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Pyruvate kinase type M2: A key regulator of the metabolic budget system in tumor cells

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ABSTRACT

Cell proliferation only proceeds when metabolism is capable of providing a budget of metabolic intermediates that is adequate to ensure both energy regeneration and the synthesis of cell building blocks in sufficient amounts. In tumor cells, the glycolytic pyruvate kinase isoenzyme M2 (PKM2, M2-PK) determines whether glucose is converted to lactate for regeneration of energy (active tetrameric form, Warburg effect) or used for the synthesis of cell building blocks (nearly inactive dimeric form). This review discusses the regulation mechanisms of pyruvate kinase M2 expression by different transcription factors as well as the regulation of pyruvate kinase M2 activity by direct interaction with certain oncoproteins, tyrosine and serine phosphorylation, binding of phosphotyrosine peptides, association with other glycolytic and non glycolytic enzymes, the promyelocytic leukemia tumor suppressor protein, as well as metabolic intermediates. An intervention in the regulation mechanisms of the expression, activity and tetramer to dimer ratio of pyruvate kinase M2 has severe consequences for metabolism as well as proliferation and tumorigenic capacity of the cells which makes this enzyme a promising target for potential therapeutic approaches. The quantification of the dimeric form of pyruvate kinase M2 (Tumor M2-PK) in plasma and stool allows early detection of tumors and therapy control. Several different mechanisms may induce a translocation of pyruvate kinase M2 into the nucleus. The role of pyruvate kinase M2 in the nucleus is complex as witnessed by evidence of its effect both as pro-proliferative as well as pro-apoptotic stimuli.

1. Introduction

The investigation of tumor metabolism (the tumor metabolome; www.metabolic-database.com) was initiated in the 1920s when Otto Heinrich Warburg first discovered that tumor cells convert large amounts of glucose to lactate even in the presence of oxygen (Warburg et al., 1924). This phenomenon has been termed the *Warburg effect* or *aerobic glycolysis*. In differentiated tissues, in the presence of oxygen, glucose is completely degraded to CO_2 and water via glycolysis, the citric acid cycle and oxidative phosphorylation (Fig. 1). In addition, differentiated tissues, such as muscle may degrade glucose to lactate. However, except in cases of mitochondrial disorders, which may be accompanied by an increase in lactate levels in body fluids in the presence of oxygen (Shoffner, 2005), healthy differentiated tissues usually only follow this pathway when oxygen levels are low. At high oxygen concentrations ATP levels are high and AMP and ADP levels are low due to a high capacity of oxygen dependent mitochondrial respiration. High ATP and low AMP and ADP levels inhibit the key glycolytic enzyme 6-phosphofructo1-kinase which is the basis of the downregulation of glycolysis in differentiated tissues in the presence of oxygen, a phenomenon commonly known as the *Pasteur effect*. At low oxygen pressures ATP levels are low and AMP and ADP levels are high, which lead to an activation of 6-phosphofructo 1-kinase and consequently to an increased glucose conversion rate. In addition high exogenous concentrations of certain hexoses (i.e. glucose and fructose but not galactose) have been found to induce an acute inhibition of mitochondrial respiration in normal proliferating cells and several cancer cell types (Melo et al., 1998). The molecular mechanism of this so called *Crabtree effect* is unknown. However, an increase in glucose 6-P, fructose 6-P, fructose 1,6-P2 and lactate as well as a decrease in the ATP/ADP ratio has been described (Burd et al., 2001; Rodríguez-Enríquez et al., 2001). The total degradation

Abbreviations: CREB, cAMP response element binding protein; ERK, extracellular signal regulated kinase; HCV, hepatitis C virus; HPV, human papillomavirus; IL, interleukin; JAK, Janus kinase; MAGUK, membrane-associated guanylate kinase; MAPK, mitogen-activated protein kinase; M2-PK, pyruvate kinase type M2; OXPHOS, oxidative phosphorylation; PEP, phosphoenolpyruvate; PI3K, phosphatidyl inositol-3-kinase; PKM2, pyruvate kinase type M2; PML, promyelocytic leukemia; RAF, rapidly growing fibroblasts; SNP, single nucleotide polymorphism; ZOP, zonula occludens protein; TEM8, tumor endothelial marker 8.

