mouse received one STZ injection for five consecutive days. Control group was injected intraperitoneally with sodium citrate buffer at the same dose. One week after the final STZ injection, animals with fasting blood glucose less than 15 mmol/L (280 mg/dL) were not considered diabetic.