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Cancer cell metabolism regulates extracellular matrix degradation by invadopodia

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ABSTRACT

Transformed cancer cells have an altered metabolism, characterized by a shift towards aerobic glycolysis, referred to as 'the Warburg phenotype'. A change in flux through mitochondrial OXPHOS and cytosolic pathways for ATP production and a gain of capacity for biomass production in order to sustain the needs for altered growth and morphodynamics are typically involved in this global rewiring of cancer cell metabolism. Characteristically, these changes in metabolism are accompanied by enhanced uptake of nutrients like glucose and glutamine. Here we focus on the relationship between cell metabolism and cell dynamics, in particular the formation and function of invadopodia, specialized structures for focal degradation of the extracellular matrix. Since we recently found presence of enzymes that are active in glycolysis and associated pathways in invadopodia, we hypothesize that metabolic adaptation and invadopodia formation are linked processes. We give an overview on the background for this idea and show for the first time that extracellular matrix degradation by invadopodia can be differentially manipulated, without effects on cell proliferation, by use of metabolic inhibitors or changes in nutrient composition of cell culture media. We conclude that cell metabolism and carbohydrate availability, especially pyruvate, are involved in fuelling of invadopodia formation and activity.

Introduction

Cellular metabolic pathways are well-known and well-studied for many fully-differentiated cells and tissues in mammals. In most non-proliferating cells, glucose is metabolized to pyruvate in the cytosol via several enzymatic breakdown steps in glycolysis, with a yield of 2 ATP molecules per molecule of glucose. Pyruvate is imported into mitochondria and further metabolized to carbon dioxide through the tricarboxylic acid (TCA) cycle. Electrons of NADH and $FADH_2$, jointly produced in the TCA, will ultimately enter the electron transport chain (ETC) of the mitochondrial oxidative phosphorylation (OXPHOS) system, and drive the production of ATP. A maximal yield of 36 moles of ATP per mole of glucose can be achieved by tight coupling of glycolytic and mitochondrial activities. A strict prerequisite for a high energy yield of glucose metabolism is the availability of oxygen, without which OXPHOS cannot take place. In absence of oxygen, a substantial portion of glucose-derived pyruvate is converted in the cytosol to lactate by lactate dehydrogenase (LDH), in a combined process called anaerobic glycolysis (Salway, 2004). In proliferating cells (including tumour cells), due to altered coupling of cytosolic and mitochondrial pathways, lactate is produced even in the presence of oxygen, through a process called aerobic glycolysis. This process was initially observed in cancer cells by Otto Warburg almost a century ago (Warburg et al., 1924). Although his observations were initially received with much criticism, the improved understanding of tumour metabolism that has been reached in the last decade, has led to the recognition that adaptations in metabolism are characteristic for cancer cells and form a distinguishing feature (now called "the Warburg phenotype"). Use of aerobic glycolysis and altered flux through associated pathways is therefore now considered a hallmark of cancer (Hanahan and Weinberg, 2011).

The Warburg effect

Based on his observations, Otto Warburg defined cancer as a disease of defective mitochondria (Warburg, 1956). This interpretation, however, turned out to be wrong and led to consequent scepticism surrounding the issue of cancer metabolism for decades. Nowadays, mitochondria are known to play a major role in cancer

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metabolism and form promising therapeutic targets (Fulda et al., 2010). Nevertheless, initial observations that led to the formulation of the Warburg effect were correct, as they have been repeatedly confirmed for many different types of tumours. The typical features persist even when tumour cells are cultured in laboratory conditions in the presence of 20% oxygen (actually a hyperoxic condition; Gatenby and Gillies, 2004). Remarkably, it was the increased use of positron emission tomography (PET) in the clinic, based on monitoring of uptake of 18-fluorodeoxy-glucose by cancer cells, which strongly contributed to the revitalization of interest in tumour metabolism. The virtually universal increase in glucose uptake by both primary and metastatic cancer lesions remains one of the most evident demonstrations of the validity of Warburg's observations (Gambhir, 2002).

Molecular regulators of the Warburg effect

The metabolic phenotype of cancer cells, typically associated with decreased dependence on OXPHOS, provides cancer cells a growth advantage in the hypoxic tumour microenvironment.