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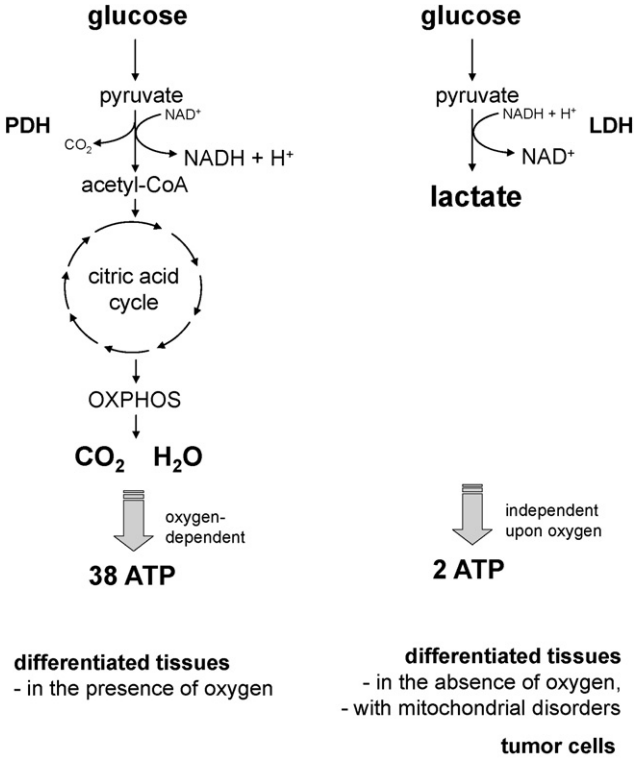

Fig. 1. Glucose degradation in differentiated tissues and tumor cells. PDH: pyruvate dehydrogenase

of glucose to CO_2 and water by mitochondrial respiration yields 38 moles of ATP per mole of glucose whereas only two moles of ATP are generated when glucose is degraded to lactate (Fig. 1). This apparently senseless waste of energy prompted Otto Warburg to formulate the hypothesis that a defect in mitochondrial respiration could be the cause of increased aerobic glycolysis in tumor cells. Indeed, in the following decades mutations in mitochondrial DNA and changes in mitochondrial enzyme activities have been described in tumor cells (Cuezva et al., 2002; Hervouet and Godinot, 2006; Irminger-Finger, 2007; Ohta, 2003; Rossignol et al., 2004). However, there have also been other reports which show that in special metabolic situations tumor cells are still able to switch back to mitochondrial respiration. Fantin et al. (2006) for example showed that knockdown of lactate dehydrogenase by siRNA leads to a decrease in lactate dehydrogenase activity and a stimulation of mitochondrial respiration. Thus, mitochondrial respiration cannot be irreversibly defective in tumor cells. Instead, bioenergetic analysis of different tumor tissues and tumor cell lines revealed a large variability in the relative contribution of glycolysis and oxidative phosphorylation (OXPHOS) to cellular ATP production. This observation led to the classification of high glycolytic, OXPHOS deficient tumor types and OXPHOS enhanced tumor types which may generate up to 80% of their energy by mitochondrial respiration (Bellance et al., 2009b). Furthermore, dihydroorotate dehydrogenase, a key enzyme within pyrimidine de novo synthesis uses prosthetic flavin and ubiquinone as proximal and cytochrome C and molecular oxygen as final electron acceptors. Thus, pyrimidine de novo synthesis is directly connected to the mitochondrial electron transport chain. Consequently any dysfunction of the mitochondrial electron transport chain, i.e. lack of oxygen and/or deficiencies of the enzyme complexes of the electron transport chain may impair UTP, CTP and TTP de novo synthesis as well as RNA and DNA synthesis (Löffler et al., 2005). As verbalized in the title of a Nature Review from 2004 more than 80 years after Otto Warburg's discovery we still ask ourselves "why do cancers have high aerobic glycolysis?" (Gatenby and Gillies, 2004). This question, however, can be understood in two different ways: 1. What is the cause of increased aerobic glycolysis in tumor cells? or 2. What is the advantage of increased aerobic glycolysis for tumor cells? From the metabolic point of view tumor cells with a high proliferation rate primarily require two things: energy and cell building blocks, such as nucleic acids, amino acids and phospholipids. Cell proliferation only proceeds when tumor metabolism is able to provide a budget of metabolic intermediates which is adequate to ensure energy regeneration as well as to provide the synthesis of cell building blocks in sufficient amounts. This requirement has been termed the *metabolic budget system* (Eigenbrodt and Glossmann, 1980; Eigenbrodt et al., 1992; Mazurek et al., 2005). This review will discuss the important metabolic role of the glycolytic pyruvate kinase isoenzyme type M2 for the regulation of the metabolic budget system.

