Chemistry & Biology
Review

Action at a Distance: Allostery and the Development of Drugs to Target Cancer Cell Metabolism

Byron DeLaBarre,[1,2] Jonathan Hurov,[1,2] Giovanni Cianchetta,[1,2] Stuart Murray,[1] and Lenny Dang[1,*]
[1]Agios Pharmaceuticals, Inc., 38 Sidney Street, Cambridge, MA 02139, USA
[2]Co-first author
*Correspondence: lenny.dang@agios.com
http://dx.doi.org/10.1016/j.chembiol.2014.08.007

Cancer cells must carefully regulate their metabolism to maintain growth and division under varying nutrient and oxygen levels. Compelling data support the investigation of numerous enzymes as therapeutic targets to exploit metabolic vulnerabilities common to several cancer types. We discuss the rationale for developing such drugs and review three targets with central roles in metabolic pathways crucial for cancer cell growth: pyruvate kinase muscle isozyme splice variant 2 (PKM2) in glycolysis, glutaminase in glutaminolysis, and mutations in isocitrate dehydrogenase 1 and 2 isozymes (IDH1/2) in the tricarboxylic acid cycle. These targets exemplify the drugging approach to cancer metabolism, with allosteric modulation being the common theme. The first glutaminase and mutant IDH1/2 inhibitors have entered clinical testing, and early data are promising. Cancer metabolism provides a wealth of novel targets, and targeting allosteric sites promises to yield selective drugs with the potential to transform clinical outcomes across many cancer types.

The development of cancer is driven by the acquisition of multiple alterations in normal human cells that allow dysregulated proliferation. These alterations encompass a broad spectrum of biological processes including cell growth, epigenetic regulation of gene expression, and cell metabolism (Hanahan and Weinberg, 2011). The specific metabolic requirements of cancer cells are driven by interplay between their genetic and epigenetic status and the microenvironment; thus, the metabolic machinery must carefully regulate energy use to maintain growth and division under conditions of varying nutrient and oxygen levels. Some of the central metabolic pathways utilized by cancer cells are illustrated in Figure 1. Neighboring stromal cells and normal tissues compete with the tumor for nutrients and paradoxically, in some cases, provide critical signals and nutrients. Additionally, genetic mutations alter the utilization of, or dependence on, nutrients according to the tumor suppressor or oncogene affected (Cairns et al., 2011; Yuneva et al., 2012). The crosstalk between the signaling pathways of these genetic alterations and the metabolic machinery of the cell is complex and only partially understood at present (Ward and Thompson, 2012).

In this review, we discuss the rationale for developing drugs that target cancer cell metabolism, and provide in-depth discussion on three cancer metabolism targets that exemplify the drugging approach: pyruvate kinase (PK) in glycolysis, glutaminase in the glutaminolysis pathway, and isocitrate dehydrogenase (IDH) in the tricarboxylic acid (TCA) cycle. We aim to highlight the common theme of allosteric modulation in designing drugs against these targets, which have central roles in metabolic pathways that are crucial for cancer cell growth, with a focus on the interface between chemistry and biology.

Rationale for Targeting Cancer Cell Metabolism

Early work in cancer metabolism includes not only the much-cited work by Otto Warburg in the 1920s, describing the propensity of cancer cells for glycolysis, but also groundbreaking work by Farber and others in the 1940s on the use of antifolates in the clinic as efficacious anticancer agents (Farber and Diamond, 1948; Warburg et al., 1927). In the 1960s and 1970s, work with the glutamine analogs, acivicin and 6-diazo-5-oxo-1-norleucine (DON), introduced the possibility of therapeutic benefit through inhibition of glutamine pathways in cancer cells (Ahluwalia et al., 1990). The 1970s also saw the emergence of asparaginase therapy for the treatment of childhood acute lymphoblastic leukemia, which is still in use today, as removal of circulating asparagine halts the growth of asparagine-dependent leukemic cells (Avramis, 2011).

