Biochemical Pharmacology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Heterogeneity of glycolysis in cancers and therapeutic opportunities

Marc O. Warmoes, Jason W. Locasale *

Division of Nutritional Sciences, Cornell University, Ithaca, NY, United States

ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form 21 July 2014
Accepted 21 July 2014
Available online xxx

Chemical compounds studied in this article:
Bromopyruvate (PubChem CID: 70684)
2-deoxyglucose (PubChem CID: 9062)
3-PO (PubChem CID: 5720233)
PGMI-004A (PubChem CID: 66521681)
TEP-46 (Chem CID: 44246499)

Keywords:
Glycolysis
Isoenzymes
Cancer
Spliceforms
Anti-glycolytic agents

ABSTRACT

Upregulated glycolysis, both in normoxic and hypoxic environments, is a nearly universal trait of cancer cells. The enormous difference in glucose metabolism offers a target for therapeutic intervention with a potentially low toxicity profile. The past decade has seen a steep rise in the development and clinical assessment of small molecules that target glycolysis. The enzymes in glycolysis have a highly heterogeneous nature that allows for the different bioenergetic, biosynthetic, and signaling demands needed for various tissue functions. In cancers, these properties enable them to respond to the variable requirements of cell survival, proliferation and adaptation to nutrient availability. Heterogeneity in glycolysis occurs through the expression of different isoforms, posttranslational modifications that affect the kinetic and regulatory properties of the enzyme. In this review, we will explore this vast heterogeneity of glycolysis and discuss how this information might be exploited to better target glucose metabolism and offer possibilities for biomarker development.

Published by Elsevier Inc.

Contents

Abbreviations: UDP-GlcNAc, uridine diphospho-N-acetylglucosamine; HK, hexokinase; BrPyr, 3-bromopyruvic acid; EC, endothelial cells; MCT, monocarboxylate transporter; 2DG, 2-deoxyglucose; F26P, fructose-2,6-bisphosphate; PFKFB, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase; O-GlcNAc, O-linked β-N-acetylglucosamine; OGT, O-linked N-acetylglucosamine (GlcNAc) transferase; AMPK, AMP-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; GLUT1, glucose transporter 1; SER, serine; PPP, pentose phosphate pathway; SAICAR, succinyl-5-aminoimidazole-4-carboxamide-1-ribose-5′-phosphate; PTM, posttranslational modification; Kpg, lysine-phosphoglycerate; 13BGP, 1,3-biphosphoglycerate; MCA, metabolic control analysis.

* Corresponding author at: 108 Savage Hall, Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, United States. Tel.: +1 607 255 5114.
E-mail address: locasale@cornell.edu (J.W. Locasale).

http://dx.doi.org/10.1016/j.bcp.2014.07.019
0006-2952/Published by Elsevier Inc.

1. Introduction

Increased glucose metabolism is one of the distinguishing features between normal cells and highly proliferating cells like cancer, stem and immune cells [1]. Recognized by Otto Warburg over 80 years ago, one of the main features is the observation that growing cells secrete lactate while consuming large amounts of glucose (the Warburg Effect) [2]. The Warburg Effect occurs both through the activity of oncogenes and tumor suppressor genes [3] and through adaptations to the tumor microenvironment [4]. Pre-clinical and clinical research into cancer metabolism has shown that small molecules that interfere with different metabolic pathways can indeed have marked effects on tumor proliferation, either alone [5–7] or in combination with longstanding chemotherapies [8–11]. In fact, some of the oldest classes of chemotherapy are anti-metabolites that interfere with one-carbon metabolism and nucleotide synthesis such as 5-fluorouracil (5-FU) and methotrexate [12]. Even though many of these drugs target one-carbon metabolism and nucleotide synthesis, the last decade has seen additional development of agents that target glycolysis. There are several rationales for targeting glycolysis. First, high glycolytic flux leads to new biology including the selective diversion of carbon into several anabolic pathways and thus provides precursors for nucleotide, protein and fatty acids synthesis and the maintenance of signal transduction processes that occur through changing the levels of metabolites [2]. Second, the rate at which glycolysis is upregulated in cancer cells compared to normal cells creates an opportunity to selectively target tumors [13]. Last, the high flux leading to lactate secretion has also been linked to promote favorable non-cell autonomous conditions for uncontrolled proliferation such as evasion of the immune system [14] and induction of angiogenesis and metastasis [14] and these processes may be targeted by altering glycolysis. Nevertheless, there are still many unanswered questions on how to target glycolysis more effectively.

