TIGAR's promiscuity

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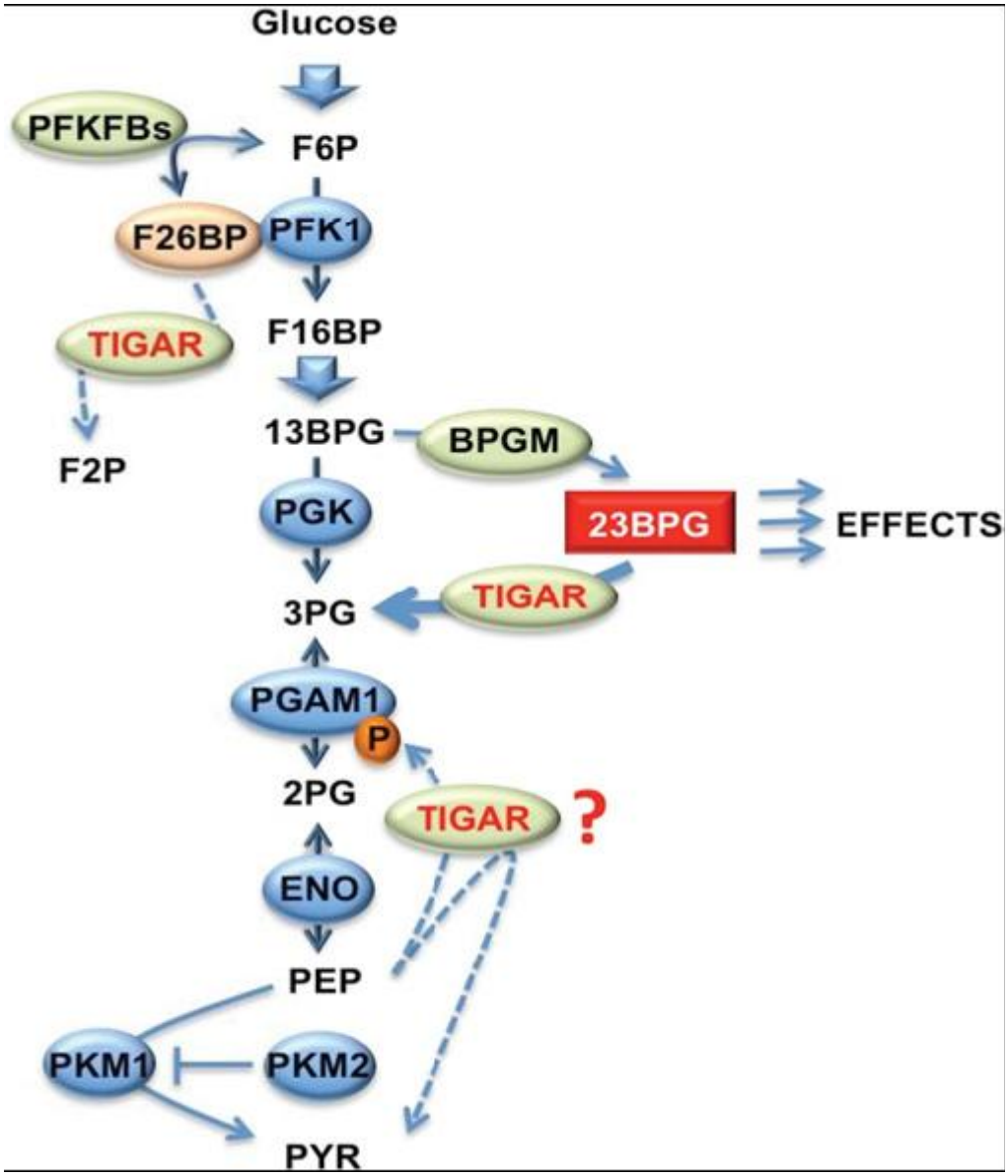
TIGAR [TP53 (tumour protein 53)-induced glycolysis and apoptosis regulator] is an important survival factor for cancer cells. The enzymatic activity supported by sequence analysis led Bensaad et al. to propose a fructose 2,6-bisphosphatase function for TIGAR. TIGAR has thus received much attention within the biochemistry and cancer research communities given the key role of its substrate F26BP (fructose 2,6-bisphosphate), both as a positive allosteric effector of PFK1 (6-phosphofructo-1-kinase) and a negative allosteric effector of fructose-1,6-bisphosphatase, two master regulators of glycolysis and gluconeogenesis respectively. Thus p53-induced TIGAR confers cancer resistance against oxidative stress, since decreased F26BP shifts glycolysis to the PPP (pentose phosphate pathway), a metabolic route that, by regenerating NADPH(H+), a glutathione reductase cofactor, maintains the antioxidant glutathione in a reduced status. Furthermore, by restraining ROS (reactive oxygen species) levels, TIGAR can induce autophagy, hence moderating the apoptotic response. Besides TIGAR, cellular levels of F26BP are controlled by PFKFB1 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 1)–PFKFB4, a family of bifunctional enzyme isoforms that differ in kinetic properties. Most PFKFB isoforms are regulated in order to equilibrate their kinase/bisphosphatase ratio to determine a particular level of F26BP. Accordingly, the extent whereby TIGAR can affect cellular F26BP concentration relies both on its own activity and on the specific PFKFB isoform that predominates in a given cell type or tissue. For instance, PFKFB3 is the isoform that has the highest (~700:1) kinase/bisphosphatase ratio; accordingly, regulating PFKFB3 protein levels determines its kinase activity. However, in a previous study aimed to decipher the crystal structure of TIGAR, it was found that the catalytic efficiency of TIGAR as a fructose-2,6-bisphosphatase is several orders of magnitude lower than that of PFKFBs, thus questioning an efficient competition between TIGAR and PFKFBs at controlling F26BP levels. In this issue of the *Biochemical Journal*, Gerin et al. clarify the substrate preferences for TIGAR. As a first attempt, Gerin et al. screened several intracellular phosphate carboxylic acid esters as putative recombinant TIGAR substrates *in vitro*. They found 23BPG (2,3-bisphosphoglycerate) to be the best substrate, which TIGAR converted into 3-phosphoglycerate (Figure 1); in addition, although to a lesser catalytic efficiency, TIGAR also dephosphorylated 2-phosphoglycerate, phosphoglycolate and phosphoenolpyruvate. Moreover, TIGAR was indeed able to dephosphorylate F26BP, but mostly (~95%) to fructose 2-phosphate, in contrast with the bisphosphatase action of PFKFBs that exclusively form fructose 6-phosphate (Figure 1); however, the catalytic efficiency of TIGAR at dephosphorylating F26BP was approximately 400-fold lower than that observed for 23BPG. To assess the physiological relevance of these results and, in view that the high 23BPG concentration in erythrocytes masks the actual 23BPG concentration in blood-contaminated tissues *in vivo*, Gerin et al. examined 23BPG and F26BP levels in cultured cancer cells upon modulation of TIGAR.

