Received: 18 March 2019 | Revised: 24 April 2019 | Accepted: 29 April 2019

DOI: 10.1002/jcb.29004

Journal of Cellular Biochemistry WILEY

VIEW POINTS

Neuroprotection by glucose-6-phosphate dehydrogenase and the pentose phosphate pathway

Bor Luen Tang[1,2]

[1]Department of Biochemistry, Yong Loo Lin School of Medicine, National University Health System, Singapore, Singapore

[2]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, Singapore

Correspondence
Bor Luen Tang, Department of Biochemistry, Yong Loo Lin School of Medicine, National University Health System, MD7, 8 Medical Drive, Singapore 117596, Singapore.
Email: bchtbl@nus.edu.sg

Abstract

Glucose-6-phosphate dehydrogenase (G6PD), the rate limiting enzyme that channels glucose catabolism from glycolysis into the pentose phosphate pathway (PPP), is vital for the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH) in cells. NADPH is in turn a substrate for glutathione reductase, which reduces oxidized glutathione disulfide to sulfhydryl glutathione. Best known for inherited deficiencies underlying acute hemolytic anemia due to elevated oxidative stress by food or medication, G6PD, and PPP activation have been associated with neuroprotection. Recent works have now provided more definitive evidence for G6PD's protective role in ischemic brain injury and strengthened its links to neurodegeneration. In *Drosophila* models, improved proteostasis and lifespan extension result from an increased PPP flux due to G6PD induction, which is phenocopied by transgenic overexpression of G6PD in neurons. Moderate transgenic expression of G6PD was also shown to improve healthspan in mouse. Here, the deciphered and implicated roles of G6PD and PPP in protection against brain injury, neurodegenerative diseases, and in healthspan/lifespan extensions are discussed together with an important caveat, namely NADPH oxidase (NOX) activity and the oxidative stress generated by the latter. Activation of G6PD with selective inhibition of NOX activity could be a viable neuroprotective strategy for brain injury, disease, and aging.

1 | INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49)[1] catalyzes the conversion of G6P into 6-phosphoglucono-δ-lactone, thus channeling glucose into the pentose phosphate pathway (PPP).[2] This rate limiting step of PPP catalyzed by the oxidoreductase is coupled to the reduction of nicotinamide adenine dinucleotide phosphate ($NADP^+$) to form NADPH. The PPP is the major source of NADPH in animals, providing the cellular reducing equivalent for various biosynthetic reactions. Importantly, NADPH is the reductant that regenerates a major cellular antioxidant, namely reduced glutathione (GSH), from its oxidized, disulfide form (GSSG).[3] Mammalian *G6PD* is localized to the X-chromosomes, and mouse hemizygous male embryos died by E10.5. Heterozygous females may also die in utero because of specific inactivation of the otherwise normal paternal allele.[4] The human *G6PD* gene is localized to Xq28. G6PD deficiency in humans[5,6] resulting from gene mutations and polymorphisms[7,8] underlies the most common form of nonimmune hemolytic anemia precipitated by infection, certain food (eg, fava beans) or medication (eg, primaquine). This condition arises

because G6PD-deficient erythrocytes could only generate NADPH through the PPP and are therefore more susceptible than other cell types to oxidative damage.[9]

Neurons are metabolically vulnerable and neuronal metabolic responses play critical roles in survival during stress and injury.[10] Oxidative stress by reactive oxygen species (ROS) occurs in multiple neuronal injuries[11] and neurodegenerative diseases.[12,13] Adequate generation of NADPH to sustain the level of GSH is therefore important for neuronal survival.[14,15] In this regard, G6PD expression is indeed regulated[16,17] by the master redox stress regulator system of Kelch-like enoyl-CoA hydratase-associated protein 1/nuclear factor erythroid 2-related factor 2 (KEAP1/NRF2).[18] For the neonate, G6PD deficiency is a major risk factor for hemolysis precipitated hyperbilirubinemia and the risk of bilirubin encephalopathy, or kernicterus.[19,20] Over the years, however, a neuroprotective role for G6PD and the PPP in adult brain injury and neurodegenerative diseases of the elderly has also been implicated by a number of studies. More definitive works in this regard have emerged recently together with clear indications of G6PD activity promoting healthspan and lifespan in animal models.[21-24] Notably, there is a high level of metabolic co-operation between neurons and astroglia in the brain.[25-28] Astrocytes exhibit a high degree of glucose consumption,[29] which could be higher than that of neurons. Importantly, astroglia also have a high flux of glucose into the PPP pathway and higher activity of the latter compared to neurons.[30,31] Thus, astroglia play an important role in ROS reduction in the brain and could provide neurons with GSH through an astrocyte-neuronal glutathione shuttle.[25] Attempts at acute reduction of hyperglycemia may in fact work against this protective function.[30,31] A perspective on G6PD and PPP-mediated neuroprotection should therefore include considerations on the activity of the enzyme and pathway in both neurons and astroglia.

There are two principle sources of cellular oxidative stress in neuronal injury and diseases, namely heightened production of ROS by dysfunctional mitochondria,[32] and ROS bursts produced by NADPH oxidases (NOXs)[33,34] (Figure 1). NOXs are electron transporting membrane proteins that produce extracellular or luminal superoxide and H_2O_2. A classical notion of the function of NOX is based on NOX2 expressed in neutrophils and macrophages, which has a key role in immune defense via the generation of ROS during oxidative bursts to destroy engulfed foreign microbial entities.[35] However, there are seven NOX isoforms in the human genome with different modes of activation,[36] and these are expressed in different central nervous system (CNS) cell types like neurons, microglia, astrocytes as well as cerebrovascular endothelial cells.[37] These NOXs, together with those carried by immune cells, collectively contribute to oxidative stress during CNS injury[34,38] and neurodegenerative diseases.[33] In this regard, microglia NOX2[39] and neuronal/endothelial NOX4 are known to be induced during CNS injury and ischemia. In particular, the latter appears to be a major contributor to neuronal death and disruption of the blood-brain barrier (BBB).[40,41] Clearly, NADPH production could be either antioxidative and beneficial, or pro-oxidant and detrimental, depending on the enzymatic processes it participates in.

The role of NOXs in ischemic injury and CNS neurodegeneration has been the subject of a number of recent excellent reviews.[33,34,38,42-45] In the paragraphs below, the focus shall be on works implicating a beneficial role for G6PD and PPP in neuroprotection. The relevant findings are summarized and discussed, together with recent advances in G6PD activation as a potential therapeutic approach, bearing in mind the important caveat of NADPH-mediated ROS production by NOXs.

