Experimental and Molecular Pathology 86 (2009) 174–179

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

Review

Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases in cancer

Abdullah Yalcin [a,b], Sucheta Telang [a], Brian Clem [a], Jason Chesney [a,*]

[a] Molecular Targets Group, Department of Medicine, Medical Oncology, James Graham Brown Cancer Center, University of Louisville, Baxter II Research Building, #204E, 580 South Preston Street, Louisville, KY, 40202, USA
[b] Department of Biochemistry, School of Veterinary Medicine, Uludag University, Bursa, Turkey

ARTICLE INFO

Available online 14 January 2009

Keywords:
Glycolysis
Fructose-2,6-bisphosphate
Ras
Neoplastic transformation
Chemotherapy
PFKFB3

ABSTRACT

A high rate of glycolytic flux, even in the presence of oxygen, is a central metabolic hallmark of neoplastic tumors. Cancer cells preferentially utilize glycolysis in order to satisfy their increased energetic and biosynthetic requirements. This metabolic phenotype has been confirmed in human studies using positron emission tomography (PET) with ^{18}F-2-fluoro-deoxy-glucose which have demonstrated that tumors take up 10-fold more glucose than adjacent normal tissues *in vivo*. The high glucose metabolism of cancer cells is caused by a combination of hypoxia-responsive transcription factors, activation of oncogenic proteins and the loss of tumor suppressor function. Over-expression of HIF-1α and *myc*, activation of *ras* and loss of p53 function each have been found to stimulate glycolysis in part by activating a family of regulatory bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB). The PFKFB enzymes synthesize fructose-2,6-bisphosphate (F2,6BP) which allosterically activates 6-phosphofructo-1-kinase (PFK-1), a rate-limiting enzyme and essential control point in the glycolytic pathway. PFK-1 is inhibited by ATP when energy stores are abundant and F2,6BP can override this inhibition and enhance glucose uptake and glycolytic flux. It is therefore not surprising that F2,6BP synthesis is stimulated by several oncogenic alterations which simultaneously cause both enhanced consumption of glucose and growth. Importantly, these studies suggest that selective depletion of intracellular F2,6BP in cancer cells may suppress glycolytic flux and decrease their survival, growth and invasiveness. This review will summarize the requirement of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases for the regulation of glycolysis in tumor cells and their potential utility as targets for the development of antineoplastic agents.

Contents

Introduction

Unlike many differentiated cells in an adult organism, tumor cells have an unrestricted capacity to divide and proliferate. Relentless biosynthesis of macromolecules that are needed for the growth of

* Corresponding author. Fax: +1 501 852 3661.
E-mail address: jason.chesney@louisville.edu (J. Chesney).

0014-4800/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexmp.2009.01.003

newly divided cells requires the uptake of glucose and other carbon sources in excess of energetic needs (Bauer et al., 2004; DeBerardinis et al., 2008). The vast majority of tumor cell types display modified rates and pathways of energetic and anabolic metabolism in comparison to their tissue of origin (Moreno-Sanchez et al., 2007). The most notorious and well-known metabolic alteration of tumor cells is increased glucose uptake and glycolytic capacity, even in the presence of a high oxygen concentration (*i.e.*, aerobic glycolysis) (Gatenby and Gillies, 2004; Moreno-Sanchez et al., 2007). The observation that tumor cells take up and catabolize glucose at a significantly higher rate than their tissue of origin was first made by Otto Warburg over 90 years ago. This phenomenon, known as "the Warburg effect", provides the basis for the most sensitive and specific imaging technique available for the diagnosis and staging of solid cancers: positron emission tomography of 2-[^{18}F]fluoro-2-deoxy-glucose uptake (Wechalekar et al., 2005).

Though still debated, the following rationales for increased glycolysis in tumor cells have been put forward: (i) rapid energy production in the form of ATP; (ii) synthesis of intermediates needed for biosynthetic pathways, including fructose-6-phosphate and glyceraldehyde-3-phosphate for shunting into *de novo* nucleic acid synthesis, and pyruvate for amino acid metabolism and further oxidation in the citric acid cycle (Boros et al., 2000; Boren et al., 2001; Boros et al., 2001); and (iii) establishment of low intracellular and extracellular pH, causing apoptosis in normal cells that express functional p53 (Williams et al., 1999; Gatenby and Gillies, 2004). Tumor cells thus may sustain high glycolysis not only for energy and biosynthetic precursor production, but also for the eradication of adjacent normal cells within organ parenchyma (Gatenby and Gillies, 2004).

Control of glycolysis in cancer

The glycolytic pathway is regulated by availability of substrates, allosteric effectors, and the activities of metabolic transporters, enzymes and regulators, which are in turn controlled by mRNA and protein expression levels and by post-translational modifications such as phosphorylation (Gatenby and Gillies, 2004; Moreno-Sanchez et al., 2007). A key characteristic that distinguishes transformed cells from normal cells is the constitutive activation of growth factor signaling pathways, and these pathways directly control cellular metabolism. Activation of oncogenes and/or inactivation of tumor suppressor genes often cause alterations in response to growth signals, resulting in a "short-circuit" or bypass of critical control steps, which ultimately leads to the constitutive activation of growth-factor stimulated pathways. Among such genes are *ras*, c-*myc*, *src* and p53, and each of these key regulators have been implicated in the Warburg phenomenon (Hue and Rousseau, 1993; Osthus et al., 2000; Kondoh et al., 2005; Vizan et al., 2005; Bensaad et al., 2006). For example, the majority of glycolytic genes are transcriptional targets of c-*myc* and several of them, including hexokinase-2, lactate dehydrogenase-A and enolase, directly interact with c-*myc* (Kim and Dang, 2006).

