Cancer Cell Metabolism: There Is No ROS for the Weary

Chi V. Dang

Summary: Using a high-throughput short-hairpin RNA library screen targeting 222 metabolic nodes, Ros and colleagues identified 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 (PFKFB4), a glycolytic enzyme that shunts glucose into the pentose phosphate pathway for NADPH production, as a critical node for the survival of prostate cancer cells. Blocking PFKFB4 induces reactive oxygen species and cancer cell death, suggesting that PFKFB4 could be therapeutically targeted.

Commentary on Ros et al., p. 328 (1).

Cancer cells are on the go, adsorbing lipids and picking up nutrients for deregulated self-replication, which frequently culminates in the death of the host. In this issue of Cancer Discovery, Ros and colleagues (1) document the dependence of prostate cancer cells on 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 (PFKFB4), a glycolytic enzyme that plays an important role in attenuating reactive oxygen species (ROS) for the weary cancer cell to survive and in lipid synthesis for the cancer cell to replicate.

Normal mammalian cells, such as cells of the gut or bone marrow, can replicate at significant rates for tissue homeostasis. Tissue stem cells or immune cells stimulated by growth factors or cytokines undergo a signal transduction and transcriptional program that permits both cell mass accumulation through macromolecular synthesis (e.g., lipid, protein, and nucleic acid synthesis) and acquisition of a commensurate bioenergetic supply. Nutrient-depleted, lowered energy conditions trigger AMP-activated protein kinase, which diminishes cellular ATP consumption and stimulates self-eating or autophagy to recycle cellular components as energy.

In nutrient-replete conditions, glucose modulates transcription through its conversion to intracellular hexose-phosphate and hexosamine, whereas glutamine import is required for activation of mTORC1 to stimulate cell growth (2, 3). Growth factor-receptor signaling further bolsters the cell growth program by activating mTORC2 and AKT, which can directly stimulate glycolysis, and early response genes such as MYC (Fig. 1A). The MYC gene produces a pleiotropic transcription factor that stimulates ribosome biogenesis, nucleotide synthesis, DNA replication, and the import of both glucose and glutamine to support the bioenergetic needs of a growing cell (4).

Cancer cells with deregulated MYC are addicted to glucose and glutamine, whereas healthy cells diminish the expression of MYC under nutrient- or oxygen-deprived conditions. Hence, the proto-oncogene responds to nutrient availability, whereas the deregulated oncogene, through gene amplification or chromosomal translocation, for example, severs its ties to the external world, inducing a constitutive growth program that is addicted to nutrients. In this regard, it is notable that MYC expression is elevated in patients with prostate cancer and that MYC plays a central role in murine models of prostate cancer and maintenance of human prostate cancer, such that knockdown of MYC expression results in diminished prostate cancer cell proliferation (5).

The import of glucose and glutamine or other amino acids is essential for growing cells to generate ATP and provides carbon skeletons as building blocks for ribosomes, membranes, various cellular organelles, and replication of the genome (Fig. 1A (3)). Glucose is converted to pyruvate and in turn into acetyl-CoA for mitochondrial oxidation, or into lactate under hypoxia. Glucose, upon conversion to glucose-6-phosphate, is also catabolized by the pentose phosphate pathway (PPP) to produce NADPH, which provides the key reductive power for biosynthesis and redox balance and ribose for nucleic acid synthesis (Fig. 1B). Glutamine, however, enters the tricarboxylic acid (TCA) cycle via conversion to glutamate and then to α-ketoglutarate, which is further catabolized by the TCA cycle (Fig. 1B (6)). The byproducts of glucose and glutamine catabolism are ROS resulting from electron leakage from the electron transport chain, carbon dioxide, water, and lactate that are all disposed by different mechanisms (Fig. 1B). ROS, being inherently genotoxic, are titrated by layers of safeguards, including NADPH-glutathione, peroxiredoxins, superoxide dismutase, and catalase. As such, rapidly proliferating cancer cells must deal with the ROS load to survive.

