Glucose-6-phosphate dehydrogenase neutralizes stresses by supporting reductive glutamine metabolism and AMPK activation

Dear Editor,

Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the oxidative pentose phosphate pathway (oxPPP) that can generate cytosolic NADPH (Fig. 1a) for biosynthesis and oxidative defence. Here, we reveal a previously unidentified function of G6PD. It, even the natural G6PD deficiency-associated mutant without the activity to maintain the normal oxPPP, can antagonize the stresses by supporting the reductive glutamine metabolism and AMPK activation, independently of the NADPH generation by the oxPPP.

We deleted G6PD in HeLa, MDA-MB-231, and HCT116 cells, and then re-expressed the wild type (WT) enzyme, some natural variants, and mechanism-based inactivated mutants (Fig. 1b and Supplementary Fig. S1a-c). G6PD-KO significantly sensitized cells to hydrogen peroxide (H$_2$O$_2$), hypoxia, and the electron transport chain (ETC) inhibition by antimycin A, which, however, was completely rescued by all the expressions except artificial mutants including H263A (Fig. 1c and Supplementary Fig. S1a-c), even though they displayed obviously decreased activities by measuring the product of 6-phosphate glucuronolactone (Fig. 1d) and the $[3-^{2}H]$-glucose-labeled NADPH m + 1 generated from oxPPP (Fig. 1e and Supplementary Fig. S1d). The NADPH/NAD$^{+}$ ratio was dramatically reduced in HeLaKO cells, but it was also totally reversed by natural mutants but not H263A (Fig. 1f). We meantime observed comparatively increased $[3-^{2}H]$-glucose-labeled NADPH m + 2 in HeLaKO and natural mutant-re-expressing cells (Fig. 1e), which resulted from the newly synthesized NADPH incorporating ribose 5-phosphated m + 2 derived from the non-oxPPP (Supplementary Fig. S1a). These data suggest that enabling the robust oxPPP is not required for the anti-stress activity of G6PD.

We then performed metabolomic profiling on HeLaKO, HeLaWT, and HeLaR257G cells, and found that, except the products of the oxPPP, most of the significantly changed metabolites in HeLaKO cells were reversed by the re-expression of R257G without detectable activity (Supplementary Fig. S2a-c). Among the significantly increased metabolites in KO cells, pyruvate and $\alpha$-hydroxyglutarate were associated with glycolysis and tricarboxylic acid cycle except $\alpha$-ketoglutarate in HeLaKO cells (Supplementary Fig. S3a,b). These metabolic changes were exactly similar to those induced by NADH accumulation under ETC dysfunction. We further measured an increased NADH/NAD$^{+}$ ratio in HeLaKO but in HeLaWT and HeLaR257G cells (Fig. 1j), suggesting that G6PD can maintain the NADH/NAD$^{+}$ homeostasis, independently of its intact activity.

Hypoxia, H$_2$O$_2$, and antimycin A treatments increased the NADH/NAD$^{+}$ ratio (Supplementary Fig. S4a). To determine whether the deregulated NADH/NAD$^{+}$ homeostasis contributed to cell death in G6PD-KO cells, we used $\alpha$-ketobutyrate, a pyruvate analogue substantially converting NADH to NAD$^{+}$, to reduce the NADH/NAD$^{+}$ ratio (Fig. 1j and Supplementary S4b). We found that $\alpha$-ketobutyrate, as well as other electron accepters, significantly protected G6PD-KO cells against antimycin A and H$_2$O$_2$ (Fig. 1k and Supplementary Fig. S4c).

Furthermore, we expressed a doxycycline-inducible LbNOX$^4$ (Fig. 1l), which transfers electrons from NADH to oxygen, in HeLaKO cells. Both cytosolic LbNOX and mitochondrial mitoLbNOX significantly decreased NADH/NAD$^{+}$ ratio (Supplementary Fig. S4d,e) and repressed antimycin A-induced cell death (Fig. 1m). Another enzyme, TPNOX, can convert NADP$^{+}$ to NADP and thus decrease the NADPH/NADP$^{+}$ ratio, but it also meantime reduced the NADH/NAD$^{+}$ ratio. Interestingly, only the cytosolic TPNOX, not mitochondrial mitoTPNOX, rescued HeLaKO cells (Fig. 1m). Therefore, G6PD-KO sensitizes cells to the deregulated NADH/NAD$^{+}$ ratio, most likely in the cytosol, not to the decreased NADPH/NADP$^{+}$.

