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How the Warburg effect supports aggressiveness and drug resistance of cancer cells?

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ABSTRACT

Cancer cells employ both conventional oxidative metabolism and glycolytic anaerobic metabolism. However, their proliferation is marked by a shift towards increasing glycolytic metabolism even in the presence of O_2 (Warburg effect). HIF1, a major hypoxia induced transcription factor, promotes a dissociation between glycolysis and the tricarboxylic acid cycle, a process limiting the efficient production of ATP and citrate which otherwise would arrest glycolysis. The Warburg effect also favors an intracellular alkaline pH which is a driving force in many aspects of cancer cell proliferation (enhancement of glycolysis and cell cycle progression) and of cancer aggressiveness (resistance to various processes including hypoxia, apoptosis, cytotoxic drugs and immune response). This metabolism leads to epigenetic and genetic alterations with the occurrence of multiple new cell phenotypes which enhance cancer cell growth and aggressiveness. In depth understanding of these metabolic changes in cancer cells may lead to the development of novel therapeutic strategies, which when combined with existing cancer treatments, might improve their effectiveness and/or overcome chemoresistance.

1. Introduction

In eukaryotic cells, cellular respiration is primarily aerobic, but in some instances, cells can produce energy anaerobically through glycolytic fermentation. These two processes share a common initial metabolic pathway called glycolysis which is regulated by phosphofructokinase1 (PFK1) and which leads to the production of pyruvate from glucose. Under aerobic conditions, pyruvate enters the mitochondria and fuels the tricarboxylic acid (TCA) cycle which produces citrate, high levels of ATP and CO_2. In contrast, under anaerobic conditions, pyruvate remains in the cytosol and is converted into lactate and markedly smaller yield of ATP.

Otto Warburg first demonstrated that proleferating cancer cells enhanced their glucose consumption and produced lactate, even under aerobic conditions (Warburg, 1930, 1956). This reprogrammed cellular metabolism is now recognized as a hallmark of cancer (Hanahan and Weinberg, 2011) and it is important to better understand its underlying molecular mechanisms for the development of new therapeutic interventions. In the current review, we attempt to explain why cancer cells reduce their oxidative metabolism in order to adapt their ability to survive. We also show how this metabolic shift towards increased glycolytic functioning sustains tumor cell proliferation and aggressiveness (resistance to various processes including hypoxia, apoptosis and immune response). As discussed in the current review, the Warburg effect also promotes many tumor characteristics that result in drug resistance including increased drug efflux and DNA damage repair, metabolic

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inactivation of drugs, epigenetic alterations, mutations in drug targets, activation of survival pathways and evasion of cell death (Holohan et al., 2013; Raz et al., 2014).

In the present review, we describe the many facets of the Warburg effect in cancer cells and discuss how a better understanding of this metabolic shift may facilitate the development of new anti-cancer drug treatments and strategies to overcome resistance to the treatments that are currently available.

2. The Warburg effect in cancer cells

2.1. The "Warburg effect" enhances glycolysis and glutaminolysis to sustain cellular proliferation

While normal cells oxidize glucose to provide ATP, cancer cells use a large fraction of their glucose to produce building blocks for cellular proliferation. For that purpose, they enhance their consumption of glucose and transform much of it into various molecules that sustain nucleotide and triglyceride biosynthesis (Assaraf, 2007; DeBerardinis and Thompson, 2012; Gonen and Assaraf, 2012; Raz et al., 2016; Vander Heiden et al., 2009). Furthermore, cancer cells consume glutamate derived from glutamine (*i.e.* glutaminolysis) to produce molecules such aspartate entering nucleotide biosynthesis and citrate furnishing acetyl-coA for fatty acid biosynthesis and protein acetylation (Cai et al., 2011; Deberardinis and Cheng, 2010; Hatzivassiliou et al., 2005; Huang et al., 2014; Mates et al., 2009; Wellen et al., 2009; Zaidi et al., 2012). Accordingly, glucose transporters (Chen et al., 2017; Macheda et al., 2005; Yu et al., 2017), glycolytic enzymes (Madhukar et al., 2015) and glutaminase 1 (GLS1) (Diaz-Moralli et al., 2013) represent a substantial proportion of the cancer metabolic proteome and metabolome.

Nutrient availability (glucose, glutamine, fatty acids, etc.) and O_2 concentration play a major role in the marked cell growth differences that may occur between various regions of the same tumor (Nakazawa et al., 2016). The nutrients are transformed into "bricks" for anabolic synthesis and/or are degraded to produce ATP required for biosynthesis and/or cell survival. The crossroad between anabolic and catabolic pathways is mainly regulated by pyruvate kinase which is re-expressed in its embryonic form PKM2 (Mazurek, 2012; Mazurek et al., 2002). When PKM2 is inactivated, anabolic synthesis (and branched pathways in glycolysis) are promoted (Lepleux et al., 2012; Locasale et al., 2011; Yang and Vousden, 2016), whereas when PKM2 is active, phosphoenolpyruvate (PEP) is transformed into pyruvate, hence producing one molecule of ATP (Mazurek, 2012). Glycolysis can deliver ATP up to 100-fold more rapidly than mitochondria in cancer cells (Pfeiffer et al., 2001) and PKM2 adjusts the concentration of ATP into the range needed for cellular proliferation and cell survival. Of note, the PKM2 reaction can be bypassed by an alternative pathway not furnishing ATP (Vander Heiden et al., 2010), this bypass may be avoiding an excess of ATP production (Fig. 1).