2 | G6PD AND BRAIN INJURY

A myriad of evidence point toward G6PD's neuroprotective function during brain injury. Glucose is the primary fuel for brain neurons and a persistent supply of glucose and oxygen is critical for neuronal survival and function.[46] In metabolic resting states, brain neurons are in fact the major glucose consuming cell type in the body. Imported by the high affinity glucose transporters GLUT1 and GLUT3 expressed in neurons,[47] glucose is converted to pyruvate by glycolysis, with the carbon subsequent channeled into the tricarboxylic acid (TCA) cycle for the production of more reducing equivalents for adenosine triphosphate (ATP) synthesis during aerobic respiration.[48] Recent works also suggest that during neuronal stimulation, direct glucose uptake and neuronal glycolysis-TCA cycle[49,50] is important for sustaining the heightened ATP requirement for brain neuronal activity. However, upon glucose conversion to G6P by hexokinase, the latter can also be channeled to the PPP.[2] This latter mode of glucose metabolism plays an important role in neuronal survival. One reason for this is that the proapoptotic activity of cytochrome *c* is increased by ROS following injury or insult, which leads to the former's oxidation and activation. In healthy neurons, cytochrome *c* is reduced and inactivated by PPP generated GSH.[51]

This bifurcation of glucose metabolism between ATP-producing glycolysis and NADPH-producing PPP is dependent on 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3). The latter synthesizes

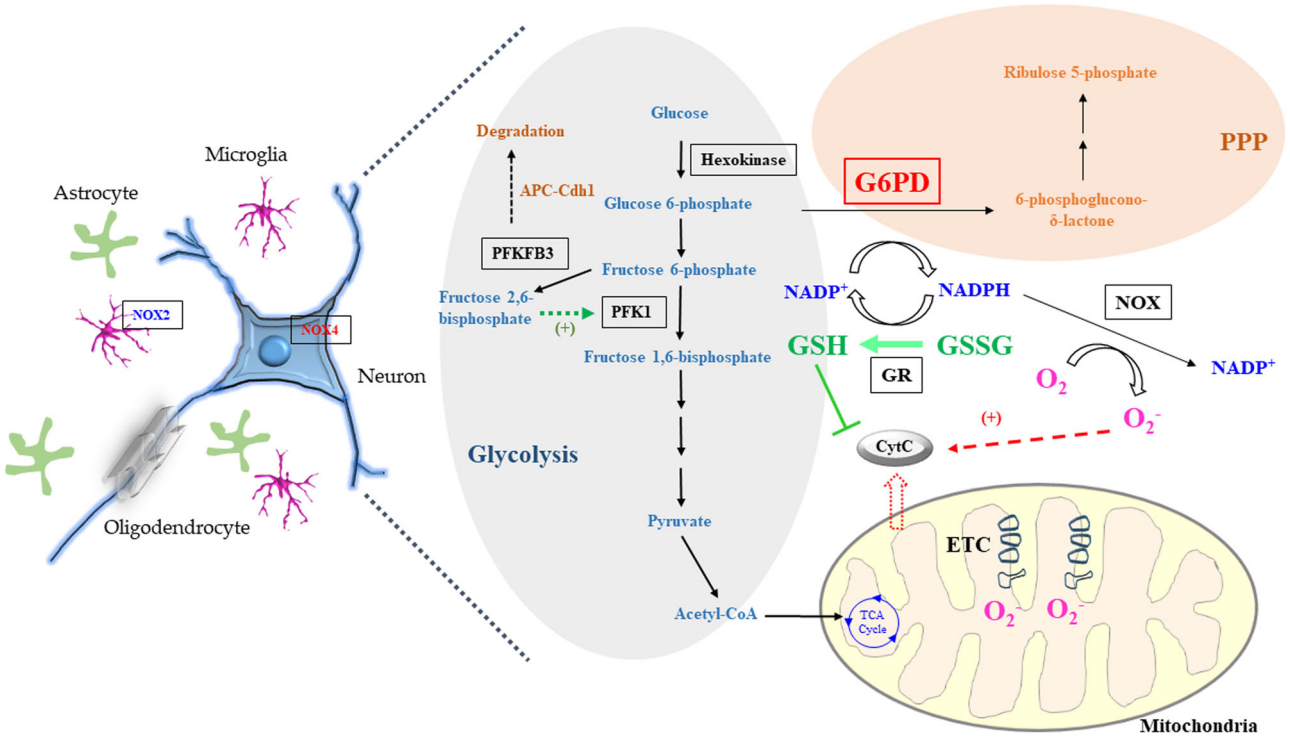

FIGURE 1 A simplified schematic diagram of the metabolic pathways and intermediates around the action of glucose-6-phosphate dehydrogenase (G6PD) in various CNS cell types. Glycolysis commitment in neurons could be limited by synthesis of fructose 2,6-bisphosphate, the allosteric activator of phosphofructokinase 1 (PFK1), by the labile 6-phosphofructo-2-kinase (PFKFB3). Neurons (but not astrocytes) have a high level of the E3 ubiquitin ligase anaphase-promoting complex-Cdh1 (APC-Cdh1), of which PFKFB3 is a substrate. G6PD's activity channels carbon from glycolysis to the pentose phosphate pathway (PPP) and reduces nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH. The latter is used by glutathione reductase (GR) to reduce the oxidized, disulfide form of glutathione (GSSG) to the sulfhydryl form (GSH). NADPH is, however, also a substrate of NADH oxidases (NOX), with NOX2 and NOX4 being expressed in microglia and neurons, respectively. NOX activity generates superoxide anion (O_2^-), a form of ROS. O_2^- is also constantly generated by the electron transport chain (ETC) activities within the mitochondria during oxidative phosphorylation, and this is heightened in dysfunctional or damaged mitochondria. G6PD's generation of NADPH and maintenance of GSH levels would be neuroprotective if NOX activities could be selectively inhibited

fructose-2,6-bisphosphate, which allosterically activates phosphofructokinase-1 (PFK-1), the enzyme catalyzing the committed or irreversible step of glycolysis (Figure 1).[52] Neurons have a high level of the E3 ubiquitin ligase anaphase-promoting complex (APC) and its coactivator Cdh1 (APC/Cdh1), of which PFKFB3 is a substrate. PFKFB3 in neurons, and consequently commitment to glycolysis for energy production, could be kept in check by its proteasomal degradation.[53] PFKFB3 activity would therefore result in a decrease in substrate channeling to the PPP, with a consequential reduction in NADPH. Neurons could thus use glucose metabolism for antioxidant function at the expense of its role in bioenergetics.[54] During injury and ischemia, neurons are particularly vulnerable to excitotoxic death due to uncontrolled glutamate release and massive activation of glutamate receptors. Activation of *N*-methyl-D-aspartate glutamate receptors (NMDARs) is shown to stabilized PFKFB3 protein levels in cortical neurons, and this NMDAR-mediated increase in PFKFB3 levels elevated glycolysis, reduced PPP's generation of NADPH leading to a consequential drop in GSH levels, thus enhancing oxidative stress and apoptotic neuronal death.[55] Importantly and interestingly, this effect could be counteracted by G6PD overexpression.[10,55] A very recent report also showed that APC/Cdh1 levels were reduced in rat primary cortical neurons under conditions of oxygen-glucose deprivation and reperfusion (OGD/R). Reperfusion enhanced glycolysis, with an elevation in PFKFB3 expression that was concomitant with a reduction in G6PD.[56] These changes switch neuronal glucose metabolism from PPP to aerobic glycolysis, effectively resulting in an increase in ROS production and apoptosis during reperfusion.