Glycolysis is probably the best-understood metabolic pathway in cancer cells. Many cancers utilize glucose for ATP production and as a means of providing carbon for the pentose phosphate, serine, and 1-carbon pathways (Figure 1). Glycolysis is regulated directly by multiple growth factor pathways and by tumor microenvironment, including hypoxia. Compelling data exist to support the investigation of several enzymes in the pathway as drug targets including hexokinase 2 (Muller et al., 2012), enolase 2 (Muller et al., 2012), and the muscle isozyme of PK (PKM) (Christofk et al., 2008a). We will focus on PKM, in particular the alternative splice variant, PKM2, which has been shown to be critical for cancer cell proliferation (Anastasiou et al., 2012; Christofk et al., 2008a; Kung et al., 2012).

In recent years, glutamine biology has become an area of intense research as a greater appreciation for its role in cancer cell homeostasis has emerged. Glutaminase has been implicated as a critical node in cancer cells, and glutamine dependence has been described in multiple settings, including triple negative breast cancer (Gross et al., 2014; Timmerman et al., 2013) and renal cell carcinoma (Metallo et al., 2012).

While PKM2 and glutaminase represent interesting therapeutic targets, the genes encoding these enzymes have yet to be

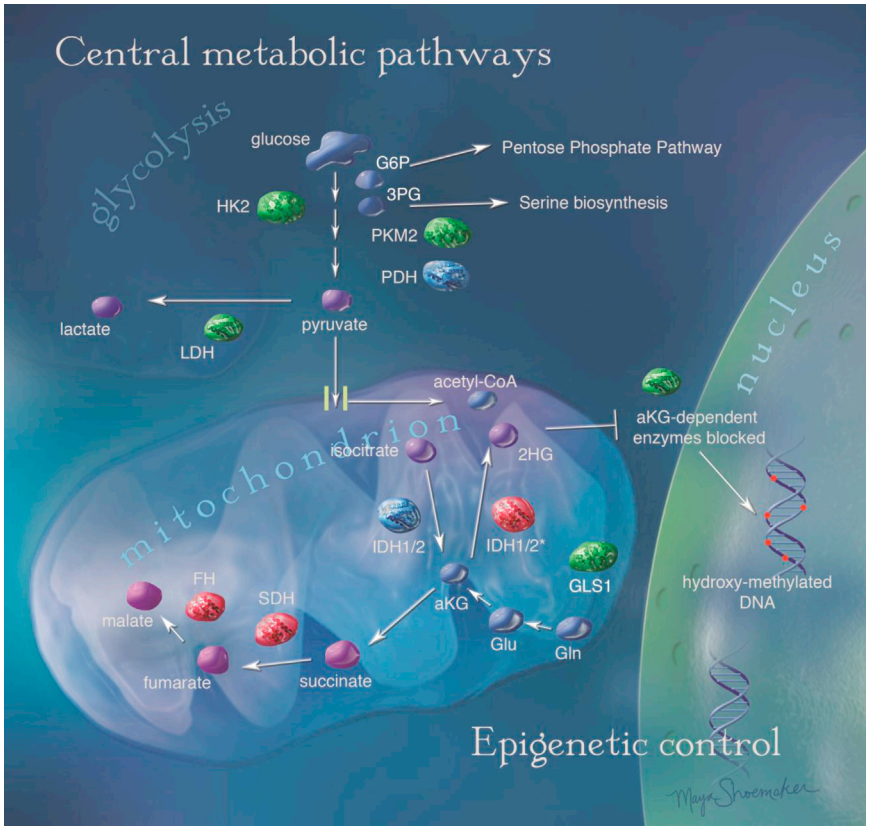

Figure 1. Central Metabolic Pathways Utilized by Cancer Cells
*denotes mutated isoenzyme.