2.2. The Warburg effect promotes a shift from oxidative to reductive metabolism

The increased consumption of glucose leads proliferating cells to reduce their oxidative metabolism, avoiding overproduction of ATP and citrate by mitochondria which would otherwise arrest glycolysis because these molecules downregulate PFK1 (Icard and Lincet, 2012; Lehninger, 1975).

The shift from oxidative to reductive metabolism (*i.e.* the fermentation of glucose into lactate) is linked to the inhibition of pyruvate dehydrogenase (PDH) by pyruvate dehydrogenase kinase 1 (PDK1) (Semenza, 2012; Papandreou et al., 2011; Semenza, 2013). PDH inhibition disconnects the TCA cycle from glycolysis and leads to a marked decrease in mitochondrial production of ATP and citrate. PDK1 is activated by hypoxia inducible factor-1 (HIF-1) and by two kinases which translocate into mitochondria: Akt (Chae et al., 2016) and the multifunctional enzyme PGK1 (Li et al., 2016b). The disconnection of the TCA cycle from glycolysis is reinforced by the decreased expression of the mitochondrial pyruvate carrier (Schell et al., 2014). HIF-1 also transactivates lactate dehydrogenase 5 (LDH5) that transforms pyruvate into lactate, which is exported by monocarboxylate transporter 4 (MCT4) (Marin-Hernandez et al., 2009; Semenza, 2013).

The main activator of the Warburg effect is HIF-1, which selects cells that are able to survive when their oxygen supply is reduced (Rohwer and Cramer, 2011; Semenza, 2016). HIF-1 allows adaptation to hypoxia by increasing glucose transport, glycolysis and lactate production, whereas it can favor autophagy (Leung et al., 2017; Zhang et al., 2008). Importantly, HIF-1 can also be upregulated in the presence of O_2 (Dhup et al., 2012; Keith et al., 2012; Lee et al., 2015; Ryu et al., 2011).

The upregulation of HIF-1 results from several processes: a) inactivation of prolyl-hydroxylase (PHD) by hypoxia, lactate, fumarate, and succinate; PHD inactivation arrests HIF-1 degradation (Ryu et al., 2011; Selak et al., 2005; Snell et al., 2014); b) increased production of reactive oxygen species (ROS) stimulated by hypoxia (Chandel et al., 2000; Ryu et al., 2011); c) inactivation of the mitochondrial tumor suppressor deacetylase sirtuin3 (SIRT-3) due to the lack of mitochondrial NAD^+, SIRT-3 promotes HIF-1 stabilization (Bell et al., 2011; Haigis et al., 2012); and d) upregulation of PKM2 which activates HIF-1 through the NF-kappaB pathway (Azoitei et al., 2016).

All these processes (activation and/or stabilization of HIF-1) create positive feedback loops which further drive glycolysis towards glucose consumption and lactate production.

It is noteworthy that the Warburg effect can be reinforced by the Crabtree effect which is the inhibition of OXPHOS when cancer cells are fed with large amounts of glucose (Crabtree, 1929; Diaz-Ruiz et al., 2008; Diaz-Ruiz et al., 2011; Redman et al., 2013; Smolkova et al., 2011). This effect should be related to the inactivation of complex IV of OXPHOS by fructose 1,6-biphosphate (F1,6-BP) (Diaz-Ruiz et al., 2008; Diaz-Ruiz et al., 2011).

2.3. The Warburg effect reduces the mitochondrial production of ATP, citrate, CO_2 and reactive oxygen species (ROS)

Excessive production of citrate and ATP by mitochondria is detrimental to cellular proliferation (Kruspig et al., 2012; Lincet et al., 2013; Lu et al., 2011; Samudio et al., 2009; Zhang et al., 2009; Zhou et al., 2012). Thus, the Warburg effect may appear as an regulatory mechanism avoiding mitochondrial overproduction of ATP and citrate, two molecules which are known to arrest glycolysis at various levels (PFK1 and PK) (Lehninger, 1975). The upregulation of ATP citrate lyase (ACLY) (Hatzivassiliou et al., 2005; Pietrocola et al., 2015; Zaidi et al., 2012) participates in the maintenance of low concentrations of ATP and citrate, promoting tumor aggressiveness i.e. resistance to apoptosis and dedifferentiation (Hanai et al., 2013; Zhou et al., 2012); for review, see (Icard and Lincet, 2017) (Fig. 1). Accordingly, mitochondrial ATP production has been found to decrease to 50% in poorly differentiated cancer cells (Nakashima et al., 1984; Pedersen, 1978). The decreased production of ATP in cancer cells could be also related to the frequent damage of the respiratory chain, primarily occurring at the ATPase (complex V) (Cuezva et al., 2002; Garcia-Heredia and Carnero, 2015; Matoba et al., 2006).

Diminished oxidative metabolism also reduces the production of CO_2. ATP and CO_2 are the two major sources of H^+ inside cells (Swietach et al., 2014) and their decrease promotes the establishment of an alkaline intracellular pH (pHi) in cancer cells (between 7.12 and 7.65), slightly elevated over that of normal cells (between $6.99 - 7.20$) (Webb et al., 2011); for a recent review see (Alfarouk et al., 2014; White et al., 2017). It should be noted that pH is a logarithmic value, and a slight variation implies a potentially large decrease in the concentration of H^+. Several membrane exchangers (in particular the