

COMMENTARY

Adapting glycolysis to cancer cell proliferation: the MAPK pathway focuses on PFKFB3

Juan P. BOLAÑOS¹

Institute of Functional Biology and Genomics (IBFG), University of Salamanca-CSIC, Zacarias Gonzalez 2, 37007 Salamanca, Spain

Besides the necessary changes in the expression of cell cycle-related proteins, cancer cells undergo a profound series of metabolic adaptations focused to satisfy their excessive demand for biomass. An essential metabolic transformation of these cells is increased glycolysis, which is currently the focus of anticancer therapies. Several key players have been identified, so far, that adapt glycolysis to allow an increased proliferation in cancer. In this issue of the *Biochemical Journal*, Novellasdemunt and colleagues elegantly identify a novel mechanism by which MK2 [MAPK (mitogen-activated protein kinase)-activated protein kinase 2], a key component of the MAPK pathway, up-regulates glycolysis in response to stress in cancer cells. The authors found that, by phosphorylating specific substrate residues, MK2

promotes both increased the gene transcription and allosteric activation of PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3), a key glycolysis-promoting enzyme. These results reveal a novel pathway through which MK2 co-ordinates metabolic adaptation to cell proliferation in cancer and highlight PFKFB3 as a potential therapeutic target in this devastating disease.

Key words: cell proliferation, glycolysis, mitogen-activated protein kinase-activated protein kinase 2 (MK2), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), p38 α mitogen-activated protein kinase (p38 α MAPK).

Reprogrammed energy metabolism to satisfy biomass growth is now considered a hallmark of cancer [1]. Therefore understanding how the expression of cell cycle-related proteins are co-ordinated with the increased metabolic rate is currently challenging. Glucose and glutamine are essential metabolic substrates for cancer cell proliferation. Accordingly, the metabolic pathways that need to be fully functional in cancer include: (i) those involving glucose utilization for biomass growth, notably glycolysis, the pentose phosphate pathway, and nucleotide, protein and lipid synthesis; and (ii) the mitochondrial machinery for energy production from glutamine, i.e. glutaminolysis, tricarboxylic acid cycle, electron transport chain and oxidative phosphorylation. However, the timing at which each of these pathways becomes critical depends on the stage of cancer or tumour progression [2]. During the highly proliferative stage of cancer cells, the glycolytic rate is rather enhanced and becomes a limiting factor, thus it seems reasonable that the proteins that switch on the cell-cycle machinery are the same that are regulating metabolic adaptation. This paradigm was partially unveiled with the elucidation of HIF-1 (hypoxia-inducible factor-1), a transcription factor that, being up-regulated in most types of cancers, induces key proteins for both glycolysis and cancer progression [3]. However, reprogramming of the cell cycle-related protein pattern in cancer is a complex process involving an additional number of stress-responsive transcription factors and signalling pathways.

The MAPK (mitogen-activated protein kinase) pathway accounts for many of the cellular reprogramming changes during tumorigenesis. The MAPK signalling family of proteins, which includes ERK (extracellular-signal-regulated kinase), JNK (c-Jun

N-terminal kinase) and p38 MAPK, regulates the transcription of IEGs (immediate-early genes) in response to a range of stress types. When active, i.e. phosphorylated, the p38 α MAPK isoform phosphorylates, and forms a complex with, its downstream MAPK MK2 (MAPK-activated protein kinase 2), a key cell cycle checkpoint kinase that drives a plethora of changes in transcription, protein synthesis, cell-surface receptor expression and cytoskeletal structure, ultimately affecting cell survival and apoptosis [4]. Novellasdemunt et al. [5] now reveal, in this issue of the *Biochemical Journal*, that PFKFB (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase) 3, a key glycolysis-promoting enzyme, is an important target of MK2 activity (Figure 1).