Thus TIGAR was first depleted in cells, which resulted in significant increases in 23BPG without (or modestly) affecting F26BP. However, this lack of effect on F26BP could be ascribed to either any residual TIGAR protein left in the depleted cells, or to the fact that cancer cells may express other PFKFB isoform(s) that could dominate over TIGAR fructose-2,6-bisphosphatase activity. Accordingly, the authors knocked out TIGAR in mouse embryonic stem cells that express high levels of TIGAR; in TIGAR-knockout cells, 23BPG levels increased by 5-fold when compared with wild-typecells, whereas levels of F26BP increased by 3.5-fold. These results strongly suggest that 23BPG is a physiological substrate of TIGAR, and that the effect of TIGAR on cellular F26BP depends on the extent of the expression and/or activity of other fructose-2,6-bisphosphatase(s) in a given physiological setting. The phosphatase activity on 23BPG is the result of the collective action of a family of phosphohistidine-intermediate phosphatases, including, besides TIGAR, PGAMs (phosphoglycerate mutases) and BPGM (bisphosphoglycerate mutase). These enzymes differ in their mechanism of action and, importantly, in their dependence on phosphoglycolate for full activity. Thus Gerin et al. next aimed to decipher which type of 23BPG phosphatase TIGAR belongs to. According to its substrate preference, TIGAR resembled that of a previously identified phosphoglycolate-independent 23BPG phosphatase]. In addition, phosphoglycolate, far from activating, inhibited TIGAR activity; and, finally, the reported phosphoglycolateindependent 23BPG phosphatase activity was exclusively present in the fraction corresponding to TIGAR activity and immunoreactivity in mouse skeletal muscle protein extracts subjected to cation-exchange chromatography. These results unambiguously demonstrate that TIGAR is the phosphoglycolateindependent 23BPG phosphatase (Figure 1). The results of Gerin et al. have important implications for several reasons. First, it appears that TIGAR is better suited to regulate 23BPG than F26BP, thus challenging the currently held notion that, biochemically, TIGAR is mainly a fructose-2,6-bisphosphatase. This is supported by: (i) the 400-fold TIGAR preference for 23BPG than for F26BP as substrate; and (ii) the higher increase in 23BPG than in F26BP concentrations in TIGAR-inhibited cells. Secondly, 23BPG phosphatase activity would increase the glycolytic intermediates phosphoenolpyruvate and 2-phosphoglycerate, which stimulates, rather than inhibits, the terminal part of the glycolytic flux. This is difficult to reconcile with the reported inhibitory effect of TIGAR on glycolysis. However, it should be noted that the glucose assay specifically quantifies the flux from glucose to triose phosphates, making it possible that an activation of the terminal part of glycolysis could remain masked. In addition, the contribution of TIGAR at modulating F26BP depends on the specific PFKFB pattern of a given cell type. For instance, provided a predominant PFKFB isoform with low kinase/bisphosphatase ratio, TIGAR would have little impact on F26BP levels; however, in cells, such as neurons exclusively expressing the high kinase/bisphosphatase ratio isoform PFKFB3, TIGAR activity would have a stronger contribution to regulating F26BP levels.