The TP53-induced glycolysis and apoptosis regulator (TIGAR)[57] inhibits glycolysis and increases the flow of glucose to the PPP.[58] Qin et al have extensively

documented TIGAR's beneficial effect against ischemic injury through its actions in neurons[59,60] as well as astrocytes.[61] TIGAR appears to be highly expressed in brain neurons and was further upregulated in response to ischemia/reperfusion.[60] The authors showed that TIGAR overexpression reduced, whereas it's silencing aggravated, ischemic neuronal injury in vivo and oxygen and glucose deprivation/reoxygenation (ODG/R)-induced injury of neurons in culture. G6PD levels are upregulated during ischemic injury and this elevation is further enhanced by TIGAR expression. In fact, TIGAR expression level changes during mouse postnatal development appear to correlate with the vulnerability of neurons of different ages to ischemic injury.[59] On the whole, TIGAR's neuroprotective activity appears to stem from its suppression of oxidative damage and neuronal death, at least partially through G6PD and PPP. A direct involvement of G6PD in alleviation of neuronal death in ischemic injury was demonstrated by the group with reciprocal overexpression and silencing of G6PD itself in vivo with an ischemia/reperfusion mouse model, as well as in vitro with ODG/R treatment of primary neurons.[62] The negative effect of G6PD silencing is in turn alleviated by exogenous NADPH. In fact, exogenous NADPH administration significantly protected neurons against ischemia/reperfusion-induced injury in mouse and rat stroke models, with beneficial effects still observed even when given 24 hours after experimental ischemia.[63]

The activation of G6PD and enhanced flux through PPP have also been demonstrated to contribute towards ischemic injury protection mechanisms associated with various neuroprotective factors. The chaperone protein HSP27 is known to be protective against ischemic brain injury[64] in a manner that is regulated by its phosphorylation.[65] In models of cerebral ischemia with[66] and without[67] reperfusion, ischemic injury resulted in an increase in HSP27 Ser85 phosphorylation and concomitant upregulation of G6PD activity, which was blocked by the ataxia telangiectasia mutated (ATM) kinase inhibitor (KU-55933).[66,67] Ischemia induced HSP27 phosphorylation may thus be an upstream signaling event that contributes towards the increase in G6PD and PPP. The ischemic injury protective effect of hypoxic preconditioning has recently been shown to also involve an inhibition of HSP27 degradation.[68] Another report has implicated the involvement of the oxidative stress regulator hypoxia-inducible factor 1 (HIF1) in maintaining the expression of G6PD that is reduced by severe hypobaric hypoxia.[69] Interestingly, cannabidiol, an FDA-approved nonpsychotropic compound from *Cannabis sativa* that has antiepileptic properties, attenuated ODG/R induced death of a cultured hippocampal neuronal cell line.[70] Cannabidiol treatment resulted in G6PD activation and maintenance of the NADPH/$NADP^+$ ratio, and could be of therapeutic interest.

On the other hand, one needs to bear in mind that NADPH produced by G6PD and PPP is notably also a substrate for NOXs.[35] The detrimental role of CNS expressed NOX4 in ischemic injury is particularly well known.[40,41,71,72] In fact, it was shown that G6PD silencing by an antisense approach reduced global cerebral ischemia-induced oxidative DNA damage and delayed neuronal cell death in rat hippocampal CA1 region.[73] However, silencing of G6PD at a later reperfusion period instead increased oxidative DNA damage and exacerbated neuronal cell death. Although it is unclear in this case as to the degree of contribution of NOXs to the ischemic injury, G6PD activation may well be a double-edged sword in brain injury and ischemia, and its effect could be complex depending on the extent of injury and the cell types involved.

3 | G6PD AND NEURODEGENERATIVE DISEASES

Oxidative stress underlies all major neurodegenerative diseases[12,21] and it is therefore unsurprising that changes and involvement of G6PD and PPP have been implicated in myriad of neurodegenerative disease as gleaned from post-mortem human brain samples and cellular/animal models. A brief summary of these findings are described and discussed below.

G6PD levels have been found to be elevated in postmortem brain samples of Alzheimer's disease (AD)[74] patients as indicated by a majority of early reports.[75-79] However, in one report in which the enzyme activity was measured, G6PD activity was found to be reduced in the hippocampi of human AD brains.[80] A more recent study found G6PD activities to be elevated in the serum of AD patients compared to age controlled subjects.[81] Despite speculations of a link between G6PD and AD,[82] there is, however, very limited direct evidence for a role for G6PD in alleviating AD pathology. In a rat aluminum neurotoxicity model, G6PD activities were shown to be reduced by direct injection of an $AlCl_3$ solution into the CA1 region of the hippocampus. Aluminium-treated animals exhibited a significantly worse response in a two-way active avoidance task, which was alleviated by co-administration with a G6PD enzyme solution.[83] The latter also appeared to reduce immunohistochemical signals for tau and amyloid-β. However, exactly how exogenously added G6PD could improve behavioral deficits and alleviate AD pathological manifestations is completely unclear.

For Parkinson's disease (PD),[84] an early report has indicated that the levels of a number of antioxidant enzymes, including G6PD, were significantly lower in the erythrocytes of PD patients compared to controls.[85] However, other reports found no significant difference in this regard.[86] While AD brains showed clear increases in NADPH in diseased areas, this was only seen in some late-stage PD cases.[75] Both the PPP's NADPH-producing enzymes, G6PD and 6-phosphogluconate, are reduced in the putamen of early-stage PD and in the cerebellum of early and late-stage PD. There are also conflicting reports on whether G6PD is neuroprotective in PD models. Transgenic overexpression of G6PD in the dopaminergic nigrostriatal pathway neurons in mice protected these from the toxic effects of the PD-associated neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), even in aged animals that are more susceptible to the neurotoxin.[87] On the contrary, the herbicide paraquat, another neurotoxin often associated with PD, elevated the PPP and G6PD levels in dopaminergic neuroblastoma cells (SK-N-SH). Overexpression of G6PD in this case selectively increased paraquat toxicity, while its inhibition with 6-aminonicotinamide attenuated the paraquat-induced oxidative stress and cell death.[88] In the context of PD, astroglia plays pivotal roles in disease pathology.[89] A recent report has indeed showed that dopamine-induced increase in PPP activity in astroglia produced a protective effect on cocultured neurons.[90]

In amyotrophic lateral sclerosis (ALS),[91] an early work did not discern any clear changes in posterior root ganglion cells of the spinal cord,[92] but G6PD activity and transcript levels were shown to be increased in biopsied quadriceps muscle samples from ALS and other neurogenic myotropy patients.[93] The activities of G6PD and other antioxidant factors were also found to be significantly reduced in erythrocytes from ALS patients compared to those from healthy subjects, with reductions in G6PD and GSH levels correlating with disease progression.[94] In a popular ALS animal model, namely mice with transgenic expression of the human superoxide dismutase 1 mutant, hSOD1-G93A, G6PD activity, and PPP were reduced in the spinal cord at disease onset.[95]

An early study with erythrocytes from a small cohort of Huntington's disease (HD)[96] patients indicated an increase in G6PD, but a decrease in GSH, in five of eight individuals.[97] In a study of bioenergetics changes in cybrids (generated by fusion of mitochondrial DNA-depleted human teratocarcinoma cells with platelets from HD individuals), an increase glycolysis rate and a decrease in G6PD levels were observed.[98] A more direct indication of a beneficial effect of G6PD in HD is demonstrated by a *Drosophila* transgenic model. For flies carrying the exon 1 of the human huntingtin gene with 93 glutamine repeats (HQ93), overexpression of G6PD was shown to significantly increased lifespan and reduced eye neurodegeneration.[99]

G6PD activity may also be disease determinants in nerve damage and neuropathy.[100] G6PD-deficient patients in the Sardinian population have a significantly decreased risk of nonarteritic anterior ischemic optic neuropathy.[101] However, a meta-analysis of published data indicated that G6PD deficiency is associated with a higher risk of diabetes, more so for men compared to women.[102] Interestingly, the messenger RNA (mRNA) and protein expression levels of G6PD in rat L4-L6 dorsal root ganglion cells were significantly decreased in diabetic rats. Furthermore, adenoviral vector-mediated G6PD overexpression markedly attenuated the pain hypersensitivity of the hindpaw of these animals,[103] which is attributed to a suppression of Toll-like receptor 4 expression. The latter is known to have CNS roles in initiation of CNS neuroimmune activation that contribute to painful neuropathy.[104]

G6PD also appears to be protective against endogenous ROS-mediated neurodegeneration during aging.[105] In comparing aged female mice which are either wild-type (*G6PD* (+/+)), heterozygous (*G6PD* (+/def)) or homozygous (*G6PD* (def/def)) with respect to a G6PD deficiency mutant allele,[106] DNA oxidation (assessed by the levels of 8-oxo-2′-deoxyguanosine, the oxidized derivative of deoxyguanosine) is increased in the G6PD-deficient mice in multiple brain regions compared to wild-type mice. The level of DNA oxidative damage corresponded with enhanced morphological changes indicative of neurodegeneration in a brain region and cell type-specific manner.[23] These findings points to the notion that hereditary G6PD deficiency may be a risk factor for aging-associated neurodegeneration.

