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NEWS AND VIEWS

Signaling for death: tyrosine phosphorylation in the response to glucose deprivation

Bryan D Bryson[1,2] and Forest M White[1,2,*]

[1] Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA and [2] Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA
* Corresponding author. Department of Biological Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Bldg 76-353F, Cambridge, MA 02139, USA. Tel.: +1 617 258 8949; Fax: +1 617 258 0225; E-mail: fwhite@mit.edu

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The shift from oxidative phosphorylation to aerobic glycolysis in cancer has focused attention on the altered metabolism of cancer cells as a means of therapeutic intervention. Metabolic dysregulation in cancer was first proposed by Warburg in the 1930s, and this topic remains an active area of research. While previous studies have explored the connection between cellular signaling and metabolism, many have focused on a small subset of components within a complex network of proteins, enzymes, and biochemical signals. In a recent article published in *Molecular Systems Biology*, Graham *et al* (2012) endeavor to better understand the relationship between metabolism and signaling at the network level. Although the question of how cancer cells respond to glucose starvation posited by the authors is relatively simple, the answer ends up being unexpectedly complex. To answer this question, the authors use mass spectrometry and other biochemical profiling techniques to demonstrate a connection between glucose levels, reactive oxygen species (ROS), and alterations in phosphotyrosine-mediated signaling in glioblastoma cell lines.

A number of previous studies have suggested a link between post-translational modifications and signaling in the cell metabolic network. For example, oncogenic tyrosine kinases can localize to the mitochondria providing an avenue whereby metabolic enzymes can be phosphorylated (Hitosugi *et al*, 2011), and phosphorylation of metabolic enzymes have been observed to regulate enzymatic activity (Christofk *et al*, 2008; Hitosugi *et al*, 2009; Fan *et al*, 2011). These findings help set the stage for the systems-level analysis performed by Graham *et al* (2012).

Graham *et al* (2012) screened multiple cancer cell lines to determine their sensitivity to glucose starvation, and then assessed the cross-talk between metabolism and signaling. Using mass spectrometry, the authors found that cells sensitive to glucose withdrawal had increased tyrosine phosphorylation levels on many proteins following glucose starvation, suggesting a link between metabolism, signaling, and cellular response. Probing the signaling network further, the authors noted increased phosphorylation on a number of tyrosine kinases. To assess the mechanism driving this unexpected result, the authors quantified ROS, which had been previously implicated in mediating the cellular response to glucose withdrawal (Ahmad *et al*, 2005). As expected, they observed increased ROS levels following glucose deprivation, and inhibition of ROS activity ablated the dynamic phosphorylation response to glucose withdrawal. To explore the mechanism underlying this effect, the authors measured reduced levels of tyrosine phosphatase activity on glucose withdrawal; this reduction is presumably due to increased oxidation of the active site cysteine, known to decrease the activation state of these enzymes. Moreover, chemically inhibiting phosphatase activity in these cells was shown to increase ROS levels, which the authors propose creates a positive feedback loop amplifying this response. Taken in sum, the authors report a mechanism where glucose withdrawal induced increased levels of ROS that in turn inactivated tyrosine phosphatases and drove supraphysiological levels of tyrosine phosphorylation, which may amplify the feedback loop of further ROS production and tyrosine kinase signaling, finally ending with cell death (Figure 1).

The increased phosphorylation, after glucose withdrawal, of activating sites on receptor tyrosine kinases is a striking result given the typical correlation between increased tyrosine kinase signaling and cell growth. Some studies, however, have suggested a delicate balance within signaling networks, including at least two that have demonstrated that activation of Erk, a pro-growth MAPK kinase, can lead to cell death in certain contexts (Huang *et al*, 2010; Tentner *et al*, 2012). The mechanism by which increased tyrosine phosphorylation reduces cell viability in these glioblastoma cells remains to be determined, and could lead to insights into a potential avenue for cancer treatment, but there remains a significant amount of work to understand this behavior broadly.

