DIABETES

A BAD portion of glucose can be good for inflamed beta cells

Exposure to high glucose under inflammatory conditions is detrimental to insulin-secreting beta cells in the pancreas. Fu and colleagues describe a metabolic axis that decreases production of the 'danger molecule' nitric oxide and improves the survival of beta cells exposed to an inflammatory milieu, thus paving the way to new interventions for diabetes.

Christian Frezza

Glucose is one of the main nutrients for cells, and its homeostasis is tightly regulated in the human body by the release of insulin by beta cells in the pancreas. Glucose greatly contributes to beta cell function and fitness[1]. As a consequence, exposure to aberrant glucose levels, compounded by the inflammatory milieu typical of many human diseases, compromises beta cell viability, thus leading to the pathogenesis of diabetes[2]. Yet, how inflammation and glucose metabolism cooperatively regulate the viability of these cells, and whether this link can be harnessed therapeutically are still unclear.

A first indication of the mechanisms through which glucose regulates cell survival came almost 20 years ago, when a landmark discovery by Danial and colleagues revealed an unanticipated molecular link between glucose metabolism and the apoptotic machinery[3]. The authors showed that the BAD protein, previously known as a proapoptotic BCL-2 family member, resides in a multiprotein complex at the mitochondrial surface, where it regulates the activity of the glycolytic enzyme glucokinase (GK)[4]. GK is a hexokinase that is expressed in the liver and pancreas, where it phosphorylates glucose to glucose-6-phosphate in the first step of glycolysis. In the pancreas, GK regulates insulin release in response to glucose, and a loss of GK activity has been implicated in diabetes[5]. In agreement with its role in regulating GK activity, the ectopic expression of a phosphomimic BAD mutant obtained by stapling its BH3 domain[6] preserves the survival of beta cells subjected to a variety of stress signals, including proinflammatory cytokines known to play a role in diabetes[7]. In contrast, glucose deprivation triggers cell death in a BAD-dependent fashion in beta cells[3]. These lines of evidence support an important role of BAD mimicry as a strategy to activate GK in beta cells and improve their function in inflammatory diseases. GK activators have long been considered a therapeutic strategy to increase insulin release and decrease glucose levels in type 2 diabetes[8]. Yet, whether different modes of GK activation have similar functional outcomes to those of BAD mimicry was unclear. In their article in *Nature Metabolism*, Fu and colleagues[9] performed an extensive comparative analysis of GK activators and found that BAD mimicry elicits a unique anti-inflammatory metabolic rewiring that starts from glucose and ends in the urea cycle, in which it modulates the synthesis of nitric oxide (NO), a key player in cytotoxic inflammation (Fig. 1).

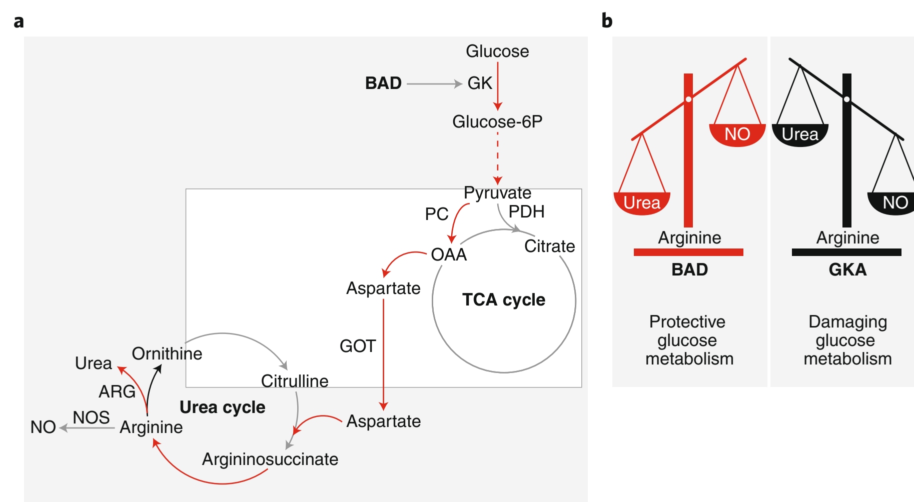

Fig. 1 | The metabolic axis elucidated by Fu and colleagues. a, BAD activates GK and elicits metabolic rewiring of glucose oxidation, which in turn activates PC and leads to the production of aspartate via the glutamate oxaloacetate transaminases (GOT). Aspartate enters the urea cycle and fuels the synthesis of arginine, which is then converted to urea, thus decreasing the danger molecule NO. **b**, Representation of the effects of BAD versus other GK activators (GKA) regarding the conversion of arginine to the products urea and NO. NOS, NO synthase; ARG, arginase; PDH, pyruvate dehydrogenase; OAA, oxaloacetate.

Fu and colleagues started their study by comparing the effects of a BAD mimetic to those of other modes of GK activation, including the expression of a gain-of-function mutation of the enzyme and a small-molecule allosteric activator on the survival of human islets exposed to proinflammatory cytokines. The first striking finding was that, although all the strategies effectively activated GK, only BAD mimicry exhibited a prosurvival function, thus suggesting that activating glucose metabolism can have opposing outcomes on beta cells. Were these findings due to a different metabolic fate of glucose after GK activation? A comparative metabolomics study was instrumental to addressing this

