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
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-nitrosylation level 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-
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