Finally, the results of Gerin et al. might explain a missing step of the glycolytic shunt (Figure 1), i.e. the pathway observed in cancer cells naturally overexpressing the form of PKM2 (pyruvate kinase M2) with low activity. Avoidance of the pyruvate kinase step allows a high glycolytic flux in the absence of a high ATP production, which is advantageous for cancer cells for biomass generation. This shunt requires the transfer of a phosphate group from phosphoenolpyruvate to PGAM1, and the phosphoenolpyruvate phosphatase activity of TIGAR may well accomplish this function; however, this still requires specific validation. Finally, given the well-known function of 23BPG as a negative allosteric effector of haemoglobin, it would be reasonable to speculate the possible role of TIGAR in regulating oxygen binding to haemoglobin. Gerin et al. discarded this possibility in viewof the unknown functional relevance of 23BPG outside the erythrocytes. Indeed, haemoglobin in adult erythrocytes is fully saturated with 23BPG, which is at ~5–10 mM in these cells. In addition, the lack of a nucleus (at least in mammals) hampers p53-induced TIGAR expression in erythrocytes. However, an increasing body of evidence now shows that haemoglobin is expressed in other cell types and tissues. For instance, both *α*- and *β*-subunits of haemoglobin are present in dopaminergic neurons, glia, macrophages, mesangial cells and glioblastoma and references cited therein). The functional relevance of haemoglobin occurrence in these cells is currently enigmatic; however, it has been suggested that it may play roles in ROS scavenging and mitochondrial biogenesis, indicating a biologically relevant function for haemoglobin in energy and redox homoeostasis control. Given that the 23BPG concentration in non-erythrocyte cells is ~0.1 mM, the saturation curve of haemoglobin for 23BPG would be within the logarithmic phase. Thus one could hypothesize that, upon conditions of p53-mediated TIGAR induction, such as cancer, decreases in 23BPG levels would increase the affinity of haemoglobin for oxygen, probably causing local hypoxia. Whether this could account for the HIF1 (hypoxia-inducible factor 1)-mediated TIGAR mitochondrial re-localization remains to be elucidated; furthermore, such a putative local hypoxia might contribute to the Warburg effect. If so, through its ability to modulate 23BPG, TIGAR would represent a novel regulator of cell oxygen sensing in cancer cell metabolism and survival.



According to the results of Gerin et al., TIGAR is a phosphoglycolate-independent 23BPG phosphatase. As such, TIGAR-mediated modulation of 23BPG levels could have as-yet unrecognized biological functions. In a lower extent, TIGAR is also a phosphoenolpyruvate (PEP) phosphatase that Gerin et al. speculate could be responsible for the previously reported enzyme activity that phosphorylates, in a phosphoenolpyruvate-dependent manner, PGAM1 as part of the cancer-cell-specific glycolytic shunt. To a much lesser extent, TIGAR was found to dephosphorylate F26BP, a potent positive allosteric effector of PFK1, confirming previous results, although this activity mainly yielded fructose 2-phosphate (F2P), instead of fructose 6-phosphate (F6P) like the bisphosphatase activity of PFKFBs. 13BPG, 1,3-bisphosphoglycerate; BPGM, 1,3-bisphosphoglycerate mutase; ENO, enolase; F16BP, fructose 1,6-bisphosphate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; PGK, phosphoglycerate kinase; PKM, pyruvate kinase M; PYR, pyvuvate.