Hypoxia-inducible factors 1 (HIF1) and 2 (HIF2), the two transcription factors regulating the downstream response to hypoxia, directly control the expression of metabolic enzymes of glycolysis, including hexokinase (HK), pyruvate dehydrogenase kinase 1 (PDK1) and lactate dehydrogenase A (LDH-A) (Dang et al., 2008; Kim et al., 2007). Next to glycolytic enzymes, the glucose transporter 1 (GLUT-1) is also upregulated by HIF, allowing increased uptake of glucose by cancer cells (Brahimi-Horn et al., 2011). Likewise, the oncogenic transcription factor MYC plays an important role in cell metabolism (Dang et al., 2009). HIF and MYC often share target genes, so that they collaborate in the activation of GLUT, LDH-A and PDK1 (Dang et al., 2008). MYC has recently been found to modulate the splicing of the enzyme involved in the final step of glycolysis, pyruvate kinase (PK), resulting in increased expression of the PK-M2 (embryonic) over the PK-M1 (adult) isoforms (David et al., 2010). Upregulation of PK-M2 is related to tumour growth (Christofk et al., 2008). MYC also induces the transcription of genes that stimulate mitochondrial biogenesis (Li et al., 2005). As mentioned above, due to the biosynthetic demand of tumour cells, large amount of biomass, such as membrane lipids, must be produced. Mitochondrial TCA intermediates are used as substrates for this purpose (see below) and as a consequence tumour cells need to increase the uptake of glutamine. Perhaps not surprisingly therefore, MYC also plays an important role in the uptake and metabolism of glutamine, a process referred to as glutaminolysis (or anaplerosis), by directly regulating the levels of both glutamine receptors and the mitochondrial enzyme glutaminase (Dang et al., 2009). In addition to HIF and MYC, the phosphoinositide-3 kinase (PI3K) signalling pathway and the transcription factor tumour suppressor p53 play key roles in the molecular regulation of cancer cell metabolism. The most prominent downstream effector of PI3K, the kinase AKT1, is an important glycolysis stimulating factor (Elstrom et al., 2004). AKT1 phosphorylates (and activates) glycolytic enzymes including HK and phosphofructokinase and also stimulates mTOR signalling and ATP citrate lyase, which results in increased protein and lipid synthesis. AKT1 can also activate HIF, even under normoxic conditions (Cairns et al., 2011; Robey and Hay, 2009). Wild type p53 inhibits glycolysis by upregulation of TIGAR (tumour protein 53-induced glycolysis and apoptosis regulator), which reduces fructose-2-6-bisphosphate levels (Bensaad et al., 2006; Vousden and Ryan, 2009). It also promotes OXPHOS and the expression of the phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase PTEN, which inhibits the PI3K pathway, and thus inhibits glycolysis (see above) (Matoba et al., 2006; Stambolic et al., 2001). Recent data suggest that p53 also directly inhibits biosynthesis by binding to the first and rate-limiting enzyme of the pentose phosphate pathway (PPP), glucose-6-phosphate dehydrogenase (G6PD) (Jiang et al., 2011). As a consequence, one might infer that loss of p53, which occurs in the majority of cancers, is a key driver of the glycolytic phenotype. In summary, in tumourigenesis, gene regulatory programmes and metabolic reactions steps are intimately – and seemingly sometimes reciprocally – linked.

Biosynthetic pathways: from TCA cycle to macromolecules

The multifaceted role of mitochondrial metabolism in tumourigenesis is also becoming more evident. Intermediates in the mitochondrial TCA cycle serve as precursors for lipids proteins and nucleic acids (DeBerardinis et al., 2008) and mitochondrial-cytosolic exchange of these compounds is therefore an important regulatory process. During cell proliferation and tumour growth, citrate is transported out of the mitochondria and serves as a carbon source for fatty acids in the synthesis of lipids and cholesterol. In the cytosol, citrate is converted back to acetyl-CoA and oxaloacetate by ATP citrate lyase. Acetyl-CoA is the precursor for lipids, while oxaloacetate is converted to pyruvate, which again can enter the TCA cycle or be secreted as lactate. The lipogenic enzymes of the fatty acid synthesis pathway, such as ATP citrate lyase and fatty acid synthase, are upregulated in tumour cells and represent interesting therapeutic targets (Hatzivassiliou et al., 2005; Vander Heiden, 2011). As a consequence of citrate export, alpha-ketoglutarate levels in the mitochondria drop. To replenish these levels, tumour cells take up high amounts of glutamine, which is converted to glutamate and subsequently to alpha-ketoglutarate in the mitochondria. Hence, although mitochondria are functional, the TCA cycle is truncated, because the amount of citrate that can be oxidised is limited. Also in tumours carrying mutations that result in defective mitochondria, the importance of providing substrates for macromolecules is maintained, via an alternative pathway including reductive metabolism of glutamine. In this way TCA intermediates still are generated in order to support tumour growth (Mullen et al., 2012). The special dependence of tumours on glutamine also represents an interesting and promising new target for cancer therapy (Wang et al., 2010). In addition, other aspects of intermediate metabolism are altered in cancer cells. For example, for the synthesis of nucleic acids, high amounts of glucose-6-phosphate enter the PPP to be converted to ribose-5-phosphate. The PPP-enzymes G6PD and transketolase, in respectively the oxidative and non-oxidative branches of the PPP, are upregulated during proliferation of tumour cells (Vizan et al., 2009). Next to the high glucose uptake, flux through the PPP and other biosynthetic pathways is increased, indicating an enlarged need for co-factors required in many metabolic conversions (like NADPH for ROS protection). In Fig. 1, the metabolic features of cancer cells are summarized in a scheme and compared to normal metabolism of non-proliferating cells.

Cancer cell metabolism and invasive growth

The tumour microenvironment is at the same time a pivotal regulator of cancer invasion (Friedl and Alexander, 2011) and metabolism. For instance, hypoxia is a strong inducer of (secreted) protease activity, needed for aggressive cancer cells to grow invasively and correlated with metastatic disease (Finger and Giaccia, 2010). A hypoxic microenvironment selects for the most aggressive tumour cells that can survive under low oxygen conditions and also results in local acidification. This acidification is directly linked to lactate production as observed in the Warburg effect, leading to the suggestion that the glycolytic phenotype is required for invasive tumour growth (Gatenby and Gillies, 2004). An acidic extracellular microenvironment is found in many tumours and low pH values are reported to stimulate invasive behaviour (Bhujwalla,