2. Heterogeneity in glycolysis

Heterogeneity in glycolysis is a typical feature of eukaryotic cells living either as interacting cell populations or as multi-cellular organisms [15]. This offers cells from various tissues or populations control to express the enzyme with the optimal kinetic and regulatory properties needed for the specific tissue or population function. Specifically, for multi-cellular organisms, this serves three main functions. Firstly, it allows for a coordinated control of glucose homeostasis within the body [16]. Second, it also allows an organisms cells to respond adequately to different forms of oxygen or glucose stress, such as hypoxia [15], ischemia [17] or changes in diet [16]. Lastly, glycolytic heterogeneity that allows for a high glycolytic rate, offers a subset of cells that need to sustain increased levels of proliferation during certain stages of physiological processes like angiogenesis [18], immune activation [19] and stem cell growth [20] (also see above). Research has shown that these isoenzymes also display a wide variety in expression according to tissue specific tumor development [21–23]. The functional basis for this tumor specific heterogeneity can to a certain degree be explained by the metabolic features of the tissue of origin [21]. There is also evidence that glycolytic isoenzymes are differentially regulated during the cell cycle [24]. Because proliferation rate and glycolysis also show a certain degree of correlation, this therefore explains another contributing factor to glycolytic heterogeneity in tumors with varying proliferation rates [21].

Many glycolytic isoenzymes are transcribed from different gene loci (see Table 1 and Fig. 1). This offers cells the first level of heterogeneity. Additional heterogeneity is introduced because a specific gene locus can also give rise to different splice-forms. Some of these splice forms have only been predicted computationally or identified from preliminary large scale RNA-sequencing and still await further experimental validation [25]. Especially, N-terminal splicing seems to be common for most glycolytic enzymes, with one of the most notable exceptions being the pyruvate kinase muscle isozyme (PKM) (see Table 1). These isoenzymes have different enzyme kinetic parameters such as Michaelis constants (see Table 1) and turnover rates. In addition many isoforms of glycolytic enzymes experience different allosteric regulation of small molecules. Furthermore, these enzymes have different regulation of their levels by promoters [26,27], non-coding RNA [28,29], expression of splice factors [30], etc. Further complexity could also be generated by the fact that glycolytic enzymes undergo a myriad of posttranslational modifications (PTMs) that are regulated by signal transduction events [31–40]. In some instances, modifications could allow for the exertion of 'moon-lighting' functionalities such as modulation of gene-expression in the nucleus [41,42] or inhibition of apoptosis [43]. According to the phosphositeplus database [44], the most prevalent modifications are serine-, threonine- and tyrosine-phosphorylation, acetylation and ubiquitination (see Table 1). Nevertheless, despite these intriguing possibilities attributed to enzyme PTMs, it is not clear whether the stoichiometry of these modifications can ever reach sufficient levels to have a substantial impact on glycolytic flux [38,45].

Together the extent of molecular diversity within glycolysis (see Fig. 1) offers the ability to rapidly tune enzyme kinetics to adapt to given environmental demands. In addition, this diversity also allows for a tremendously heterogeneous array of possibilities for flux through glycolysis and its control.

3. Therapeutic opportunities in glycolysis

Targeting glucose metabolism can occur both through altering systemic metabolism and through directly targeting enzymes in the diseased cell. For systemic metabolism examples include suppressing of hepatic gluconeogenesis by anti-diabetic agents like metformin [46–48], or the administration of the ketogenic diet [49–51] which enforces increased reliance on lipid oxidation as an energy source. Targeting glucose metabolism directly in the tumor is an alternative to this approach and aims to alter the biosynthetic, bioenergetic, and signaling processes that are differentially occurring as a result of the enhanced cell autonomous glucose metabolism [2,52]. Both approaches are complementary and are actively being explored in the clinic [49,53–55].

One approach to target glycolysis directly is to consider a single anti-glycolytic agent that targets a single enzyme with high specificity. This approach is promising in that it would offer potentially less toxicity but leads to difficulties in establishing whether there is sufficient efficacy, especially if tumors can develop resistance by expressing an alternate isoform of the targeted enzyme or establishing some other bypass mechanism. An additional approach is to consider anti-glycolytic agents that have multiple targets, both within the glycolysis pathway and in other pathways [56]. In response to this, some have argued for the development of "dirty drugs" or cocktails of specific molecules that are able to simultaneously target multiple nodes within the network and will be more effective [57,58].

Often it is difficult to classify (anti-glycolytic) agents clearly into either class because specificity of small molecules for their proposed targets is usually dose dependent [59]. Moreover, identifying off targets effects or exact mechanism of action within a cell is also impeded by technical limitations [59,60]. In the light of this uncertainty, it is interesting to describe the research into the mechanism of action of 3-bromopyruvic acid (BrPyr). Originally

Please cite this article in press as: Warmoes MO, Locasale JW. Heterogeneity of glycolysis in cancers and therapeutic opportunities. Biochem Pharmacol (2014), http://dx.doi.org/10.1016/j.bcp.2014.07.019