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Glyceraldehyde-3-Phosphate, a Glycolytic Intermediate, Prevents Cells from Apoptosis by Lowering *S*-Nitrosylation of Glyceraldehyde-3-Phosphate Dehydrogenase

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Glyceraldehyde-3-phosphate (G-3-P), the substrate of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is a key intermediate in several metabolic pathways. Recently, we reported that G-3-P directly inhibits caspase-3 activity in a reversible noncompetitive mode, suggesting the intracellular G-3-P level as a cell fate decision factor. It has been known that apoptotic stimuli induce the generation of NO, and NO *S*-nitrosylates GAPDH at the catalytic cysteine residue, which confers GAPDH the ability to bind to Siah-1, an E3 ubiquitin ligase. The GAPDH–Siah-1 complex is translocated into the nucleus and subsequently triggers the apoptotic process. Here, we clearly showed that intracellular G-3-P protects GAPDH from *S*-nitrosylation at above a certain level, and consequently maintains the cell survival. In case G-3-P drops below a certain level as a result of exposure to specific stimuli, G-3-P cannot inhibit *S*-nitrosylation of GAPDH anymore, and consequently GAPDH translocates with Siah-1 into the nucleus. Based on these results, we suggest that G-3-P functions as a molecule switch between cell survival and apoptosis by regulating *S*-nitrosylation of GAPDH.

Keywords: Apoptosis, GAPDH, glyceraldehyde-3-phosphate, *S*-nitrosylation

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme in the glycolytic pathway and commonly used as a loading control in gene expression and protein studies [3]. However, it has been known that GAPDH is a multifunctional protein with various intracellular localizations and performs diverse activities independent of its traditional role in glycolysis [4–6]. These new functions include regulation of the cytoskeleton, membrane fusion and transport, and glutamate accumulation into presynaptic vesicles. A role of GAPDH in the nucleus is also suggested on the basis of its ability to activate transcription in neurons, export nuclear RNA, and affect DNA repair [12]. Recently, it has been reported that GAPDH is *S*-nitrosylated at the catalytic cysteine residue upon exposure to stressors [13, 15], resulting in the loss of catalytic activity but conferring the ability to bind to Siah-1, an E3 ubiquitin ligase. Siah-1 has a nuclear localization signal and escorts GAPDH to the nucleus where GAPDH increases the stability of Siah-1. This enables Siah-1 to degrade various protein targets eliciting cell death. Recently, we reported that glyceraldehyde-3-phosphate (G-3-P), a substrate of GAPDH, plays a crucial role as a cell fate decision factor *via* directly inhibiting caspase activity [7]. We proposed G-3-P as a potent molecular switch determining the cell fate. In this study, we investigated whether G-3-P has influence on GAPDH nitrosylation, since G-3-P is a substrate of GAPDH and nitrosylation occurs in the catalytic cysteine residue.

As an initial step, we checked the GAPDH localization upon apoptotic stimuli using subcellular fractionation (Fig. 1A) and confocal microscopic analysis (Fig. 1B). As shown, G-3-P suppressed the translocation of GAPDH into the nucleus upon apoptotic stimuli using etoposide. Deprenyl (selegiline, hereafter designated deprenyl) used in the therapy of Parkinson's disease (PD) also prevents the *S*-nitrosylation of GAPDH and subsequently represses the

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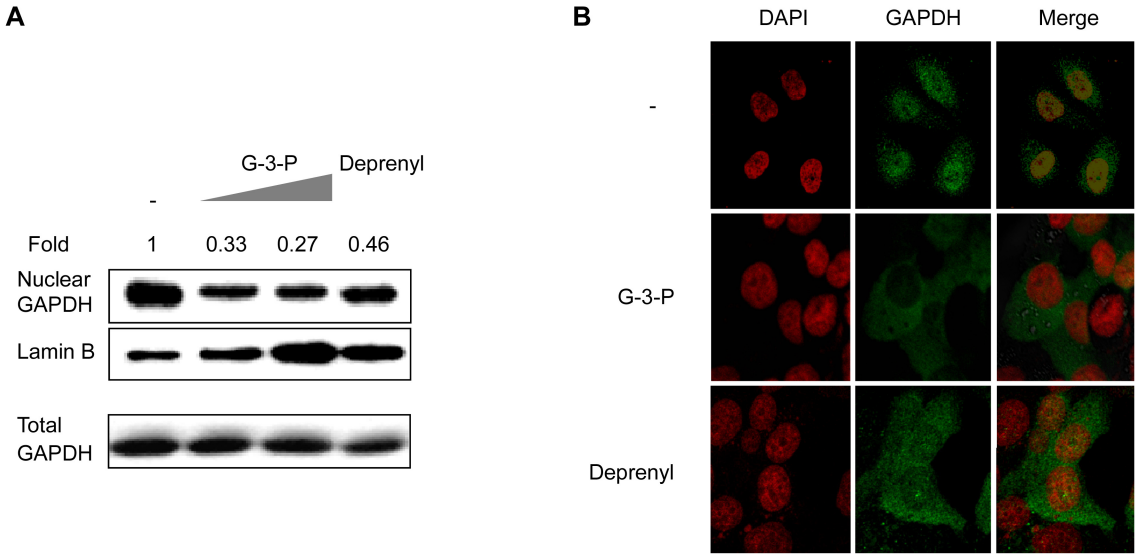

