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PARIS reprograms glucose metabolism by HIF-1α induction in dopaminergic neurodegeneration

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ABSTRACT

Our previous study found that PARIS (ZNF746) transcriptionally suppressed transketolase (TKT), a key enzyme in pentose phosphate pathway (PPP) in the substantia nigra (SN) of AAV-PARIS injected mice. In this study, we revealed that PARIS overexpression reprogrammed glucose metabolic pathway, leading to the increment of glycolytic proteins along with TKT reduction in the SN of AAV-PARIS injected mice. Knock-down of TKT in differentiated SH-SY5Y cells led to an increase of glycolytic enzymes and decrease of PPP-related enzymes whereas overexpression of TKT restored PARIS-mediated glucose metabolic shift, suggesting that glucose metabolic alteration by PARIS is TKT-dependent. Inhibition of PPP by either PARIS overexpression or TKT knock-down elevated the level of H_2O_2, and diminished NADPH and GSH levels, ultimately triggering the induction of HIF-1α, a master activator of glycolysis. In addition, TKT inhibition by stereotaxic injection of oxythiamine demonstrated slight decrement of dopaminergic neurons (DNs) in SN but not cortical neurons in the cortex, suggesting that TKT might be a survival factor of DNs. In differentiated SH-SY5Y, cell toxicity by GFP-PARIS was partially restored by introduction of Flag-TKT and siRNA-HIF-1α. We also observed the increase of HIF-1α and glycolytic hexokinase 2 in the SN of Parkinson's disease patients. Taken together, these results suggest that PARIS accumulation might distort the balance of glucose metabolism, providing clues for understanding mechanism underlying selective DNs death by PARIS.

1. Introduction

The selective loss of dopaminergic neurons (DNs) in the substantia nigra pars compacta (SNpc) is a hallmark of Parkinson's disease (PD), characterized by non-motor and motor disorder [1]. In order to understand the mechanism underlying selective DNs toxicity, the researchers have been studying several PD-associated genes, including parkin, α-synuclein, LRRK2, DJ-1, PINK1, and ATP13A2 [2]. Our previous study demonstrated that the accumulation of PARIS (PARkin-Interacting Substrate) was observed in the SN of conditional parkin knockout mice and post-mortem PD patient brains, contributing to DNs degeneration and mitochondrial dysfunction [3,4]. In order to understand how PARIS achieved brain region-specific toxicity, we applied LC-MS/MS-based quantitative proteomic analysis to profile the global proteomic alteration in the cortex, striatum, and SN of AAV-PARIS injected mice, revealing that PARIS transcriptionally suppressed transketolase (TKT) in SN-specific manner [5].

TKT is a key enzyme in the non-oxidative branch of the pentose phosphate pathway (PPP), which is responsible of producing NADPH and pentoses (5-carbon sugars) [6]. Since TKT is a key enzyme for maintaining NADPH level and connects the PPP to glycolysis, its malfunction has been linked to many diseases such as cancer, diabetes, Alzheimer's disease, and Wernicke-Korsakoff's syndrome, a latent genetic neurological disorder [7–10]. In DNs, the increased levels of reactive oxygen species (ROS) are thought to be generated during dopamine metabolism and exacerbated by low levels of reduced glutathione (GSH) [11]. Since NADPH is required

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for reducing glutathione and antioxidant process, TKT seems to be important to prevent oxidative stress in neuronal survival [12]. Notably, accumulating evidence suggests that glucose metabolism is perturbed in PD, *i.e.* PPP dysregulation was observed in post-mortem sporadic PD brain samples [13].

In this study, we found that the reduction of TKT by PARIS accumulation led to the disruption of PPP enzymes as well as increase of glycolytic proteins. These findings suggest that PARIS might be involved in metabolic reprogramming via TKT suppression, contributing to PARIS-mediated selective DNs death in PD pathogenesis.

2. Material and methods

2.1. Antibodies

The primary antibodies used: rabbit anti-TKT (sc-67120, Santa Cruz), rabbit anti-G6PD (sc-67165, Santa Cruz), mouse anti-PGD (sc-398977, Santa Cruz), rabbit anti-GPI (sc-33777, Santa Cruz), rabbit anti-HIF-1α (sc-10790, Santa Cruz), mouse anti-GFP (sc-9996, Santa Cruz), rabbit anti-HK2, PFKP, LDHA (Glycolysis Antibody Sampler Kit, 8337, Cell signaling), mouse anti-PARIS (MABN476, Millipore), mouse anti-Flag (F3165, Sigma), and mouse anti-actin-HRP (ab49900, Abcam).

2.2. Brain tissues from mouse and PD patients

Sungkyunkwan University Ethical Committee approved all animal experiments in accordance with international guidelines. Information of the brain samples of PD patients was previously described [5].