key question. Indeed, the largest difference among cells treated with different modes of GK activation exposed to inflammatory cytokines was in the metabolism of arginine, an amino acid in the urea cycle (Fig. 1a). Performing elegant tracing studies using stable carbon and nitrogen isotopes, the authors confirmed that BAD mimicry affects the abundance of two urea-cycle intermediates, increasing urea and in parallel decreasing the levels of NO, a key mediator of cytotoxic inflammation (Fig. 1b). Importantly, this metabolic switch was not underpinned by changes in the expression of the enzymes that convert arginine into urea or NO—arginase and nitric oxide synthase, respectively—thus indicating that the activity of these enzymes, rather their expression, is responsible for this metabolic axis. The remaining piece of the puzzle was the link between glucose metabolism and the urea cycle. The authors focused on aspartate, a metabolite that fuels the urea cycle and can be derived from glucose via the production of oxaloacetate in the mitochondria (Fig. 1a). Tracing experiments using ^{13}C-labelled glucose showed that GK activation by the BAD mimetic increases the incorporation of carbons in aspartate. Furthermore, inhibition of the synthesis of aspartate diminished urea formation in cells treated with the BAD mimetic. The authors then investigated in detail how aspartate is generated from glucose-derived carbons in the mitochondria. Carbons from glucose can enter the mitochondria via the oxidation of pyruvate to acetyl-CoA by the enzyme pyruvate dehydrogenase, or via the carboxylation of pyruvate to oxaloacetate by the enzyme pyruvate carboxylase (PC). These two fates of glucose give rise to a specific labelling pattern of the carbons of tricarboxylic-acid-cycle intermediates that can help to distinguish them via mass spectrometry. The authors found that incubation with the BAD mimetic specifically enhances PC activity, which is essential to generate aspartate for the urea cycle. In the final part of their work, Fu and colleagues provide further evidence that this metabolic axis is relevant in vivo, using experimental models of islet stress. Strikingly, the authors found that GK activation via BAD mimicry, but not the other modes of GK activation, protects human beta cells during islet transplantation into the mouse kidney capsule. Furthermore, the overexpression of PC is sufficient to protect these cells from inflammation-induced death and protects human islets from glucotoxicity.

The work by Fu et al. has multiple implications both for understanding cellular metabolism and for developing new therapies to which deregulated glucose metabolism contributes. First, they elucidated a novel metabolic link between glucose metabolism and the urea cycle, mediated by the enzyme PC, which spares cells from the formation of the damage molecule NO. Although aspartate is an established nitrogen donor for the urea cycle, the authors elucidated the source of its carbon backbone, glucose, and propose that aspartate is preferentially generated via pyruvate carboxylation, rather than pyruvate oxidation, in the mitochondria when cells are exposed to BAD mimicry. Why this protective type of glucose metabolism is elicited by BAD only when proinflammatory cytokines are present remains to be elucidated. The cytokines tested are those normally elevated in type 1 diabetes[10]. Investigating whether and how different sets of cytokines shape this metabolic BAD-dependent axis would be interesting. In addition, why do different modes of GK activation have such different functional outcomes? Do these small molecules affect different pools of GK within the cell? Although the authors have shown that BAD does not physically interact with PC, the mechanism underlying this specificity might potentially be due to BAD's activation of the mitochondrial pool of GK[3]. This mitochondrial complex, in which BAD and GK reside, may be in proximity to PC and arginase. Of note, arginase exists in two isoforms, and ARG2, which is responsible for urea production in these settings, is mitochondrial. Second, the authors showed that substrate availability, rather than changes in enzyme expression, orchestrates the metabolic axis triggered by BAD. Whether these changes in metabolic fluxes are due to alterations in the physical organization of the enzymes, as argued above, or are sustained by post-translational modifications, remains to be determined. For instance, investigating whether changes in NO abundance may fine-tune N-nitrosylation of some of the enzymes that participate in the axis described here would be interesting. Finally, this work calls for a deeper understanding of cellular metabolism to infer the biological and functional outcomes of pharmacological agents, and it may explain the failure of some GK activators to prevent beta cell demise.

Overall, Fu and colleagues report a previously unappreciated metabolic dialogue between glucose metabolism and the urea cycle that regulates inflammation, stress and eventually cell survival. Who would have imagined that almost 20 years after the seminal discovery of its role as a key player at the interface between glucose metabolism and apoptosis[3], BAD would be identified as an integrator of glucose metabolism and inflammation? ❐

Christian Frezza ✉
MRC Cancer Unit, University of Cambridge, Cambridge, UK.

✉e-mail: cf366@MRC-CU.cam.ac.uk

Published online: 11 May 2020
https://doi.org/10.1038/s42255-020-0198-5

References

1. Dadon, D. et al. *Diabetes Obes. Metab.* **14**(Suppl. 3), 101–108 (2012).
2. Donath, M. Y., Dalmas, É., Sauter, N. S. & Böni-Schnetzler, M. *Cell Metab.* **17**, 860–872 (2013).
3. Danial, N. N. et al. *Nature* **424**, 952–956 (2003).
4. Danial, N. N. et al. *Nat. Med.* **14**, 144–153 (2008).
5. Matschinsky, F. M. & Wilson, D. F. *Front. Physiol.* **10**, 148 (2019).
6. Szlyk, B. et al. *Nat. Struct. Mol. Biol.* **21**, 36–42 (2014).
7. Ljubicic, S. et al. *Cell Rep.* **10**, 497–504 (2015).
8. Matschinsky, F. M. *Nat. Rev. Drug Discov.* **8**, 399–416 (2009).
9. Fu, A. et al. *Nat. Metab* https://doi.org/10.1038/s42255-020-0199-4 (2020).
10. Donath, M. Y., Størling, J., Berchtold, L. A., Billestrup, N. & Mandrup-Poulsen, T. *Endocr. Rev* **29**, 334–350 (2008).

Competing interests

C.F. is a member of the scientific advisory board of Owlstone Medicals and a scientific advisor of Istesso.