identified as mutated or amplified in cancer to any significant degree. In contrast, several metabolic genes have been identified as frequently mutated in cancer. For example, homozygous loss of function mutations in the TCA cycle enzymes succinate dehydrogenase and fumarate hydratase are observed in multiple cancers (Raimundo et al., 2011), and accumulation of their metabolites (fumarate and succinate) in deficient tumors are thought to impact pathways capable of driving tumorigenesis (Adam et al., 2014; Kaelin, 2011). Mutations in another TCA cycle enzyme, IDH, have also garnered much interest and excitement. IDH mutations are prevalent in glioma (~70% of low-grade gliomas and secondary glioblastomas) (Yan et al., 2009) and acute myeloid leukemia (AML; up to 20% of all patients) (McKenney and Levine, 2013), and have been identified in other cancers including lymphoma (Cairns et al., 2012), cholangiocarcinoma (Borger et al., 2012), and chondrosarcoma (Amary et al., 2011). IDH mutations are often identified in founding clones of AML and are likely to be disease initiating, highlighting their importance as drug targets (Welch et al., 2012). IDH1/2 inhibitor discovery efforts are ongoing, as the first wave of these drugs has recently entered clinical trials, as detailed later in this review.

Based on knowledge acquired to date, there is no doubt that cancer metabolism provides a wealth of novel therapeutic targets and multiple innovative ways in which to exploit metabolic vulnerabilities for therapeutic benefit. More comprehensive reviews cover the breadth of metabolic targets that are currently under investigation (Stine and Dang, 2013; Vander Heiden, 2011). The following sections of this review focus on PKM2, glutaminase, and mutated IDH1/2 as exemplary metabolism targets under investigation for development of cancer therapies.

Drugging Glycolysis: Targeting Pyruvate Kinase Muscle Isozyme Alternative Splice Variant 2

PK catalyzes the last step of glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate, while producing one molecule of ATP. The reaction encompasses two chemical steps: the first involves a phosphoryl transfer from PEP to ADP, forming an enolate intermediate and ATP, and the second involves protonation of the enolate intermediate, forming pyruvate (Robinson and Rose, 1972).

PKM2 is one of four PK isoforms in humans. PKM1 and PKM2 result from the alternative splicing of exons 9 and 10 of the *PKM* gene, which encode a stretch of amino acids that differ at 23 positions between PKM1 and PKM2. PKM1 is constitutively active in skeletal muscle and brain tissue, but is not allosterically regulated. PKM2 is expressed in fetal and proliferating tissues, has low basal activity compared with PKM1, and is allosterically regulated. R-type pyruvate kinase (PKR) and L-type pyruvate kinase (PKL) are transcribed via different promoters from the *PKLR* gene. PKR is expressed in erythrocytes and PKL in the liver. PKR, PKL, and PKM1 exist as stable tetramers, whereas PKM2 forms tetramers (high activity form), dimers (low activity form), and monomers (Mazurek, 2011).

Pyruvate Kinase Muscle Isozyme Alternative Splice Variant 2 in Cancer Cell Metabolism

Cancer cells predominantly express PKM2, which can be downregulated by tyrosine kinase growth factor signaling pathways, allowing metabolic flexibility. Phosphotyrosine peptides have been shown to suppress PKM2 activity by binding tightly to PKM2, thereby catalyzing the release of fructose 1,6-bisphosphate (FBP), resulting in a switch to the low activity dimer state (Christofk et al., 2008b; Hitosugi et al., 2009). This downregulation is thought to support tumor growth and proliferation by allowing for the shunting of glycolytic intermediates toward other biosynthetic pathways (i.e., pentose phosphate and serine pathways). In keeping with this model, the activation of PKM2 in cancer cells using small molecule agonists resulted in serine auxotrophy (Kung et al., 2012). Consistent with the hypothesis that PKM2 is a critical metabolic switch, there is growing evidence that, depending on the cellular stress environment, PKM2 activity can be regulated by posttranslational modification such as acetylation (Lv et al., 2011), phosphorylation (Hitosugi