The studies summarized above suggest a link between G6PD and neurodegenerative diseases, likely through the former's role in PPP channeling and maintenance of GSH levels. However, definitive proof that G6PD elevation is beneficial to neurodegenerative disease progression, particularly in mammalian models, is still lacking.

4 | G6PD AND HEALTHSPAN/LIFESPAN EXTENSION

Given that G6PD deficiency could impact upon aging-associated neurodegeneration,[23] it is therefore likely that G6PD levels impacts upon aging, health-span and lifespan. Emerging evidence in this regard is discussed in the paragraphs below.

Early observations made with *Drosophila* indicated that long-lived strains generally have higher levels of G6PD activities.[107] That G6PD activity has a lifespan

extension effect in flies was demonstrated by transgenic overexpression of G6PD under the control of either ubiquitous or neuron-specific promoters.[108] All G6PD transgenic flies made exhibited an extended mean lifespan, with the broad overexpressing strains being longer living than those with neuronal overexpression. Transgenic G6PD expression is associated with an increase in the levels of NADPH and GSH/GSSG ratio, with the flies demonstrating increased resistance to challenges with hyperoxic conditions and paraquat.[108] In another recent report which revisited the mechanistic basis of a previous observation of lifespan extension in *Drosophila* by the c-Jun N-terminal kinase (JNK),[109] Jasper's laboratory found that flies carrying a loss-of-function allele for *puckered* (puc^{E69}) (which encodes a JNK phosphatase) exhibit a delay in aging-related decline in proteostasis.[21] Interestingly, metabolite analyses showed a significant reduction of glucose 6-phosphate in the *puc* mutant fly brains, accompanied by an elevated carbon flux into the PPP due to G6PD induction by the elevated JNK activity.[21] Optimal proteostasis is critical for neuronal functions, as the rate of protein turnover declines with aging, and dysfunction in proteostasis underlies neurodegenerative disease pathologies.[110] Interestingly, overexpression of G6PD in fly neurons is sufficient to phenocopy the JNK activity-induced metabolic and proteostatic changes, as well as the lifespan extension. G6PD and PPP therefore has a definitive lifespan extension effect in *Drosophila*.

In mammals, G6PD levels impacted embryonic and early postnatal survival, as litters from mutant mice with a hereditary G6PD deficiency had increased prenatal (manifested as fetal resorptions) and postnatal death.[106] As discussed in the section above, G6PD-deficient mice have an elevated levels of DNA oxidation damage and increased neurodegenerative morphologies with aging compared to wild-type animals.[23] In the rat cerebral cortex, the levels and activities of G6PD and other glutathione regenerating enzymes were shown to decline with age.[111] A demonstration of G6PD conferring a positive effect in mammalian lifespan extension is offered by a mouse model with transgenic expression of a human *G6PD* genomic fragment carrying its own promoter. This resulted in a moderate (~twofold) increase in G6PD mRNA and protein across multiple tissues, as well as derived embryonic fibroblasts (MEFs).[24] The G6PD transgenic mice have elevated levels of NADPH in several tissues including the brain, and their MEFs exhibited an increased resistance to the oxidant diamide. Both G6PD transgenic mice and MEFs were, however, more sensitive to paraquat toxicity, which is in line with findings in dopaminergic SK-N-SH cells discussed above.[88] Interestingly, there is a significant increase in the median lifespan of the G6PD transgenic female mice, but not in the males. The transgenic mice tend to be leaner with age, and the males are more glucose-tolerant and have higher insulin sensitivity at 1 year old. The females performed significantly better than controls at 1.5 to 2 years of age in the rotarod test (males showed a trend that was not statistically significant). Of note, both aged male and female transgenic mice showed a diminished accumulation of 8-hydroxyguanosine in liver and brain, and liver from 2-year old mice presented an elevated GSH levels compared to control. Taken as a whole, a moderate global increase in G6PD levels extended the healthspan and, albeit in a sexually dimorphic manner, the lifespan of mice. The sexual dimorphism observed is likely linked the well-known fact that estrogen induces G6PD activity.[112,113]

On the contrary, it should be noted that human genetics and epidemiological studies have suggested an opposite notion, namely the association of G6PD deficiency with beneficial effects that might contribute towards longevity.[9,114,115] Sardinians have a prevalence of G6PD deficiency[116] but a disproportionately large number of male centurians.[117,118] G6PD deficiency has been associated with a lower risk in cancer,[114,119] vascular diseases[120] and malaria.[121] G6PD deficiency has also been associated with an alleviation of insulin resistance in obesity by reducing adipose inflammation.[122] Of course, longevity as a phenotype has polygenic contributions and at the moment it is difficult to clearly discern a causal relationship between G6PD deficiency and lifespan extension.

5 | G6PD AS A THERAPEUTIC TARGET FOR BRAIN INJURY AND DISEASES

The preceding discussions point toward beneficial effects of elevating G6PD activity and the ensuing PPP-based production of NADPH and maintenance of GSH in terms of neuronal injury, neurodegenerative diseases, as well as possibly other aging-associated disorders. It is therefore tempting to consider G6PD as a target for possible therapeutic intervention for neurological disorders beyond the treatment of clinical symptoms arising from G6PD deficiency.[6] There has been, however, no effective way of selectively elevating G6PD activity. In this regard, the recent report of a small molecule G6PD activator is particularly exciting. Hwang et al[22] screened for a possible agonist for the Canton variant of G6PD mutation (R459L), which has both reduced activity and stability. An identified compound, AG1, both activates and stabilizes the mutant, and also has the capacity to

TABLE 1 Some key recent findings directly implicating the activation of G6PD and PPP in neuroprotection and longevity

Models	Key findings	References
Ischemia/reperfusion model in mice and OGD/reoxygenation treatment of primary neurons	Overexpression and silencing of G6PD confers protection and exacerbated injury and neuronal death, respectively.	62
Drosophila transgenic model carrying the exon 1 of the human huntingtin gene with 93 glutamine repeats (HQ93)	Overexpression of G6PD extended lifespan of transgenic HD fly significantly	99
Drosophila transgenic model	Overexpression of G6PD in *Drosophila* neurons delayed proteostasis decline with age and extend lifespan	21
Transgenic mice with moderate expression of G6PD	Moderate transgenic overexpression of G6PD in mice extended healthspan and median lifespan of females	24
Development of a G6PD agonist	G6PD agonist AG1 effectively activated G6PD and reduced oxidative stress in G6PD-deficient human fibroblasts and zebrafish embryo	22

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; OGD, oxygen-glucose deprivation; PPP, pentose phosphate pathway.

increase the activity of the wild-type protein. AG1 is shown to reduce oxidative stress in G6PD-deficient human fibroblasts and zebrafish embryo, as well as hemolysis of human erythrocytes induced by chloroquine and diamide. This compound therefore appears therapeutically promising, at least as a lead compound for the eventual development of clinically viable G6PD activating drugs.