Fig. 1. Inhibitory effect of G-3-P on translocation of GAPDH into the nucleus after apoptotic stimuli.
(**A**) Decrease in the nuclear GAPDH level by G-3-P treatment. The added G-3-P concentrations were 0.5 and 1 mM. The nuclear GAPDH level was monitored by Western blot analysis with anti-GAPDH antibody after subcellular fractionation. Lamin B served as a nuclear-specific marker. Deprenyl treatment was used as a positive control for inhibitory effect of GAPDH nuclear translocation. (**B**) The nuclear GAPDH level was assessed after treatment with G-3-P by confocal microscopic analysis using anti-GAPDH antibody. Figures are partially adapted from Ref. [7] with permission.

nuclear translocation of GAPDH (Fig. 1) [5]. Next, we examined the effect of G-3-P on *in vitro* nitrosylation of GAPDH. GST-tagged GAPDH was purified through a glutathione-Sepharose column (GE HealthCare). The purified GAPDH was preincubated with G-3-P or deprenyl in 1 ml of binding buffer (50 mM Tris, 150 mM NaCl, pH 7.4) at 4°C for 2 h. After that, *S*-nitrosoglutathione (GSNO) (final 2 mM), a NO donor, was added and incubated for 2 h. Then, *S*-nitrosylation biotin switch assay was performed as described previously [5]. As shown, the preincubation of G-3-P with GAPDH prevented the *S*-nitrosylation of GAPDH, like deprenyl (Fig. 2A). Next, we investigated the effect on the interaction between GAPDH and Siah-1 by treatment with G-3-P. Flag-tagged Siah-1 was transfected into HeLa cells and then immunoprecipitated by Flag-antibody after treatment with etoposide. The Western blotting using anti-GAPDH antibody clearly showed that G-3-P as well as deprenyl significantly suppressed the interaction between GAPDH and Siah-1 (Fig. 2B). As shown, *S*-nitrosylated GAPDH only interacted with Siah-1. These results clearly suggested that G-3-P, in the resting state, protects cell from death *via* lowering of the *S*-

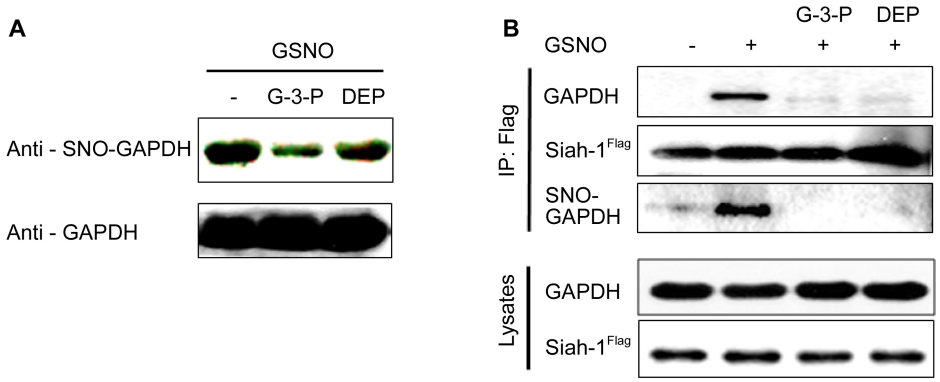

Fig. 2. G-3-P suppresses the *S*-nitrosylation of GAPDH.
(**A**) G-3-P prevents GAPDH from *S*-nitrosylation upon treatment with GSNO. The purified recombinant GST-GAPDH was preincubated with G-3-P (final 1 mM) or deprenyl, and then GSNO (final 2 mM) was added. After 2 h, the level of GAPDH *S*-nitrosylation was monitored using the biotin switch assay [5]. (**B**) G-3-P lowers *S*-nitrosylation of GAPDH and thereby reduces the interaction with Siah-1. The Flag-tagged Siah-1 was transfected into HeLa cells. After immunoprecipitation using Flag-antibody, Western blot analysis was carried out using anti-GAPDH. To assess the *S*-nitrosylation level of GAPDH, the biotin switch assay was also performed.

nitrosylation level of GAPDH and thereby inhibits translocation of GAPDH with Siah-1 into the nucleus. In case G-3-P drops below a certain level as a result of exposure to apoptotic stimuli, the *S*-nitrosylation of GAPDH occurs and apoptosis begins.