Solid tumors develop hypoxic cores, which is caused by rapid cell proliferation that outpaces the supply of oxygen from the newly formed blood vessels. Adaptation to hypoxia is largely mediated by hypoxia-inducible factor-1 (HIF-1), a transcription factor critical for tumor angiogenesis, glycolysis, and metastasis (Semenza, 2003; Huang, 2008). Nearly all glycolytic genes are transcriptional targets of HIF-1 and the transcriptional expression of the subunit HIF-1α is under the control of growth-factor signaling pathways such as the PI3K/Akt/mTOR and Raf/MAPK pathways (Jiang et al., 2001; Lopez-Lazaro, 2006; DeBerardinis et al., 2008). Under normoxia, HIF-1α is constantly degraded. However, the stabilization of the HIF-1α protein is a common occurrence in tumors even under nonhypoxic conditions (Semenza, 2003; Kim and Dang, 2006; Dang et al., 2008) and can be caused by the oncogenic activation of PI3K/Akt, *src* and *ras*, or inactivation of the tumor suppressor VHL, leading to the Warburg phenotype (Kim and Dang, 2006; DeBerardinis et al., 2008). Reducing the levels of HIF-1 therefore may be an appealing anticancer strategy (Semenza, 2003; Robey et al., 2005; Lopez-Lazaro, 2006; Bartrons and Caro, 2007).

Rate-limiting step of glycolysis and its deregulation in tumor cells

The conversion of fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F1,6BP) by 6-phosphofructo-1-kinase (PFK-1) is the first committed step of glycolysis and this reaction is essentially irreversible (Weber, 1977; Marshall et al., 1978). Thus, PFK-1 is an important control point in the glycolytic pathway and this enzyme is widely held to dictate the pace of glycolytic flux (Weber, 1977; Van Schaftingen et al., 1981). PFK-1 activity is inhibited by ATP, citrate, and fatty acids, of which the most potent negative regulator, ATP, can directly inhibit PFK-1 in order to cause negative feedback when oxygen and therefore energy is abundant (*i.e.* the Pasteur Effect). PFK-1 is a complex tetrameric enzyme consisting of three subunits: Muscle (M), liver (L), and platelet (P). Each subunit is encoded by a separate structural locus on chromosomes 1 (M), 21 (L), and 10 (P). In tumors, the subunits P and L prevail over M (Vora et al., 1985; Sanchez-Martinez and Aragon, 1997). The kinetic and regulatory properties of the heterotetrameric PFK-1 depend on the type and proportion of the different subunits (Moreno-Sanchez et al., 2007). For example, PFK-1 in rat thyroid carcinomas and human gliomas is less sensitive to inhibition by ATP in comparison to normal tissue counterparts. On the other hand, PFK-1 from human gliomas is five times more sensitive to activation by fructose 2,6-bisphosphate, the most potent allosteric activator of PFK-1 (discussed in more detail below) (Staal et al., 1987; Moreno-Sanchez et al., 2007). The high requirement of neoplastic cells for increased glycolysis suggests that a rate-limiting enzyme such as PFK-1 may serve as an essential control point during neoplastic transformation. This hypothesis is supported by the following observations: (i) PFK-1 activity is markedly increased in cancer cell lines and primary tumor tissues *in situ* (Hennipman et al., 1987; Hennipman et al., 1988; Sanchez-Martinez and Aragon, 1997); and (ii) the oncogenes *ras* and *src* activate PFK-1 in immortalized cells (Bosca et al., 1986; Kole et al., 1991). Accordingly, understanding the regulation of PFK-1 activity during neoplastic transformation should facilitate the identification of protein targets that are selectively required to activate glycolysis in cancer.

Discovery of fructose-2,6-bisphosphate

Three decades ago, an examination of the effect of glucagon on gluconeogenesis in liver extracts led to the discovery of fructose-2,6-bisphosphate (F2,6BP), which was characterized as an inhibitor of the gluconeogenic enzyme, fructose-1,6-bisphosphatase (F1,6BPase), and a powerful activator of the glycolytic enzyme PFK-1 (Van Schaftingen et al., 1980b, 1980a). Unlike the fructose-1,6-bisphosphate isomer (F1,6BP), F2,6BP is extremely acid labile and can readily be hydrolyzed to F6P and inorganic phosphate at 0 °C in the presence of 10 μM HCl. Later studies have revealed that F2,6BP, which is not a glycolytic intermediate itself, is by far the most potent positive effector of PFK-1. It greatly increases the affinity of PFK-1 for F6P by shifting the conformational equilibrium of PFK-1 from a low to a high affinity state for its substrate (Van Schaftingen et al., 1980b), and it releases the inhibition caused by ATP. On the other hand, it inhibits F1,6BPase by competing with its substrate. These effects are synergistic with those of AMP, which is also an activator of PFK-1 and inhibitor of F1,6BPase. When assayed under identical conditions, F1,6BP exerts a similar positive effect on PFK-1 but only at a 1000-fold greater concentration (well above its physiological concentration) (Hers, 1983, 1984), leaving little doubt that, in the liver (the tissue originally used to study the kinetics of F2,6BP), it is F2,6BP and not F1,6BP that regulates glycolysis/gluconeogenesis at the opposing PFK-1/F1,6BPase step.