However, as for all CNS drugs, bioavailability and the ability to cross the BBB to reach brain tissues would be critical. Furthermore, in attempting to activate G6PD systemically, one should need to be mindful of various negative implications. G6PD is a regulator of vascular smooth muscle contraction[123,124] and its acute systemic activation may result in pulmonary hypertension.[125] Excessive generation of NADPH by G6PD and PPP may of course lead to superoxide production by NOXs, which has been implicated in oxidative and inflammatory signaling in vascular cells,[126] airway epithelial cells[45] and adipose tissues,[122] and could contribute to further damages of relevant diseased tissues. Importantly, NADPH could be used by CNS NOXs[37,44] for ROS production, which exacerbates neuronal injury and death. The ability to produce more NADPH while inhibiting key NOXs within CNS cell types and those carried by invading immune cells would be critical for achieving a beneficial outcome. In this regard, a recent report on the combination of a NOX inhibitor apocynin and NADPH conferring greater benefits than either alone in an ischemia/reperfusion-induced brain inflammation and neuronal injury model seems rather encouraging.[127] With the development of better isoform-specific NOX inhibitors,[128] these maybe tailored to different CNS disease conditions in conjunction with G6PD activation to achieve more synergistic beneficial effects.

6 | EPILOGUE

In the paragraphs above, the deciphered and implicated roles of G6PD and PPP in protection against brain injury and neurodegenerative diseases, as well as documented health-span/lifespan extension are discussed (see summary in Table 1). Recent advances that point toward possible pharmacological activation of G6PD are also discussed along with the caveat of NADPH being a substrate for ROS production by CNS NOXs. On the whole, efficient G6PD agonizts, in combination with selective NOX inhibitors, offer a reasonable therapeutic option for neuronal injury and neurodegenerative diseases in the adult or aged brain.

The benefits of G6PD elevation provides a cell autonomous production of NADPH and maintenance of GSH level in neurons that would guard against acute and chronic oxidative stresses. However, benefits may also be conferred indirectly, as G6PD activation in glial cells would likely attenuate stress-induced inflammatory responses[129-131] and reactive gliosis[132] that could enhance neuronal demise and inhibit regeneration. Therefore, activation of G6PD may also create a regeneration conducive post-injury CNS environment. This would likely be a notable additional benefit that attests to the therapeutic value of G6PD activation in neuronal injury and diseases.

ACKNOWLEDGMENT

Bor Luen Tang is supported by the NUS Graduate School for Integrative Sciences and Engineering (NGS) and the

Office of Deputy President, Research & Technology (ODPRT) of the National University of Singapore.

AUTHOR CONTRIBUTION

BLT conceived and drafted the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Bor Luen Tang http://orcid.org/0000-0002-1925-636X

REFERENCES

1. Kletzien RF, Harris PK, Foellmi LA. Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. *FASEB J*. 1994;8:174-181.
2. Stincone A, Prigione A, Cramer T, et al. The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biol Rev Camb Philos Soc*. 2015;90:927-963.
3. Diaz-Vivancos P, de Simone A, Kiddle G, Foyer CH. Glutathione-linking cell proliferation to oxidative stress. *Free Radic Biol Med*. 2015;89:1154-1164.
4. Longo L, Vanegas OC, Patel M, et al. Maternally transmitted severe glucose 6-phosphate dehydrogenase deficiency is an embryonic lethal. *EMBO J*. 2002;21:4229-4239.
5. Luzzatto L, Nannelli C, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. *Hematol Oncol Clin North Am*. 2016;30:373-393.
6. Belfield KD, Tichy EM. Review and drug therapy implications of glucose-6-phosphate dehydrogenase deficiency. *Am J Health Syst Pharm*. 2018;75:97-104.
7. Vulliamy TJ, D'Urso M, Battistuzzi G, et al. Diverse point mutations in the human glucose-6-phosphate dehydrogenase gene cause enzyme deficiency and mild or severe hemolytic anemia. *Proc Natl Acad Sci USA*. 1988;85:5171-5175.
8. Gómez-Manzo S, Marcial-Quino J, Vanoye-Carlo A, et al. Glucose-6-phosphate dehydrogenase: Update and analysis of new mutations around the world. *Int J Mol Sci*. 2016;17:2069.
9. Manganelli G, Masullo U, Passarelli S, Filosa S. Glucose-6-phosphate dehydrogenase deficiency: disadvantages and possible benefits. *Cardiovasc Hematol Disord Drug Targets*. 2013;13:73-82.
10. Rodriguez-Rodriguez P, Almeida A, Bolaños JP. Brain energy metabolism in glutamate-receptor activation and excitotoxicity: role for APC/C-Cdh1 in the balance glycolysis/pentose phosphate pathway. *Neurochem Int*. 2013;62:750-756.
11. Rodríguez-Rodríguez A, Egea-Guerrero JJ, Murillo-Cabezas F, Carrillo-Vico A. Oxidative stress in traumatic brain injury. *Curr Med Chem*. 2014;21:1201-1211.
12. Liu Z, Zhou T, Ziegler AC, Dimitrion P, Zuo L. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. *Oxid Med Cell Longev*. 2017;2017:2525967.
13. Niedzielska E, Smaga I, Gawlik M, et al. Oxidative stress in neurodegenerative diseases. *Mol Neurobiol*. 2016;53:4094-4125.
14. Stanton RC. Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. *IUBMB Life*. 2012;64:362-369.
15. Bolaños JP, Almeida A. The pentose-phosphate pathway in neuronal survival against nitrosative stress. *IUBMB Life*. 2010;62:14-18.
16. Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res*. 2002;62:5196-5203.
17. Lee JM, Calkins MJ, Chan K, Kan YW, Johnson JA. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *J Biol Chem*. 2003;278:12029-12038.
18. Suzuki T, Yamamoto M. Molecular basis of the Keap1-Nrf2 system. *Free Radic Biol Med*. 2015;88:93-100.
19. Cunningham AD, Hwang S, Mochly-Rosen D. Glucose-6-phosphate dehydrogenase deficiency and the need for a novel treatment to prevent Kernicterus. *Clin Perinatol*. 2016;43:341-354.
20. Kaplan M, Hammerman C, Bhutani VK. The preterm infant: a high-risk situation for neonatal hyperbilirubinemia due to glucose-6-phosphate dehydrogenase deficiency. *Clin Perinatol*. 2016;43:325-340.
21. Wang L, Davis SS, Borch Jensen M, et al. JNK modifies neuronal metabolism to promote proteostasis and longevity. *Aging cell*. 2019;18(3):e12849. https://doi.org/10.1111/acel.12849
22. Hwang S, Mruk K, Rahighi S, et al. Correcting glucose-6-phosphate dehydrogenase deficiency with a small-molecule activator. *Nat Commun*. 2018;9:4045.
23. Jeng W, Loniewska MM, Wells PG. Brain glucose-6-phosphate dehydrogenase protects against endogenous oxidative DNA damage and neurodegeneration in aged mice. *ACS Chem Neurosci*. 2013;4:1123-1132.
24. Nóbrega-Pereira S, Fernandez-Marcos PJ, Brioche T, et al. G6PD protects from oxidative damage and improves healthspan in mice. *Nat Commun*. 2016;7:10894.
25. Bolaños JP. Bioenergetics and redox adaptations of astrocytes to neuronal activity. *J Neurochem*. 2016;139(suppl 2):115-125.
26. Tang BL. Brain activity-induced neuronal glucose uptake/glycolysis: is the lactate shuttle not required? *Brain Res Bull*. 2018;137:225-228.
27. Jha MK, Morrison BM. Glia-neuron energy metabolism in health and diseases: new insights into the role of nervous system metabolic transporters. *Exp Neurol*. 2018;309:23-31.
28. Mason S. Lactate shuttles in neuroenergetics-homeostasis, allostasis and beyond. *Front Neurosci*. 2017;11:43.
29. Ivanov AI, Malkov AE, Waseem T, et al. Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. *J Cereb Blood Flow Metab*. 2014;34:397-407.
30. Takahashi S, Izawa Y, Suzuki N. Astroglial pentose phosphate pathway rates in response to high-glucose environments. *ASN Neuro*. 2012;4:AN20120002.
31. Takahashi S, Izawa Y, Suzuki N. Astrogliopathy as a loss of astroglial protective function against glycoxidative stress under hyperglycemia. *Rinsho Shinkeigaku*. 2012;52:41-51.