Ros and colleagues (1) undertook a genetic screen with a short-hairpin RNA library targeting 222 genes involved in metabolism to determine whether prostate cancer cell lines, as compared with a normal prostate cell line, are dependent on critical metabolic nodes. They found that among 3 prostate cancer cell lines (DU145, PC3, and LNCaP) that all display a dependency on glucose for viability, 2 critical genes were required for their survival under lipid-limiting culture conditions. One gene is PRKAB1, encoding the β1 subunit
ligands, such as the negative regulator citrate produced from the TCA cycle or the positive regulator 2,6-fructose bisphosphate (2,6-FBP) produced by the PFK2 family members. The PFK2 enzymes have dual kinase and phosphatase activities, producing or degrading 2,6-FBP.

There are 4 known PFK2 members [PFKFB1-4 (7)]. PFKFB3 and PFKFB4 are both activated by hypoxia, but their biochemical functions are distinctly different, with MYC acting as a positive regulator and PFKFB3 and PFKFB4 acting as negative regulators. MYC stimulates ribosome biogenesis and biomass accumulation for cell proliferation, while hypoxia activates PFKFB3 and PFKFB4, leading to increased glycolysis and reduced oxidative phosphorylation.

Figure 1. A, diagram depicting receptor signaling and nutrient import. Receptor signaling, through phosphoinositide 3-kinase (PI3K), stimulates mTORC2 and AKT as well as the MYC oncogene, which stimulates ribosome biogenesis and biomass accumulation for cell proliferation. Glucose and glutamine import stimulated by MYC induces mTORC1 and a collateral pathway of activating protein synthesis and ribosome biogenesis. Glutamine is shown converted to glutamate and then to α-ketoglutarate for further mitochondrial oxidation. Glucose is converted to lactate or to acetyl-CoA, which is further oxidized in the mitochondrion. Glucose is also shown shunted to the PPP to produce NADPH and ribose-5-phosphate. B, glycolytic conversion of glucose to pyruvate and its conversion to ribose-5-phosphate via the pentose phosphate pathway. Glucose is transported into the cell and phosphorylated to glucose-6-phosphate by hexokinases, and then to fructose-6-phosphate by glucose phosphate isomerase. Fructose-6-phosphate is phosphorylated by PFK1, which is positively allosterically regulated by fructose-2,6-phosphate (F2,6P), to fructose-1,6-phosphate and subsequently metabolized to pyruvate for further oxidation in the mitochondrion, a rich source of ROS. The levels of F2,6P are regulated by the PFK2 family members, PFKFB4 and TIGAR (a target of p53) that dephosphorylates F2,6P and PFKFB3 that generates F2,6P from fructose-2-phosphate. Increased PFKFB4 or TIGAR lowers F2,6P and hence decreases PFK1 activity, shunting glucose-6-phosphate into the pentose phosphate pathway for production of ribose-5-phosphate and NADPH. NADPH reduces glutathione and thereby inhibits ROS.
P FKFB3 having more kinase activity and PFKFB4 having more phosphatase activity (Fig. 1B). This duo therefore provides a yin-yang control over the levels of 2,6-FBP that potently activates PFK1 to drive glycolysis; in the absence of 2,6-FBP, glucose is shunted away from PFK1 toward the PPP. The PFK2 node is also regulated by p53, which transactivates TIGAR, producing a PFK2 member that also has greater phosphatase than kinase activity (7). Hence, p53 activation shunts glucose toward the PPP (Fig. 1B).

The PPP converts glucose to ribulose-5-phosphate through glucose-6-phosphate dehydrogenase and 2 other enzymes in the oxidative phase of the PPP, which produces NADPH from NADP+ (Fig. 1). Ribulose-5-phosphate is then converted in the nonoxidative phase of the PPP to ribose-5-phosphate for nucleic acid synthesis or to other intermediates such as glyceraldehyde-3-phosphate or fructose-6-phosphate, which can enter glycolysis. As such, the PFK2 enzymes play an important role in the switch away from glycolysis toward the pentose phosphate pathway, resulting in the production of NADPH, which is important both for dampening oxidative stress through increased glutathione levels and for fatty acid synthesis. The unbiased screen reveals that PFKFB4 is critical for prostate cancer cell line survival, particularly for redox homeostasis, because treatment of prostate cancer cell lines with an antioxidant could partially rescue their demise from diminished PFKFB4 expression (1).