In conjunction with earlier report [7], the present results suggest that the G-3-P level is a key indicator of cell energy level status and plays crucial roles as a cell fate decision factor *via* (i) directly inhibiting the enzymatic activity of caspase(s); and (ii) lowering the *S*-nitrosylation level of GAPDH. Apoptosis is a highly regulated process leading to cell death and contains a number of ATP-dependent steps, including caspase activation, formation of bleb and apoptotic body, and enzymatic hydrolysis of macromolecules [1, 8, 10]. Therefore, it is reasonable that there is a link between apoptosis and cellular energy metabolism. In this study, we suggest that G-3-P is the indicator of the cell metabolism state and plays a role as a critical factor for determining cell fate.

Dysregulation of apoptosis can lead to a number of pathological conditions including cancers and neurodegenerative diseases [2, 9]. Furthermore, the pathological roles for nuclear GAPDH have been suggested in several neurodegenerative disorders [12, 14, 15]. Accumulation of nuclear GAPDH was found in fibroblasts and postmortem brains of patients with Huntington's disease, Parkinson's disease (PD), and Alzheimer's disease (AD) [3, 11]. Thus, the effective inhibition of abnormal apoptosis is one of the methods for treatment of neurodegenerative diseases. Therefore, G-3-P and its derivatives could be used as candidate drugs for the treatment of degenerative diseases.

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REFERENCES

1. Bao, Q. and Y. Shi. 2007. Apoptosome: A platform for the activation of initiator caspases. *Cell Death Diff.* **14:** 56–65.
2. Brown, G. C. 2010. Nitric oxide and neuronal death. *Nitric Oxide* **23:** 153–165.
3. Chuang, D.-M., C. Hough, and V. V. Senatorov. 2005. Glyceraldehyde-3-phosphate dehydrogenase, apoptosis, and neurodegenerative diseases. *Annu. Rev. Pharmacol. Toxicol.* **45:** 269–290.
4. Hara, M. R., M. B. Cascio, and A. Sawa. 2006. GAPDH as a sensor of NO stress. *Biochim. Biophys. Acta* **1762:** 502–509.
5. Hara, M. R., B. Thomas, M. B. Cascio, B.-I. Bae, L. D. Hester, V. L. Dawson, *et al*. 2006. Neuroprotection by pharmacological blockade of the GAPDH death cascade. *Proc. Natl. Acad. Sci. USA* **103:** 3887–3889.
6. Ishitani, R., K. Sunaga, A. Hirano, P. Saunders, N. Katsube, M. Tanaka, and D. M. Chuang. 1996. Evidence that glyceraldehyde-3-phosphate dehydrogenase is involved in age-induced apoptosis in mature cerebellar granule neurons in culture. *J. Neurochem*. **66:** 928–935.
7. Jang, M., H. J. Kang, S. Y. Lee, S. J. Chung, S. Kang, S. W. Chi, *et al*. 2009. Glyceraldehyde-3-phosphate, a glycolytic intermediate, plays a key role in controlling cell fate *via* inhibition of caspase activity. *Mol. Cells* **28:** 559–563.
8. Jang, M., B. C. Park, S. Kang, S.-W. Chi, S. Cho, S. J. Chung, *et al*. 2009. Far upstream element-binding protein-1, a novel caspase substrate, acts as a cross-talker between apoptosis and the *c-myc* oncogene. *Oncogene* **28:** 1529–1536.
9. Kim, E. Y., T.-S. Yoon, Y. J. Bahn, D. G. Jeong, M. R. Park, S. J. Chung, *et al*. 2010. Proteomic analysis of oxidative stress-induced neuronal cell death by two-dimensional fluorescence difference gel electrophoresis. *Int. J. Mol. Med.* **26:** 829–835.
10. Kumar, S. 2007. Caspase function in programmed cell death. *Cell Death Diff.* **14:** 32–43.
11. Li, C., Z. Xiao, X. Zhang, J. Li, X. Li, H. Yi, *et al*. 2006. Proteome analysis of human lung squamous carcinoma. *Proteomics* **6:** 547–558.
12. Nicholls, C., H. Li, and J. P. Liu. 2011. GAPDH: A common enzyme with uncommon functions. DOI: 10.1111/j.1440-1681.2011.05599.x.
13. Sen, N., M. R. Hara, M. D. Kornberg, M. B. Cascio, B.-I. Bae, N. Shahani, *et al*. 2008. Nitric oxide-induced nuclear GAPDH activates p300/CBP and mediates apoptosis. *Nature Cell Biol.* **10:** 866–873.
14. Shahani, N. and A. Sawa. 2011. Nitric oxide signaling and nitrosative stress in neurons: Role for *S*-nitrosylation. *Antioxid. Redox Signal.* **14:** 1493–1504.
15. Tristan, C., N. Shahani, T. W. Sedlak, and A. Sawa. 2011. The diverse functions of GAPDH: Views from different subcellular compartments. *Cell Signal.* **23:** 317–323.