2. The four pyruvate kinase isoenzymes

Pyruvate kinase (ATP: pyruvate 2-O-phosphotransferase, EC 2.7.1.40) catalyzes the dephosphorylation of phosphoenolpyruvate (PEP) to pyruvate and is responsible for net ATP production within the glycolytic sequence. In contrast to mitochondrial respiration ATP regeneration by pyruvate kinase is independent of oxygen supply and allows the survival of organs under hypoxic conditions.

Depending upon the different metabolic functions of the tissues different isoenzymes of pyruvate kinase are expressed which differ widely in their kinetic characteristics and regulation mechanisms (Table 1). The pyruvate kinase isoenzyme type L (L-PK) is expressed in tissues with gluconeogenesis, such as liver, kidney and intestine (Brinck et al., 1994; Domingo et al., 1992; Steinberg et al., 1999). The expression of L-PK is regulated by nutrition. Whereas a carbohydrate-rich diet enhances L-PK expression, hunger reduces the synthesis of L-PK. The PK isoenzyme type L has a low affinity to its substrate PEP. L-PK is allosterically activated by fructose 1,6-P2 and ATP and phosphorylated by a c-AMP-dependent protein kinase under the control of glucagon (Yamada and Noguchi, 1999a). The phosporylation of L-PK leads to an inactivation of the enzyme under physiological conditions. The pyruvate kinase isoenzyme type R (R-PK) is found in erythrocytes and is very similar to L-PK with respect to its kinetic characteristics and regulation mechanisms (Table 1) (Rodriguez-Horche et al., 1987). The R- and L-PK isoenzymes are encoded by the same gene and are expressed under the control of different tissue specific promoters (Noguchi et al., 1987). The pyruvate kinase isoenzyme type M1 (M1-PK) is characteristic of all tissues in which large amounts of energy have to be rapidly provided, such as muscle and brain (Table 1) (Carbonell et al., 1973; Reinacher et al., 1979; Yamada and Noguchi, 1999b). The M1-PK isoenzyme has the highest affinity to its substrate PEP and is not allosterically regulated and is not phosphorylated. The pyruvate kinase isoenzyme type M2 (current abbreviations: M2-PK, PKM2; former abbreviations: type I, type III, type A, type C, type K and type K4) is expressed in some differentiated tissues, i.e. fat tissue, lung, retina, pancreatic islets and is the characteristic isoenzyme of all cells with a high rate of nucleic acid synthesis, i.e. all proliferating cells, such as normal proliferating cells, embryonic cells, adult stem cells and especially tumor cells (Table 1) (Reinacher and Eigenbrodt, 1981; Reinacher et al., 1979; Staal and Rijksen, 1991; Yamada and Noguchi, 1999b). Accordingly, during embryogenesis, the M2-PK isoenzyme is progressively replaced by the respective tissue specific isoenzyme. Conversely, during tumorigenesis, the tissue specific isoenzymes of pyruvate kinase, i.e. L-PK in the liver or M1-PK in the brain disappear and the M2-PK isoenzyme is expressed (Hacker et al., 1998; Reinacher and Eigenbrodt, 1981; Steinberg et al., 1999; Yamada and Noguchi, 1999b). The PEP-affinity and activity of the M2-PK isoenzyme depend on