Intriguingly, the necessity of PFKFB4 for cell survival extends beyond prostate cancer because other cancer cell lines are also sensitive to knockdown of PFKFB4 expression. Why PFKFB4 levels are elevated in prostate and other cancer cell lines is not known. Diminished PFKFB4 expression, however, does not seem to dramatically affect growth of the normal human prostate epithelial cell line RWPE1. Until there are further studies, it seems premature to suggest that PFKFB4 is not essential for normal cell proliferation in light of a significant overlap in the metabolic profiles of normal T cells and that of lymphoma. MYC induces glucose and glutamine metabolism in normal T-cell mitogenesis and proliferation of neoplastic lymphocytes, suggesting a significant overlap between normal and neoplastic cell metabolism (8). The difference, however, is that deregulated oncogene expression renders tumor cells addicted to nutrients (4).

The article by Ros and colleagues (1) further documents a difference in the metabolism of prostate cancer cells versus the normal prostate, which exhibits a truncated TCA cycle that produces vast amounts of citrate. Citrate is secreted into the seminal fluid, presumably providing some protective measures to the sperm cells. All 3 prostate cancer cell lines studied by Ros and colleagues (1) are highly glycolytic. It is notable, however, that RWPE1 is also glycolytic and does not display the truncated TCA cycle of normal prostate. Ros and colleagues (1) also report the lack of significant dependence of these prostate cancer cell lines on glutamine, which contrasts with some previous published studies; particularly, Gao and colleagues (9) documented the dependency of PC3 cells on glutamine and particularly on glutaminase, which shunts glutamine into the TCA cycle.

It is also notable that glutamine appears to be a significant substrate for the TCA cycle, particularly under hypoxia (10–13). In fact, glutamine can contribute to a significant fraction of lipids through its conversion to α-ketoglutarate and subsequent reductive carboxylation to isocitrate, which is then converted to citrate for lipid synthesis via ATP citrate lyase (10, 12). Furthermore, under limiting glucose levels in the hypoxic tumor microenvironment, glutamine fills in the TCA cycle as well as supports the synthesis of glutathione (13). Notwithstanding these nuances, the article by Ros and colleagues (1) and earlier studies from the laboratories of Cheng and Vousden (7) and Clem and colleagues (14) underscore the importance of the PFK2 family of enzymes in tumor metabolism, which might be exploitable for cancer therapy.

Indeed, Clem and colleagues (14) suggested that inhibition of PFKFB3 could be therapeutic via diminishing glycolytic flux, but Ros and colleagues (1) could not corroborate the importance of PFKFB3 for prostate cancer cell survival. In this regard, it will be of significant interest whether highly specific inhibitors of PFKFB4 could be therapeutic in a subset of human prostate and other cancers. The challenge for targeting cancer cell metabolism is being able to precisely profile the cancer cell metabolome according to the type of cancer and to identify the metabolic Achilles’ heels. In fact, the oncogenic drive and organ site profoundly influence the cellular usage of glucose or glutamine, as recently reported by Yuneva and colleagues (15). The heterogeneous tumor microenvironment and the ability of cancer cells to handle ROS through endogenous antioxidant systems are expected to profoundly affect the response of cancer cells to metabolic inhibitors. Hence, there is no good ROS for the weary cancer cell, which also produces various toxic byproducts such as lactate and carbon dioxide that have been exploited for therapy through inhibition of lactate transporters, MCT1 and MCT4, or carbonic anhydrase CA9 (16). Insights from previous studies and the article by Ros and colleagues (1) suggest that blocking the cellular exhaust pipes is, therefore, a compelling strategy to interrupt cancer cell metabolism, in addition to disrupting the fuel lines (3).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Author Contributions

Writing, review, and/or revision of the manuscript: C. V. Dang

Acknowledgments

The author thanks Brian Altman for his comments.

Grant Support

This work is supported by the National Cancer Institute, the Leukemia and Lymphoma Society of America, a Stand Up To Cancer Dream Team Translational Cancer Research Grant, a Program of the Entertainment Industry Foundation Foundation (SU2C-AACR-DT0509), and the Abramson Family Cancer Research Institute.

Received February 20, 2012; accepted February 21, 2012; published online April 11, 2012.

REFERENCES


Cancer Cell Metabolism: There Is No ROS for the Weary

Chi V. Dang


Updated version
Access the most recent version of this article at:
http://cancerdiscovery.aacrjournals.org/content/2/4/304

Cited articles
This article cites 16 articles, 2 of which you can access for free at:
http://cancerdiscovery.aacrjournals.org/content/2/4/304.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerdiscovery.aacrjournals.org/content/2/4/304.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.