PFKFB3 is the product of one of the four *PFKFB* genes encoding PFKFB1 to PFKFB4. All PFKFB isoforms catalyse both the synthesis and the degradation of F26BP (fructose-2,6-bisphosphate), the most potent allosteric activator of PFK1 (6-phosphofructo-1-kinase), a master regulator of the glycolytic pathway. PFKFB3 is particularly interesting when considering that: (i) its activity is almost exclusively that of a kinase (i.e. F26BP-forming); (ii) its abundance is increased in cancer; and (iii) it is widely amenable to regulation by gene transcription, the stability of mRNA and protein, and allosteric activation by phosphorylation at different residues. Ser⁴⁶¹ of human PFKFB3 is of special interest, as it has been observed to be phosphorylated in human cancers and is a target of the cellular energy sensor AMPK (5'-AMP-activated protein kinase). In addition, the *PFKFB3* gene contains, in its 3'-untranslated region, multiple copies of the pattern AUUUA, a nucleotide sequence conferring instability

Abbreviations used: AMPK, 5'-AMP-activated protein kinase; APC/C–Cdh1, anaphase-promoting complex/cyclosome–Cdh1; F26BP, fructose-2,6-bisphosphate; HIF-1, hypoxia-inducible factor-1; IEG, immediate-early gene; MAPK, mitogen-activated protein kinase; MEF, mouse embryonic fibroblast; MK2, MAPK-activated protein kinase 2; PFK1, 6-phosphofructo-1-kinase; PFKFB, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; SRE, serum response element; SRF, serum response factor.

¹ email jbolanos@usal.es

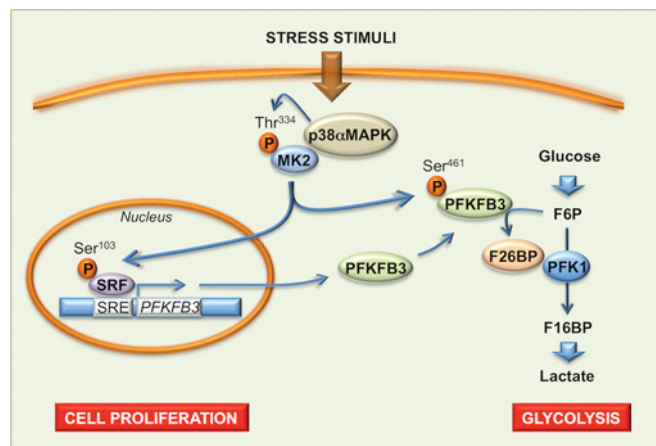


Figure 1 MK2 links glycolysis to cell proliferation

Novellasedemunt et al. [5] demonstrated that exposure of cancer cells to a wide range of known stress stimuli that promote cell proliferation, p38 α MAPK phosphorylated (P) MK2 at Thr³³⁴, which in turn resulted in both a transcriptional and a direct activation of PFKFB3. Thus, MK2, by promoting phosphorylation of Ser¹⁰³ of SRF, activated an SRE in the *PFKFB3* gene promoter region resulting in increased PFKFB3 transcription. In addition, the authors found that phosphorylated MK2 also promoted the direct phosphorylation of PFKFB3 at Ser⁴⁶¹, resulting in increased PFKFB3 activity and F26BP formation; this led to PFK1 allosteric activation and the subsequent enhancement of glycolytic rate. These results suggest that MK2 is a co-ordinator of the glycolytic adaptation to cell proliferation. F6P, fructose 6-phosphate; F16BP, fructose 1,6-bisphosphate.

