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Research article

Oxygen-glucose deprivation inducing B1 RNA inhibits neuronal cells metabolic activity by NLRP3 and associated proinflammatory cytokines production

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HIGHLIGHTS

- Double-stranded RNAs, B1 and B2, are increased in neuronal cells undergoing OGD.
- OGD and exogenous B1 RNA promote NLRP3, IL-1β and TNF-α production in HT22 cells.
- NLRP3 knockdown blocks the cellular effects induced by B1 RNA and OGD.
- B1 RNA and NLRP3 mediate the cellular effects in neuronal cells within OGD treatment.

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ABSTRACT

Cerebral ischemia occurs when blood flow to part of the brain is obstructed, which can result in oxygen and glucose deprivation (OGD) and neuronal damage. However, the mechanisms remain poorly understood. The present study investigated the production and effects of double-stranded RNA (dsRNA) induced by OGD in neuronal cells. By confocal microscopy, dsRNA containing B1 and B2 RNA, was found accumulating in HT22 cells under OGD treatment. The sequence of B1 RNA was identified and transfected into HT22 cells. Interestingly, B1 RNA induced transcription and expression of NLRP3, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α, which was similar to the effects of OGD treatment. Moreover, HT22 cell growth inhibition and proinflammatory cytokines production induced by OGD and B1 RNA treatment were down-regulated by NLRP3 knock-down. These findings suggest that B1 RNA induced by OGD forms as dsRNA and inhibits neuronal cell metabolic activity by regulating the NLRP3 and associated proinflammatory cytokines production.

1. Introduction

Cerebral ischemia (stroke) is an extreme condition of metabolic stress originating from OGD that most commonly occurs when the blood supply to part of the brain is suddenly blocked [3]. This cerebral ischemic injury leads to the oxidative stress, inflammatory reaction, and eventually cell death. Until recently, no effective treatment could be used to promote recovery from stroke. Therefore, the understanding of the molecular mechanisms of cellular damage involved is an important focus of research to find new strategies to tackle this damage.

It has been widely accepted that inflammation and apoptosis are primarily involved in the pathophysiology of cerebral ischemia

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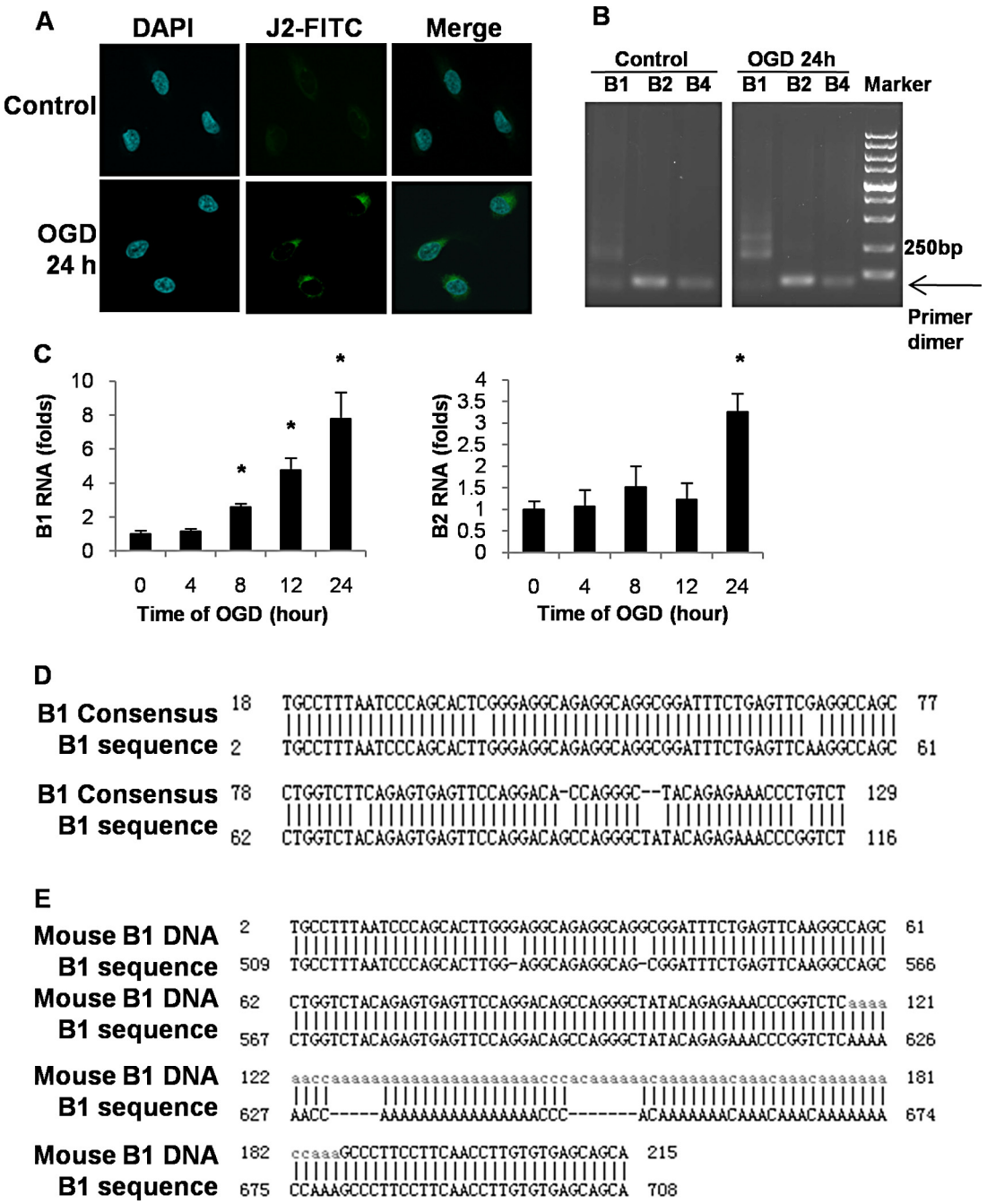