2.3. Stereotaxic injection

Eight weeks old male C57BL/6N mice (Orient) were used for stereotaxic injection of AAV-GFP or AAV-PARIS. For oxythiamine injection, 1 μl of 50 mM oxythiamine in PBS (O4000, Sigma) was injected into SN and CTX of 8-week old male C57BL/6N mice. Additional details were previously described [5].

2.4. Cell culture

Human neuroblastoma SH-SY5Y cells were purchased from KCLB (https://cellbank.snu.ac.kr) and cultured in DMEM (10% FBS and 1% (v/v) penicillin/streptomycin) in a humidified 5% CO_2/95% air atmosphere at 37 °C. To differentiate SH-SY5Y cells, we used an existing protocol [14].

2.5. Vectors and transfection

Flag-TKT plasmid was generously given by Dr. Gerard Boss (University of California San Diego). GFP-PARIS and Flag-PARIS vectors were described previously [3]. Indicated plasmids (Flag-TKT, GFP-PARIS) were transfected to cells using Lipofectamine 2000 (11668027, Thermo) according to manufacturer's protocol. siRNA-TKT (sc-45591) and siRNA-HIF-1α (sc-35561) were purchased from Santa Cruz. Efficiency of RNA interference was confirmed by qRT-PCR and immunoblot analysis.

2.6. Real-time quantitative PCR (qRT-PCR)

Total RNA of cells or tissue was extracted with Trizol (15596018, Thermo). mRNA expressions were quantified by Rotor-gene Q and Rotor-gene SYBR green PCR kit (Qiagen). Semi-quantitative PCR was performed for post-mortem tissues due to poor dissociation curve in qRT-PCR. The primer sequences are available in Supplementary Table S1.

2.7. Measurement of NADPH, GSH, and H_2O_2 levels

NADPH assay was performed according to manufacturer's instruction (AS-72205, Anaspec). Briefly, Extraction buffer was used for cell lysis and supernatant was collected to monitor NADPH level at 565 nm absorbance. GSH levels were measured according to the manufacture's protocol (703002, Cayman). Sample was lysed with ice-cold buffer (50 mM phosphate, 1 mM EDTA). Deproteinization was performed by PCA. Supernatant was used to check GSH level at 414 nm absorbance.

H_2O_2 assay was performed using H_2O_2 Colorimetric/Fluorometric Assay Kit (K265-200, Biovision). Samples lysates were centrifuged at 13,000 g for 5 min at 4 °C and supernatants were deproteinized by PCA. H_2O_2 levels were measured by 570 nm absorbance.

2.8. Cell viability analysis

Differentiated SH-SY5Y cells were transfected with GFP-PARIS, Flag-TKT, and siRNA-HIF-1α as indicated. Eve™ (nanoENTEK) was used to count live and dead cells as described [15].

2.9. Immunohistochemical analysis

To perform immunohistochemistry, mice were perfused with 4% paraformaldehyde and brain was sliced with 35 μm thickness. For tyrosine hydrolase (TH) staining, coronal sections were incubated with rabbit anti-TH (NB300-109, Novus) at 4 °C for overnight, followed by biotinylated anti-rabbit IgG for 1 h at RT. Next, sections were incubated with streptavidin-conjugated HRP (PK-4000, Vector Laboratories) and visualized with 3,3′-diaminobenzidine (D4293, Sigma). For Nissl staining, mouse brain slices were dehydrated and stained by 0.1% cresyl violet (C5042, Sigma) and washed by alcohol and distilled water.

2.10. Statistics

Statistical comparisons were performed by GraphPad Prism version 5.03. Quantitative data were expressed as the mean ± SEM. Statistical significance was tested by using an unpaired two-tailed Student's t-test or an ANOVA test with Tukey's post-hoc analysis. Differences were considered significant at $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

3. Results

3.1. PARIS accumulation reprograms glucose metabolism

As previously shown, we observed that PARIS transcriptionally suppressed TKT in SN-specific manner [5]. TKT is a crucial metabolic enzyme in the PPP for the production of NADPH and connects the PPP to glycolytic pathway [16]. Therefore, we determined the levels of glucose metabolism-related proteins to evaluate if glucose metabolic enzymes are responsive to PARIS overexpression: glycolysis enzymes - hexokinase 2 (HK2), glucose-6-phosphate isomerase (GPI), phosphofructokinase (PFKP), and lactate dehydrogenase A (LDHA), PPP enzymes - glucose-6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase (PGD), and TKT (Fig. 1A).

We monitored protein expressions of glucose metabolism in the SN and CTX of AAV-PARIS injected mice, revealing that glycolytic and PPP proteins were up- and down-regulated in the SN of AAV-

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