32. Nissanka N, Moraes CT. Mitochondrial DNA damage and reactive oxygen species in neurodegenerative disease. *FEBS Lett*. 2018;592:728-742.
33. Tarafdar A, Pula G. The role of NADPH oxidases and oxidative stress in neurodegenerative disorders. *Int J Mol Sci*. 2018;19:3824.
34. Ma MW, Wang J, Dhandapani KM, Wang R, Brann DW. NADPH oxidases in traumatic brain injury—promising therapeutic targets? *Redox Biol*. 2018;16:285-293.
35. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev*. 2007;87:245-313.
36. Brandes RP, Weissmann N, Schröder K. Nox family NADPH oxidases: molecular mechanisms of activation. *Free Radic Biol Med*. 2014;76:208-226.
37. Nayernia Z, Jaquet V, Krause KH. New insights on NOX enzymes in the central nervous system. *Antioxid Redox Signal*. 2014;20:2815-2837.
38. Yao H, Ago T, Kitazono T, Nabika T. NADPH oxidase-related pathophysiology in experimental models of stroke. *Int J Mol Sci*. 2017;18:2123.
39. Vilhardt F, Haslund-Vinding J, Jaquet V, McBean G. Microglia antioxidant systems and redox signalling. *Br J Pharmacol*. 2017;174:1719-1732.
40. Casas AI, Geuss E, Kleikers PWM, et al. NOX4-dependent neuronal autotoxicity and BBB breakdown explain the superior sensitivity of the brain to ischemic damage. *Proc Natl Acad Sci USA*. 2017;114:12315-12320.
41. Nishimura A, Ago T, Kuroda J, et al. Detrimental role of pericyte Nox4 in the acute phase of brain ischemia. *J Cereb Blood Flow Metab*. 2016;36:1143-1154.
42. Shen J, Rastogi R, Geng X, Ding Y. Nicotinamide adenine dinucleotide phosphate oxidase activation and neuronal death after ischemic stroke. *Neural Regen Res*. 2019;14:948-953.
43. Sorce S, Stocker R, Seredenina T, et al. NADPH oxidases as drug targets and biomarkers in neurodegenerative diseases: what is the evidence? *Free Radic Biol Med*. 2017;112:387-396.
44. von Leden RE, Yauger YJ, Khayrullina G, Byrnes KR. Central nervous system injury and nicotinamide adenine dinucleotide phosphate oxidase: oxidative stress and therapeutic targets. *J Neurotrauma*. 2017;34:755-764.
45. Nadeem A, Al-Harbi NO, Ahmad SF, Ibrahim KE, Siddiqui N, Al-Harbi MM. Glucose-6-phosphate dehydrogenase inhibition attenuates acute lung injury through reduction in NADPH oxidase-derived reactive oxygen species. *Clin Exp Immunol*. 2018;191:279-287.
46. Peters A. The selfish brain: competition for energy resources. *Am J Hum Biol*. 2011;23:29-34.
47. Simpson IA, Vannucci SJ, Maher F. Glucose transporters in mammalian brain. *Biochem Soc Trans*. 1994;22:671-675.
48. Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends Neurosci*. 2013;36:587-597.
49. Díaz-García CM, Mongeon R, Lahmann C, Koveal D, Zucker H, Yellen G. Neuronal stimulation triggers neuronal glycolysis and not lactate uptake. *Cell Metab*. 2017;26:361-374. e4
50. Lundgaard I, Li B, Xie L, et al. Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nat Commun*. 2015;6:6807.
51. Vaughn AE, Deshmukh M. Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. *Nat Cell Biol*. 2008;10:1477-1483.
52. Mor I, Cheung EC, Vousden KH. Control of glycolysis through regulation of PFK1: old friends and recent additions. *Cold Spring Harb Symp Quant Biol*. 2011;76:211-216.
53. Estévez-García IO, Cordoba-Gonzalez V, Lara-Padilla E, et al. Glucose and glutamine metabolism control by APC and SCF during the G1-to-S phase transition of the cell cycle. *J Physiol Biochem*. 2014;70:569-581.
54. Herrero-Mendez A, Almeida A, Fernández E, Maestre C, Moncada S, Bolaños JP. The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat Cell Biol*. 2009;11:747-752.
55. Rodriguez-Rodriguez P, Fernandez E, Almeida A, Bolaños JP. Excitotoxic stimulus stabilizes PFKFB3 causing pentose-phosphate pathway to glycolysis switch and neurodegeneration. *Cell Death Differ*. 2012;19:1582-1589.
56. Li Z, Zhang B, Yao W, Zhang C, Wan L, Zhang Y. APC-Cdh1 regulates neuronal apoptosis through modulating glycolysis and pentose-phosphate pathway after oxygen-glucose deprivation and reperfusion. *Cell Mol Neurobiol*. 2019;39:123-135.
57. Geng J, Yuan X, Wei M, Wu J, Qin ZH. The diverse role of TIGAR in cellular homeostasis and cancer. *Free Radic Res*. 2018;52:1240-1249.
58. Green DR, Chipuk JE. p53 and metabolism: inside the TIGAR. *Cell*. 2006;126:30-32.
59. Cao L, Chen J, Li M, et al. Endogenous level of TIGAR in brain is associated with vulnerability of neurons to ischemic injury. *Neurosci Bull*. 2015;31:527-540.
60. Li M, Sun M, Cao L, et al. A TIGAR-regulated metabolic pathway is critical for protection of brain ischemia. *J Neurosci*. 2014;34:7458-7471.
61. Chen J, Zhang DM, Feng X, et al. TIGAR inhibits ischemia/reperfusion-induced inflammatory response of astrocytes. *Neuropharmacology*. 2018;131:377-388.
62. Cao L, Zhang D, Chen J, et al. G6PD plays a neuroprotective role in brain ischemia through promoting pentose phosphate pathway. *Free Radic Biol Med*. 2017;112:433-444.
63. Li M, Zhou ZP, Sun M, et al. Reduced nicotinamide adenine dinucleotide phosphate, a pentose phosphate pathway product, might be a novel drug candidate for ischemic stroke. *Stroke*. 2016;47:187-195.
64. Stetler RA, Cao G, Gao Y, et al. Hsp27 protects against ischemic brain injury via attenuation of a novel stress-response cascade upstream of mitochondrial cell death signaling. *J Neurosci*. 2008;28:13038-13055.
65. Stetler RA, Gao Y, Zhang L, et al. Phosphorylation of HSP27 by protein kinase D is essential for mediating neuroprotection against ischemic neuronal injury. *J Neurosci*. 2012;32:2667-2682.
66. Yamamoto Y, Hosoda K, Imahori T, et al. Pentose phosphate pathway activation via HSP27 phosphorylation by ATM kinase: A putative endogenous antioxidant defense mechanism during cerebral ischemia-reperfusion. *Brain Res*. 2018;1687:82-94.
67. Imahori T, Hosoda K, Nakai T, et al. Combined metabolic and transcriptional profiling identifies pentose phosphate pathway