on mRNAs and, importantly, a target of MK2. Accordingly, Novellasedemunt et al. [5] initially reasoned that the elevated expression of PFKFB3 usually observed in cancer cells would be a consequence of an associated enhanced activity of MK2. To test this hypothesis, the authors subjected two human cancer cell lines (HeLa and T98G) to MAPK pathway stress stimuli and found that *PFKFB3* mRNA was rapidly increased. Using a battery of known and specific inhibitors of several MAPK protein activities, they concluded that this effect was due to the p38 α MAPK/MK2 pathway. Moreover, MK2-deficient MEFs (mouse embryonic fibroblasts) failed to support the increase in *PFKFB3* mRNA abundance and, on the contrary, overexpression of constitutively active MK2 was sufficient to increase the *PFKFB3* transcript. Surprisingly, actinomycin D did not prevent the increase in mRNA abundance in the presence of the stressors, hence suggesting that MK2 acted through *PFKFB3* gene induction rather than the expected mRNA stabilization. To ascertain how MK2 induced the *PFKFB3* gene, Novellasedemunt et al. [5] focused on a putative 30 nucleotide SRE (serum response element) sequence, which they found to be present in the 5'-flanking region of *PFKFB3* promoter. Interestingly, the SRE is another target of MK2 through some of the IEGs, notably SRF (serum response factor). Accordingly, the authors performed ChIP (chromatin immunoprecipitation) analysis, and demonstrated that the 30 nucleotide SRE element in the *PFKFB3* promoter directly bound to phospho-SRF (Ser¹⁰³). These set of experiments elegantly demonstrated that the *PFKFB3* gene is transcriptionally activated by the p38 α MAPK/MK2 pathway through phospho-SRF (Ser¹⁰³) acting on its target SRE in the *PFKFB3* gene promoter (Figure 1).

Not satisfied sufficiently, Novellasedemunt et al. [5] further pursued how the levels of the PFKFB3 product, F26BP, which were consistently monitored upon treatment with the stressors, increased faster than the *PFKFB3* mRNA. They observed that the levels of F26BP correlated with those of phospho-PFKFB3 (Ser⁴⁶¹), as identified by MS upon the stimuli of the stressors.

Given that inhibition of the p38 α MAPK/MK2 pathway prevented PFKFB3 (Ser⁴⁶¹) phosphorylation, the authors hypothesized that MK2 was also responsible for PFKFB3 phosphorylation, leading to enzyme activation. To confirm this, the authors used wild-type and MK2-knockout MEFs and found that the MK2-knockout cells were unable to form phospho-PFKFB3 (Ser⁴⁶¹) after stimulus with the stressors. Furthermore, MK2 directly phosphorylated PFKFB3 *in vitro*, and the phosphorylated PFKFB3 had increased V_{max} and decreased K_m values for fructose 6-phosphate. Finally, using site-directed PFKFB3 mutants either unable to phosphorylate Ser⁴⁶¹ (S461A-PFKFB3) or constitutively active (phospho-mimetic S461E-PFKFB3), they demonstrated an increased F26BP-synthesizing activity and lactate formation by phospho-PFKFB3 (Ser⁴⁶¹) in cancer cells (Figure 1). These data strongly suggest that PFKFB3 is a *bona fide* MK2 substrate undergoing allosteric activation upon Ser⁴⁶¹ phosphorylation, as is evident by both *in vitro* studies and in intact cells that rapidly up-regulate glycolysis in cancer cells.

The work by Novellasedemunt et al. [5] is ground-breaking for at least three reasons. First, it describes a bimodal mechanism of glycolytic up-regulation in cancer that involves both a rapid post-translational and a long-term transcriptional adaptive response. This further adds complexity to the regulation of PFKFB3 activity, taking the responsibility of a double role in glycolysis regulation. By phosphorylation, PFKFB3 almost instantaneously adapts cells to glycolytic demand; by modulating its abundance, PFKFB3 executes long-term cellular adaptations to proliferation. This occurs not only at the transcriptional level by MK2, as Novellasedemunt et al. [5] identified, but also by mRNA [6] and protein stability via the E3 ubiquitin ligase APC/C–Cdh1 (anaphase-promoting complex/cyclosome–Cdh1) [7]. Secondly, PFKFB3 excellently mimics cellular situations of long-term glycolytic demands compatible with high proliferative status. Whether the levels of PFKFB3 protein should be considered as a hallmark of cancer remains to be shown. Nonetheless, Novellasedemunt et al. [5] add the MAPK pathway to the shortlist of signalling pathways adapting glycolysis to cell proliferation, notably HIF-1, PI3K (phosphoinositide 3-kinase)/Akt, LKB1/AMPK/mTOR (mammalian target of rapamycin), p53, cMyc or APC/C–Cdh1 [8]. Thirdly, this work convincingly pinpoints PFKFB3 as a key therapeutic target in cancer; it would be interesting to show that PFKFB3 up-regulation provides selective advantage to cancer cells over normal proliferative cells *in vivo*. If so, the development of selective PFKFB3 pharmacological inhibitors for clinical studies would be a promising next step in cancer therapy.

