主論文の要約

Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes

（サーチュイン１はアストロサイトのスーパーオキシドジスムターゼ２とカタラーゼの発現上昇を介して酸化ストレスを低減する）

名古屋大学大学院医学系研究科　細胞情報医学専攻
高次神経統御学講座　神経免疫学分野
（指導：錫村 明生　教授）
程　祎

Introduction

Sirtuin 1 (SIRT1) is a NAD-dependent histone deacetylase involved in induction of lifespan extension by calorie restriction (CR) in many organisms, including yeast, worms, flies, and mice. SIRT1 suppresses pro-apoptotic factors and pro-inflammatory factors by downregulating p53 and nuclear factor-kappa B (NF-κB), whereas it upregulates a select set of proteins related to energy metabolism and pro-survival signals, such as peroxisome proliferator–activated receptor-gamma coactivator 1 alpha (PGC-1α) and forkhead box class O transcription factors (FOXOs). These transcription factors activate antioxidant enzymes such as superoxide dismutase 2 (SOD2) and catalase to reduce levels of reactive oxygen species (ROS).

Astrocytes are the most abundant cells in the central nervous system (CNS) and play a crucial role in the maintenance of CNS homeostasis. Astrocytes exert neuroprotective functions during CNS trauma, stroke and cerebrovascular disease, infection, seizure disorders, multiple sclerosis, autoimmune inflammatory disorders, and neurodegenerative disease. Expression of SIRT1 in neurons contributes to neuroprotective functions, but the precise roles of astrocytic SIRT1 are still unclear. In this study, we investigated the roles of astrocytic SIRT1 in anti-inflammatory and anti-oxidant functions. Our findings suggest that astrocytic SIRT1 may be a potential therapeutic target for treatment of neurologic diseases such as multiple sclerosis and Alzheimer's disease.

Methods

The protocols for animal experiments were approved by the Animal Experiment Committee of Nagoya University. Mouse primary astrocytes (the purity > 95%) were prepared from newborn C57BL/6J mice. To mimic calorie restriction *in vitro*, astrocytes were treated with glucose deprivation. To inhibit SIRT1, cells were treated with SIRT1 inhibitor III (Calbiochem). Expression levels of mRNAs were evaluated by TaqMan quantitative PCR (qPCR) (Applied Biosystems). The protein expression levels were examined by Western blotting. The acetylation of transcriptional factors were detected by Immunoprecipitation and Western blotting. The ratio of NAD+ to NADH in astrocytes was determined using the NAD+/NADH Quantification Kit (BioVision). MitoSOX™ and CellROX™ fluorescence dyes (Invitrogen) were used to detect astrocytic production of superoxide and ROS, respectively. Statistical significance was analyzed with Student's *t*-test or one-way analysis of variance followed by post-hoc Tukey's test, using GraphPad Prism version 5.0 (GraphPad Software).

Results

We first sought to determine whether glucose deprivation activates the NAD-SIRT1 pathway in astrocytes. As shown in Fig. 1A, the NAD+/NADH ratio in astrocytes increased significantly, in a time-dependent manner, upon glucose deprivation. Glucose deprivation also significantly upregulated SIRT1 both in mRNA expression level (Fig. 1B) and protein level (Fig. 1C).

We next investigated whether SIRT1 upregulation affects the pro-inflammatory function of astrocytes under pathologic conditions. Whereas LPS stimulation increased the expression levels of inflammatory cytokines such as TNF-α and IL-1β, glucose deprivation dramatically downregulated these cytokines (Fig. 2A and B). The effects of glucose deprivation were partially reversed by addition of SIRT1 inhibitor (Fig. 2A and B). Next, we investigated whether SIRT1 activation affects ROS production in astrocytes. When astrocytes were deprived of glucose, ROS production did not detectably change (Fig. 3A and B). However, the addition of SIRT1 inhibitor significantly increased ROS production (Fig. 3A and B). Previous studies have shown that glucose deprivation itself induced dual and opposing effects on ROS production, and SIRT1 inhibition revealed the effect of SIRT1 under glucose deprivation. Taken together, these results indicated that SIRT1 is an important negative regulator of inflammation and oxidative stress in astrocytes.

Glucose deprivation significantly increased the expression levels of SOD2 and catalase; addition of SIRT1 inhibitor significantly downregulated these proteins (Fig. 4). These data indicated that SOD2 and catalase are the main downstream effectors by which SIRT1 activity suppresses ROS production in astrocytes.

SIRT1 deacetylates histone and non-histone substrates, including PGC-1α, FOXO1, FOXO3a, and FOXO4. Therefore, we examined the acetylation of these proteins under glucose deprivation and SIRT1 inhibition. Glucose deprivation did not obviously influence FOXO4 acetylation, but the addition of SIRT1 inhibitor significantly increased FOXO4 acetylation (Fig. 5). By contrast, SIRT1 did not affect deacetylation of PGC-1α, FOXO1, or FOXO3a (data not shown). Therefore, our data suggested that FOXO4 deacetylation is downstream of SIRT1 in astrocytes.

Discussion

In this study, we showed that SIRT1 attenuates oxidative stress and inflammation in astrocytes via upregulation of the antioxidant enzymes SOD2 and catalase. As shown in Fig. 6, signals from calorie restriction increase the NAD+/NADH ratio, leading to upregulation of SIRT1. SIRT1 deacetylates FOXO4, which upregulates SOD2 and catalase activities, leading ultimately to suppression of ROS production. Excessive ROS production has been implicated in a variety of neurologic disorders such as Alzheimer's disease, Parkinson's disease, and other neurodegenerative diseases, as well as in aging itself. Antioxidant therapies have been proven effective against neuronal damage in these diseases. Therefore, our results underscore the potential therapeutic value of astrocytic SIRT1 in protecting against oxidative stress in the CNS.

Conclusion

This study demonstrated that SIRT1 suppresses ROS production in astrocytes via deacetylation of FOXO4, leading to upregulation of SOD2 and catalase, suggesting that

astrocytic SIRT1 may play a role in neuroprotection by exerting anti-oxidative and anti-inflammatory effects.