activation by HSP27 phosphorylation during cerebral ischemia. *Neuroscience*. 2017;349:1-16.
68. Zhan L, Liu L, Li K, et al. Neuroprotection of hypoxic postconditioning against global cerebral ischemia through influencing posttranslational regulations of heat shock protein 27 in adult rats. *Brain Pathol*. 2017;27:822-838.
69. Vetrovoy O, Sarieva K, Galkina O, et al. Neuroprotective mechanism of hypoxic post-conditioning involves HIF1-associated regulation of the pentose phosphate pathway in rat brain. *Neurochem Res*. 2018. https://doi.org/10.1007/s11064-018-2681-x
70. Sun S, Hu F, Wu J, Zhang S. Cannabidiol attenuates OGD/R-induced damage by enhancing mitochondrial bioenergetics and modulating glucose metabolism via pentose-phosphate pathway in hippocampal neurons. *Redox Biol*. 2017;11:577-585.
71. Kleinschnitz C, Grund H, Wingler K, et al. Post-stroke inhibition of induced NADPH oxidase type 4 prevents oxidative stress and neurodegeneration. *PLoS Biol*. 2010;8:e1000479.
72. Radermacher KA, Wingler K, Langhauser F, et al. Neuroprotection after stroke by targeting NOX4 as a source of oxidative stress. *Antioxid Redox Signal*. 2013;18:1418-1427.
73. Zhao G, Zhao Y, Wang X, Xu Y. Knockdown of glucose-6-phosphate dehydrogenase (G6PD) following cerebral ischemic reperfusion: the pros and cons. *Neurochem Int*. 2012;61:146-155.
74. Scheltens P, Blennow K, Breteler MMB, et al. Alzheimer's disease. *Lancet*. 2016;388:505-517.
75. Dunn L, Allen GF, Mamais A, et al. Dysregulation of glucose metabolism is an early event in sporadic Parkinson's disease. *Neurobiol Aging*. 2014;35:1111-1115.
76. Ansari MA, Scheff SW. Oxidative stress in the progression of Alzheimer disease in the frontal cortex. *J Neuropathol Exp Neurol*. 2010;69:155-167.
77. Martins RN, Harper CG, Stokes GB, Masters CL. Increased cerebral glucose-6-phosphate dehydrogenase activity in Alzheimer's disease may reflect oxidative stress. *J Neurochem*. 1986;46:1042-1045.
78. Palmer AM. The activity of the pentose phosphate pathway is increased in response to oxidative stress in Alzheimer's disease. *J Neural Transm*. 1999;106:317-328.
79. Russell RL, Siedlak SL, Raina AK, Bautista JM, Smith MA, Perry G. Increased neuronal glucose-6-phosphate dehydrogenase and sulfhydryl levels indicate reductive compensation to oxidative stress in Alzheimer disease. *Arch Biochem Biophys*. 1999;370:236-239.
80. Bigl M, Brückner MK, Arendt T, Bigl V, Eschrich K. Activities of key glycolytic enzymes in the brains of patients with Alzheimer's disease. *J Neural Transm*. 1999;106:499-511.
81. Evlice A, Ulusu NN. Glucose-6-phosphate dehydrogenase a novel hope on a blood-based diagnosis of Alzheimer's disease. *Acta Neurol Belg*. 2017;117:229-234.
82. Ulusu NN. Glucose-6-phosphate dehydrogenase deficiency and Alzheimer's disease: partners in crime? The hypothesis. *Med Hypotheses*. 2015;85:219-223.
83. Jovanović MD, Jelenković A, Stevanović ID, et al. Protective effects of glucose-6-phosphate dehydrogenase on neurotoxicity of aluminium applied into the CA1 sector of rat hippocampus. *Indian J Med Res*. 2014;139:864-872.
84. Kalia LV, Lang AE. Parkinson's disease. *Lancet*. 2015;386:896-912.
85. Abraham S, Soundararajan CC, Vivekanandhan S, Behari M. Erythrocyte antioxidant enzymes in Parkinson's disease. *Indian J Med Res*. 2005;121:111-115.
86. Gao L, Mir P, Díaz-Corrales FJ, et al. Glucose-6-phosphate dehydrogenase activity in Parkinson's disease. *J Neurol*. 2008;255:1850-1851.
87. Mejías R, Villadiego J, Pintado CO, et al. Neuroprotection by transgenic expression of glucose-6-phosphate dehydrogenase in dopaminergic nigrostriatal neurons of mice. *J Neurosci*. 2006;26:4500-4508.
88. Lei S, Zavala-Flores L, Garcia-Garcia A, et al. Alterations in energy/redox metabolism induced by mitochondrial and environmental toxins: a specific role for glucose-6-phosphate-dehydrogenase and the pentose phosphate pathway in paraquat toxicity. *ACS Chem Biol*. 2014;9:2032-2048.
89. Joe EH, Choi DJ, An J, Eun JH, Jou I, Park S. Astrocytes, microglia, and Parkinson's disease. *Exp Neurbiol*. 2018;27:77-87.
90. Mashima K, Takahashi S, Minami K, et al. Neuroprotective role of astroglia in Parkinson disease by reducing oxidative stress through dopamine-induced activation of pentose-phosphate pathway. *ASN Neuro*. 2018;10:1759091418775562. https://doi.org/10.1177/1759091418775562
91. Al-Chalabi A, Hardiman O, Kiernan MC, Chiò A, Rix-Brooks B, van den Berg LH. Amyotrophic lateral sclerosis: moving towards a new classification system. *Lancet Neurol*. 2016;15:1182-1194.
92. Hayashi H. Enzymatic analysis of individual posterior root ganglion cells in olivopontocerebellar atrophy, amyotrophic lateral sclerosis and Duchenne muscular dystrophy. *J Neurol Sci*. 1985;70:13-20.
93. Konagaya M, Konagaya Y, Horikawa H, Iida M. Pentose phosphate pathway in neuromuscular diseases—evaluation of muscular glucose 6-phosphate dehydrogenase activity and RNA content. *Rinsho Shinkeigaku*. 1990;30:1078-1083.
94. Babu GN, Kumar A, Chandra R, et al. Oxidant-antioxidant imbalance in the erythrocytes of sporadic amyotrophic lateral sclerosis patients correlates with the progression of disease. *Neurochem Int*. 2008;52:1284-1289.
95. Tefera TW, Bartlett K, Tran SS, Hodson MP, Borges K. Impaired pentose phosphate pathway in the spinal cord of the hSOD1 G93A mouse model of Amyotrophic Lateral Sclerosis. *Mol Neurobiol*. 2019. https://doi.org/10.1007/s12035-019-1485-6
96. Jimenez-Sanchez M, Licitra F, Underwood BR, Rubinsztein DC. Huntington's disease: mechanisms of pathogenesis and therapeutic strategies. *Cold Spring Harb Perspect Med*. 2017;7:a024240.
97. Zanella A, Izzo C, Meola G, et al. Metabolic impairment and membrane abnormality in red cells from Huntington's disease. *J Neurol Sci*. 1980;47:93-103.
98. Ferreira IL, Cunha-Oliveira T, Nascimento MV, et al. Bioenergetic dysfunction in Huntington's disease human cybrids. *Exp Neurol*. 2011;231:127-134.
99. Besson MT, Alegría K, Garrido-Gerter P, Barros LF, Liévens JC. Enhanced neuronal glucose transporter expression reveals metabolic choice in a HD Drosophila model. *PLoS One*. 2015;10:e0118765.
100. Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol*. 2012;11:521-534.