Fig. 1. dsRNA was induced by OGD in HT22 cells.
(A) HT22 cells were treated by OGD for 24 h. The dsRNA was stained by J2 antibody labeled with FITC. DAPI was used to indicate the location of nuclei. (B) IP assay was performed using J2 antibody, which binds to dsRNA specifically. B1, B2 and B4 RNAs were amplified by RT-PCR assay using the cDNA from IP assay elution as a template. The PCR production of B1 RNA was a smear from about 200 bp. Control is untreated HT22 cells, OGD 24 h is OGD treated HT22 cells for 24 h. (C) qRT-PCR was used to detect the changes of B1 and B2 RNA level in HT22 cells after OGD induction. Values are mean ± SEM from at least four independent experiments. $^{*}P < 0.05$ compared with 0 h. (D) BLAST results of the sequence of B1 DNA (B1 sequence) from B1 RNA induced by OGD with B1Consensus using http://blast.ncbi.nlm.nih.gov/Blast.cgi [15] showed they were 94% identical. (E) BLAST results of the sequence of B1 DNA with mouse B1 DNA (mouse repetitive sequence B1 DNA, GenBank: M18260.1) by http://blast.ncbi.nlm.nih.gov/Blast.cgi showed they were 93% identical.

[10]. Soon after cerebral ischemia, proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 are produced and secreted from brain cells of injured tissue and mediate the inflammation and result in the injury of ischemic cerebral tissues [2,4]. The NLRP3 inflammasome has been identified as a crucial complex in the pathology of cerebral cell damage [16]. The NLRP3 inflammasome promotes the activation of caspase-1, which cleaves pro- IL-1β and pro- IL-18 into secreted forms which prime receptor activation, specifically mediating the inflammation damages of various cells [1,15]. However, the activation of the NLRP3 inflammasome is not fully understood in ischemic cerebral injury.

Short interspersed elements (SINEs) were thought of as "junk DNA," and were primarily used for analyzing phylogenetic relationships between organisms and speciation probing [12]. The SINEs family includes Alu (about 11% in human [13]), B1, and B2 retroelements (in mouse) [8]. B1 sequence is shown to be scattered throughout the whole genome of mouse, and only about a quarter has the double-stranded configuration in pre-mRNA [8]. B2 RNA