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

Citation
Bryson, Bryan D, and Forest M White. “Signaling for Death: Tyrosine Phosphorylation in the Response to Glucose Deprivation.” Molecular Systems Biology 8 (2012).

As Published
http://dx.doi.org/10.1038/msb.2012.27

Publisher
Nature Publishing Group

Version
Final published version

Citable link
http://hdl.handle.net/1721.1/77993

Terms of Use
Creative Commons Attribution-Noncommercial-Share Alike 3.0

Detailed Terms
http://creativecommons.org/licenses/by-nc-sa/3.0/
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 deprivation posed 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 phosphoryrosine-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 (Christotk 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
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.

References
Ahmad I M, Aykin-Burns N, Sim JE, Walsh SA, Higashikubo R, Buettner GR, Venkataraman S, Mackey MA, Flanagan SW, Oberley LW, Spitz DR (2005) Mitochondrial O2− and H2O2 mediate glucose deprivation-induced stress in human cancer cells. J Biol Chem 280: 4254–4263
Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, Fleming MD, Schreiber SL, Cantley LC (2008) The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 452: 230–233
Fan J, Hitosugi T, Chung TW, Xie J, Ge Q, Gu TL, Polakiewicz RD, Chen GZ, Boggton TJ, Lomial S, Khuri FR, Kang S, Chen J (2011) Tyrosine phosphorylation of lactate dehydrogenase A is important for NADH/NAD(+)-redox homeostasis in cancer cells. Mol Cell Biol 31: 4938–4950
Graham NA, Tahmasian M, Kohli B, Komisopoulou E, Zhu M, Vivanco I, Teitell MA, Wu H, Ribas A, Le RS, Mellinghoff IK, Mischel PS, Graeber TG (2012) Glucose deprivation activates a metabolic and signaling amplification loop leading to cell death. Mol Syst Biol 8: 589
Hitosugi T, Fan J, Chung TW, Lythgoe K, Wang X, Xie J, Ge Q, Gu TL, Polakiewicz RD, Roesel JL, Chen GZ, Boggton TJ, Lomial S, Fu M, Khuri FR, Kang S, Chen J (2011) Tyrosine phosphorylation of mitochondrial pyruvate dehydrogenase kinase 1 is important for cancer metabolism. Mol Cell 44: 864–877
Huang PH, Mitaldi ER, Xu AM, Kundukulam VA, Del Rosario AM, Flynn RA, Cavenee WK, Furnari FB, White FM (2010) Phosphotyrosine signaling analysis of site-specific mutations on EGFRvIII identifies determinants governing glioblastoma cell growth. Mol Biosyst 6: 1227–1237
Matschinsky FM (1990) Glucokinase as glucose sensor and metabolic signal generator in pancreatic beta-cells and hepatocytes. Diabetes 39: 647–652
Tentner AR, Lee MJ, Ostheimer GJ, Samson LD, Lauffenburger DA, Yaffe MB (2012) Combined experimental and computational analysis of DNA damage signaling reveals context-dependent
roles for Erk in apoptosis and G1/S arrest after genotoxic stress. Mol Syst Biol 8: 568
Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, Yao J, Zhou L, Zeng Y, Li H, Li Y, Shi J, An W, Hancock SM, He F, Qin L, Chin J, Yang P, Chen X, Lei Q et al (2010) Regulation of cellular metabolism by protein lysine acetylation. Science 327: 1000–1004