101. Pinna A, Solinas G, Masia C, Zinellu A, Carru C, Carta A. Glucose-6-phosphate dehydrogenase (G6PD) deficiency in nonarteritic anterior ischemic optic neuropathy in a Sardinian population, Italy. *Invest Ophthalmol Vis Sci*. 2008;49:1328-1332.
102. Lai YK, Lai NM, Lee SWH. Glucose-6-phosphate dehydrogenase deficiency and risk of diabetes: a systematic review and meta-analysis. *Ann Hematol*. 2017;96:839-845.
103. Sun Q, Zhang BY, Zhang PA, Hu J, Zhang HH, Xu GY. Downregulation of glucose-6-phosphate dehydrogenase contributes to diabetic neuropathic pain through up-regulation of toll-like receptor 4 in rats. *Mol Pain*. 2019;15:1744806919838659. https://doi.org/10.1177/1744806919838659
104. Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. *Proc Natl Acad Sci USA*. 2005;102:5856-5861.
105. Sbodio JI, Snyder SH, Paul BD. Redox mechanisms in neurodegeneration: from disease outcomes to therapeutic opportunities. *Antioxid Redox Signal*. 2019;30:1450-1499.
106. Nicol CJ, Zielenski J, Tsui LC, Wells PG. An embryoprotective role for glucose-6-phosphate dehydrogenase in developmental oxidative stress and chemical teratogenesis. *FASEB J*. 2000;14:111-127.
107. Luckinbill LS, Riha V, Rhine S, Grudzien TA. The role of glucose-6-phosphate dehydrogenase in the evolution of longevity in *Drosophila melanogaster*. *Heredity*. 1990;65(Pt 1):29-38.
108. Legan SK, Rebrin I, Mockett RJ, et al. Overexpression of glucose-6-phosphate dehydrogenase extends the life span of *Drosophila melanogaster*. *J Biol Chem*. 2008;283:32492-32499.
109. Wang MC, Bohmann D, Jasper H. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell*. 2005;121:115-125.
110. Kurtishi A, Rosen B, Patil KS, Alves GW, Møller SG. Cellular proteostasis in neurodegeneration. *Mol Neurobiol*. 2018;56:3676-3689.
111. Dukhande VV, Isaac AO, Chatterji T, Lai JCK. Reduced glutathione regenerating enzymes undergo developmental decline and sexual dimorphism in the rat cerebral cortex. *Brain Res*. 2009;1286:19-24.
112. Gordon G, Mackow MC, Levy HR. On the mechanism of interaction of steroids with human glucose 6-phosphate dehydrogenase. *Arch Biochem Biophys*. 1995;318:25-29.
113. Ibim SE, Randall R, Han P, Musey PI. Modulation of hepatic glucose-6-phosphate dehydrogenase activity in male and female rats by estrogen. *Life Sci*. 1989;45:1559-1565.
114. Schwartz AG, Pashko LL. Dehydroepiandrosterone, glucose-6-phosphate dehydrogenase, and longevity. *Ageing Res Rev*. 2004;3:171-187.
115. Cocco P, Todde P, Fornera S, Manca MB, Manca P, Sias AR. Mortality in a cohort of men expressing the glucose-6-phosphate dehydrogenase deficiency. *Blood*. 1998;91:706-709.
116. De Vita G, Alcalay M, Sampietro M, Cappelini MD, Fiorelli G, Toniolo D. Two point mutations are responsible for G6PD polymorphism in Sardinia. *Am J Hum Genet*. 1989;44:233-240.
117. Deiana L, Ferrucci L, Pes GM, et al. AKEntAnnos. The Sardinia study of extreme longevity. *Aging (Milano)*. 1999;11:142-149.
118. Passarino G, Underhill PA, Cavalli-Sforza LL, et al. Y chromosome binary markers to study the high prevalence of males in Sardinian centenarians and the genetic structure of the Sardinian population. *Hum Hered*. 2001;52:136-139.
119. Cocco P. Does G6PD deficiency protect against cancer? A critical review. *J Epidemiol Community Health*. 1987;41:89-93.
120. Meloni L, Manca MR, Loddo I, et al. Glucose-6-phosphate dehydrogenase deficiency protects against coronary heart disease. *J Inherit Metab Dis*. 2008;31:412-417.
121. Mbanefo EC, Ahmed AM, Titouna A, et al. Association of glucose-6-phosphate dehydrogenase deficiency and malaria: a systematic review and meta-analysis. *Sci Rep*. 2017;7:45963.
122. Ham M, Choe SS, Shin KC, et al. Glucose-6-phosphate dehydrogenase deficiency improves insulin resistance with reduced adipose tissue inflammation in obesity. *Diabetes*. 2016;65:2624-2638.
123. Gupte RS, Ata H, Rawat D, et al. Glucose-6-phosphate dehydrogenase is a regulator of vascular smooth muscle contraction. *Antioxid Redox Signal*. 2011;14:543-558.
124. Gupte RS, Rawat DK, Chettimada S, et al. Activation of glucose-6-phosphate dehydrogenase promotes acute hypoxic pulmonary artery contraction. *J Biol Chem*. 2010;285:19561-19571.
125. Chettimada S, Gupte R, Rawat D, Gebb SA, McMurtry IF, Gupte SA. Hypoxia-induced glucose-6-phosphate dehydrogenase overexpression and activation in pulmonary artery smooth muscle cells: implication in pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2015;308:L287-L300.
126. Peiró C, Romacho T, Azcutia V, et al. Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway. *Cardiovasc Diabetol*. 2016;15:82.
127. Qin YY, Li M, Feng X, et al. Combined NADPH and the NOX inhibitor apocynin provides greater anti-inflammatory and neuroprotective effects in a mouse model of stroke. *Free Radic Biol Med*. 2017;104:333-345.
128. Altenhöfer S, Radermacher KA, Kleikers PWM, Wingler K, Schmidt HHHW. Evolution of NADPH oxidase inhibitors: Selectivity and mechanisms for target engagement. *Antioxid Redox Signal*. 2015;23:406-427.
129. Abderrazak A, Syrovets T, Couchie D, et al. NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. *Redox Biol*. 2015;4:296-307.
130. Stephenson J, Nutma E, van der Valk P, Amor S. Inflammation in CNS neurodegenerative diseases. *Immunology*. 2018;154:204-219.
131. Ma MW, Wang J, Dhandapani KM, Brann DW. NADPH oxidase 2 regulates NLRP3 inflammasome activation in the brain after traumatic brain injury. *Oxid Med Cell Longev*. 2017;2017:6057609-6057618.
132. Pekny M, Pekna M. Reactive gliosis in the pathogenesis of CNS diseases. *Biochim Biophys Acta*. 2016;1862:483-491.

How to cite this article: Tang BL. Neuroprotection by glucose-6-phosphate dehydrogenase and the pentose phosphate pathway. *J Cell Biochem*. 2019;120:14285-14295. https://doi.org/10.1002/jcb.29004