Although lipid biosynthesis was thought to require a large amount of NADPH mainly afforded by the oxPPP, we found no difference in lipids and $^{13}$C$_{5}$-glucose-labeled fatty acids between HeLaKO and HeLaWT cells (Supplementary Fig. S5a,b). However, our tracing results of $^{13}$C$_5$-glutamine indicated that the metabolic flux of glutamine to fatty acids actually decreased in HeLaKO cells (Supplementary Fig. S5c), although its contribution was much less than glucose in the normal condition. Glutamine carbon was usually used to synthesize fatty acid through the reductive pathway (Supplementary Fig. S5d), which was specifically promoted by ETC dysfunction. Our metabolic flux analyses of $^{13}$C$_5$-glutamine in HeLaWT, HeLaKO, and HeLaR257G cells showed that antimycin A obviously boosted reductive glutamine metabolism in HeLaWT and HeLaR257G but not HeLaKO cells, indicated by the increased $^{13}$C$_5$-glutamine-labeled fractions of isocitrate m + 5 (Fig. 1n).

Although the fraction and content of $^{13}$C$_5$-glutamine-derived acetyl-CoA m + 2 were not promoted in HeLaKO cells by antimycin A, the cellular level of acetyl-CoA did not decrease...
(Supplementary Fig. S5e,f), suggesting the presence of a compensative pathway, most likely from glucose metabolism. As expected, upon the antimycin A treatment, we observed an increase in 13C6-glucose-derived malate m + 3, citrate m + 3 and m + 5, and acetyl-CoA m + 2 in HeLaKO cells (Supplementary Fig. S6a-d), demonstrating that HeLaKO cells still utilize glucose, instead of glutamine, to synthesize acetyl-CoA even under ETC inhibition.
The biosynthetic process of acetyl-CoA from glucose produces NADH, whereas that from glutamine via the reductive pathway did not. This could underlie the deregulated NADH/NAD$^+$ ratio in G6PD-KO cells.

We next deleted IDH1, as well as its mitochondrial isoform IDH2, to block reductive glutamine metabolism in HeLa cells, and observed the increased NADH/NAD$^+$ ratio (Supplementary Fig. S7a). However, HeLaR166H and HeLaR257G cells did not sensitize to antimycin A (Fig. S7a). It suggests that blocking reductive glutamine metabolism is insufficient to explain the susceptibility of G6PD-KO cells to the stresses.

We then observed that antimycin A quickly induced the phosphorylations of AMPK and its substrate ACC1 in HeLa WT and HeLaR257G, but not in HeLa KO and HeLaR262A cells (Fig. 1o and Supplementary Fig. S7b), suggesting that G6PD, independently of its intact activity, can effectively support AMPK activation. Furthermore, 5-amino-4-imidazolcarboxamide ribonucleoside (AICAR) was used to activate AMPK in HeLa, MDA-MB-231, and HTTi166H cells, and confirmed that the phosphorylations of AMPK and ACC1 in G6PD-KO cells were much slower than those in G6PD-WT cells (Supplementary Fig. S7c). As expected, AICAR pre-treatment only protected G6PD-KO cells against death induced by antimycin A or H$_2$O$_2$ (Fig. 1p and Supplementary Fig. S7d), which was completely blocked by AMPK inhibitor compound C (Fig. 1p and Supplementary Fig. S7e). These data demonstrate that AMPK activation is also necessary for cells to cope with the stresses.

At last, we found that the increased NADH/NAD$^+$ ratio could delay AMPK activation (Supplementary Fig. S8a-c), and verified that simultaneously blocking AMPK and reductive glutamine metabolism mimicked G6PD knockout in HeLa cells under the stress condition (Fig. 1q). Since the oxPPP products were not significantly detected in cells expressing R257G-G6PD, traces of metabolites derived from the leaking activity, or an alternatively unidentified function, of naturally mutated G6PD could function as signaling molecules to trigger cascades to activate AMPK and reductive glutamine metabolism, which is required to neutralize the stresses (Fig. 1r and Supplementary Fig. S8d). Taken together, our findings help us to better understand the physiological roles of G6PD and its association with human diseases.

DATA AVAILABILITY

The data that support the findings of this study are available within the Article and its Supplementary Information or from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

B.L. and C.C. conceived and designed the study; B.Z., D.J. and Y.H. performed the experiments; B.Z. and D.J. analyzed the data; L.L., L.Q., R.Y., and X.J. did some Western experiments; B.Z. and D.J. performed the statistical analysis; B.L. and C.C. conceived and designed the study; B.Z., D.J. and Y.H. performed and analyzed the data; B.Z., D.J. and Y.H. wrote the paper; B.Z., D.J. and Y.H. discussed and finalized the paper.

ADDITIONAL INFORMATION

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