Finally, the notable dual effect of MK2 on PFKFB3, allosteric activation and increased transcription, is intriguing. Proliferation, both in normal and in cancer cells, appears to be an adaptive relatively slow process not requiring the impetus of the switching on of glycolysis that is observed upon acute bioenergetic deficit. The results of Novellasedemunt et al. [5] might reflect a key function of PFKFB3 activity on the proliferative machinery; PFKFB3 contains a nuclear-leading sequence that directs it to the nucleus where it synthesizes F26BP to co-activate mitotic cyclin-dependent kinases [9]. Interestingly, MK2 is subjected to a dynamic import/export sequence of events depending upon the phosphorylation status of its C-terminal residue Thr³³⁴ by p38 α MAPK [10]. It would be interesting to know whether, by providing F26BP within the nucleus, early PFKFB3 activation by p38 α MAPK–MK2-mediated Ser⁴⁶¹ phosphorylation triggers the transition from quiescence to proliferation, whereas delayed induction of PFKFB3 ensures the necessary metabolic adaptations.

FUNDING

The Bolaños laboratory is funded by the Spanish Ministry of Economy and Competitiveness [grant numbers SAF2010-20008 and RETICEF-RD12/0043/0021] and the European FEDER Fund.

REFERENCES

- 1 Hanahan, D. and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646–674
- 2 Yuan, H. X., Xiong, Y. and Guan, K. L. (2013) Nutrient sensing, metabolism, and cell growth control. *Mol. Cell* **49**, 379–387
- 3 Semenza, G. L. (2012) Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol. Sci.* **33**, 207–214
- 4 Reinhardt, H. C. and Yaffe, M. B. (2009) Kinases that control the cell cycle in response to DNA damage: Chk1, Chk2, and MK2. *Curr. Opin. Cell Biol.* **21**, 245–255
- 5 Novellademunt, L., Bultot, L., Manzano, A., Ventura, F., Rosa, J. L., Vertommen, D., Rider, M. H., Navarro-Sabate, A. and Bartrons, R. (2013) PFKFB3 activation in cancer cells by the p38/MK2 pathway in response to stress stimuli. *Biochem. J.* **452**, 531–543
- 6 Chesney, J., Mitchell, R., Benigni, F., Bacher, M., Spiegel, L., Al-Abed, Y., Han, J. H., Metz, C. and Bucala, R. (1999) An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: role in tumor cell glycolysis and the Warburg effect. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3047–3052
- 7 Herrero-Mendez, A., Almeida, A., Fernandez, E., Maestre, C., Moncada, S. and Bolaños, J. P. (2009) The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat. Cell Biol.* **11**, 747–752
- 8 Buchakjian, M. R. and Kornbluth, S. (2010) The engine driving the ship: metabolic steering of cell proliferation and death. *Nat. Rev. Mol. Cell Biol.* **11**, 715–727
- 9 Yalcin, A., Clem, B. F., Simmons, A., Lane, A., Nelson, K., Clem, A. L., Brock, E., Siow, D., Wattenberg, B., Telang, S. and Chesney, J. (2009) Nuclear targeting of 6-phosphofructo-2-kinase (PFKFB3) increases proliferation via cyclin-dependent kinases. *J. Biol. Chem.* **284**, 24223–24232
- 10 Meng, W., Swenson, L. L., Fitzgibbon, M. J., Hayakawa, K., Ter Haar, E., Behrens, A. E., Fulghum, J. R. and Lippke, J. A. (2002) Structure of mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 suggests a bifunctional switch that couples kinase activation with nuclear export. *J. Biol. Chem.* **277**, 37401–37405

Received 22 April 2013; accepted 25 April 2013

Published on the Internet 31 May 2013, doi:10.1042/BJ20130560