One question unaddressed by the authors is the mechanism by which the cells are receiving and transmitting the low-glucose signal. Some studies have implicated glucokinase and AMPK as critical mediators of glucose sensation (Matschinsky, 1990). Although no differences were observed in phosphorylation of acetyl-CoA carboxylase, a well-characterized substrate of AMPK, the activation state of AMPK was not directly tested, and therefore cannot be ruled out as a glucose-response trigger. It is likely that an alternate sensor may be responsible for transmitting the low-glucose signal to the enzymes involved in the generation of ROS, but at this point the identity of this sensor is still unknown. Activation of this sensor may be

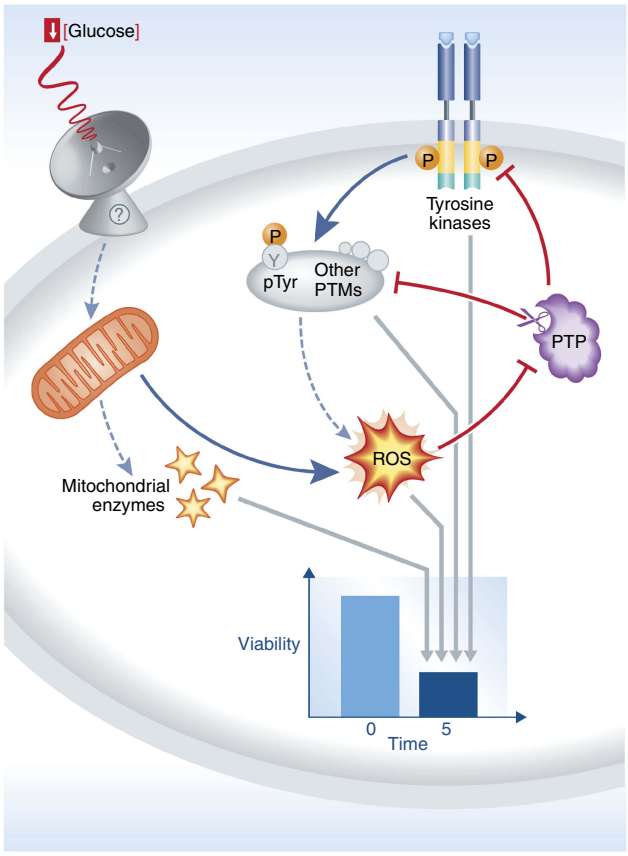

Figure 1 Glucose withdrawal initiates a complex molecular response resulting in decreased cell viability. Cells detect decreased glucose levels through an unknown sensor, which stimulates an increase in reactive oxygen species from the mitochondria. Increased ROS modify and inhibit protein tyrosine phosphatases, causing increased tyrosine phosphorylation on tyrosine kinases and other proteins. Increased phosphotyrosine signaling leads to increased ROS in a positive feedback loop, ultimately resulting in cell death. The gray and dashed lines represent connections that are hypothetical or proceed by currently unknown mechanisms.

an interesting mechanism to transmit a low-glucose signal into cells as a potential therapeutic intervention.

Other important questions remain, including whether a similar response can be observed in normal cells, and what molecular differences determine sensitivity and resistance to glucose withdrawal. The authors provide evidence that PTEN contributes to sensitivity in the LN229 cell line, but they do not fully address the molecular determinants of resistance. In addition, the mechanism underlying cell death in the sensitive cells will require more investigation, since the current experiments cannot distinguish between necrotic and apoptotic cells.

While the glucose reduction experiment demonstrates the possibility of killing cancer cells through glucose deprivation *in vitro*, recapitulating this putative therapeutic response *in vivo* is quite difficult. To this end, it will be important to identify the signals responsible for reduction in cell viability. Future studies should consider the integration of the high-content phosphorylation data sets generated by techniques such as quantitative mass spectrometry with computational approaches to identify specific phosphorylation sites correlated with reduced viability. Recent studies have also implicated other post-translational modifications such as lysine acetylation as critical regulators of metabolic enzyme activity (Zhao *et al*, 2010). In the future, it will be important to incorporate additional PTMs into the signaling framework underlying glucose deprivation response. Once identified, perturbation of these signals may prove to be the more tenable means of biochemically mimicking the effects of glucose withdrawal.

In summary, Graham *et al* (2012) demonstrate the novel and intriguing finding that perturbation of glucose levels leads to altered tyrosine phosphorylation signaling mediated via a ROS-phosphatase axis. Future studies aiming to link signaling and metabolism will be able to harness these findings to gain further insight into cancer metabolism and potential therapeutics.

Conflict of interest

The authors declare that they have no conflict of interest.

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