

Tumor Metabolism

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Tumor cells are unusual metabolically in that they display high rates of aerobic glycolysis, a phenomenon that was recognized by Otto Warburg in the 1920's and which has been called the Warburg effect. While the tumor microenvironment, which is frequently hypoxic, might select for cells that are glycolytic¹, and thus can generate ATP in the absence of oxygen, it has become increasingly clear that this and the other metabolic changes observed in tumor cells are driven by oncogene activation and tumor suppressor loss². Moreover, while these metabolic changes are important for generating ATP under the often anaerobic conditions found in the tumor microenvironment they also have other important functions, such as the generation of metabolic intermediates for biosynthetic pathways, such as lipid and nucleic acid synthesis. This altered metabolism may offer new therapeutic opportunities, but more importantly from the perspective of this meeting, new ways of detecting tumors and their responses to treatment using metabolic imaging.

Hypoxia-inducible factor (HIF)

Tumor vasculature is frequently erratic, unstable and insufficient¹. The resulting hypoxia leads to stabilization of hypoxia-inducible factor, HIF, which initiates a transcriptional programme that acts to counteract hypoxia by stimulating angiogenesis (the formation of new blood vessels) through the up-regulation of several factors, including vascular endothelial growth factor (VEGF). HIF also modifies the metabolism of the tumor cells so that they become more glycolytic, which it does by inhibiting mitochondrial respiration and by increasing the expression of the glycolytic enzymes and glucose transporters³. HIF inhibits mitochondrial respiration by increasing the expression of Pyruvate Dehydrogenase Kinase 1 (PDK1), which inhibits pyruvate dehydrogenase (PDH) by phosphorylating the E1 subunit (PDH catalyzes the oxidative decarboxylation of pyruvate to form acetyl CoA). HIF up-regulates expression of lactate dehydrogenase A (LDHA), which catalyzes the reduction of pyruvate to lactate and regenerates NAD⁺, thus allowing glycolysis to continue. HIF also increases the expression of the plasma membrane monocarboxylate transporter (MCT4), which transports lactate out of the cell.

Oncogenes drive altered tumor metabolism

The Warburg effect, the uncoupling of glycolysis from tissue oxygen concentration, cannot wholly be explained by hypoxia-dependent up-regulation of HIF. HIF1 accumulates in tumor cells after activation of oncogenes such as Ras, Src and phosphoinositide 3-kinase (PI3K), moreover these oncogenes can have a direct effect on the expression of glycolytic enzymes. The PI3K-Akt pathway, which is frequently activated in tumor cells, up-regulates expression of the glucose transporter GLUT1, stimulates the association of hexokinase isoforms with the

mitochondria, where they more readily phosphorylate glucose, and enhances the rate of glycolysis by promoting the expression of glycolytic enzymes through HIF1 α . c-Myc, which is overexpressed in ~70% of human tumors, up-regulates the expression of various metabolic genes, including those encoding GLUT1, and many of the glycolytic enzymes. A picture is emerging in which these cellular oncogenes drive cell growth and proliferation and at the same time up-regulate metabolic pathways, such as glycolysis, to provide energy and in particular metabolic intermediates for biosynthesis.

Provision of intermediates for biosynthesis

As well as having high rates of aerobic glycolysis and increased rates of protein and DNA synthesis, a third hallmark of the transformed phenotype is increased *de novo* fatty acid synthesis⁴. Tumors and their precursor lesions show high levels of endogenous fatty acid biosynthesis irrespective of the levels of extracellular lipids. The bulk of these fatty acids (FA) is esterified predominantly into phospholipids and are incorporated into membranes. This increased lipogenesis is driven by the increased expression of three enzymes: ATP citrate lyase, which produces cytosolic acetyl coenzyme A (CoA) from mitochondrial-derived citrate; Acetyl CoA carboxylase, the “rate-limiting” enzyme for long-chain FA synthesis that catalyzes the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA and fatty acid synthase (FASN), a multi-enzyme complex that is responsible for the terminal catalytic step in FA synthesis, the condensation of acetyl-CoA and malonyl-CoA to produce the saturated FA palmitate. Increased FASN expression is due to growth factor stimulation and activation of the phosphatidylinositol 3 kinase (PI3K)–Akt signaling pathway. As well as providing fatty acids for membrane biosynthesis the early activation of FASN in pre-malignant lesions has been suggested as a survival strategy to compensate for hypoxia; the synthesis of fatty acids providing a sink for reduced NAD(P)H in the cell.

Tumor cells may also enhance their biosynthetic capacity by expressing pyruvate kinase (PKM2), which catalyzes the terminal step of the glycolytic pathway, converting phosphoenolpyruvate (PEP) to pyruvate and in the process generating ATP. Rather unexpectedly this isoform of the enzyme shows a lower activity. This results in a build up in intermediates in the glycolytic pathway, which can then be shunted into lipid synthesis⁵. More recent work on PKM2 has shown that we may have to re-write the textbook descriptions of glycolysis⁶. Expression of PKM2 results in an increase in the steady state level of its substrate PEP. Remarkably this results in the PEP-dependent phosphorylation of an active site histidine residue of phosphoglycerate mutase (PGAM1), an upstream enzyme in the glycolytic pathway that catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate. The enzyme responsible for this phosphorylation has yet to be identified. Spontaneous hydrolysis of this phosphohistidine residue in PGAM1 leads to a net reaction in which PEP is converted to P_i and pyruvate. Contrast this with the glycolytic pathway in normal cells, where PKM1 uses PEP to phosphorylate ADP to produce ATP and pyruvate. Thus in tumor cells PKM2 expression uncouples glycolytic flux from ATP production.

Increased biosynthesis can explain why tumors show high rates of glucose uptake under aerobic conditions, but does not explain the increased lactate production. It has long been known that tumor cells have a high requirement for glutamine and recently this has been shown to be

converted into lactate, via the Krebs cycle, where it produces NADPH for lipid synthesis and oxaloacetate for replenishment of the Krebs cycle intermediates ⁷.

p53 and metabolism – Protecting the cell

The p53 tumor suppressor gene, which is mutated in many human tumors, plays a key role in cancer development. p53 responds to a wide range of stress signals in the cell, including DNA damage and hypoxia and mediates a variety of responses including cell cycle arrest and DNA repair to apoptosis and senescence. In the face of cellular damage it mediates processes that attempt to repair the damage and if this is too severe then it will kill the cell ⁸. The metabolic effects mediated by p53 appear to play a key role in this process. Activation of p53 in response to DNA damage leads to increased expression of TIGAR (TP53-induced glycolysis and apoptosis regulator), which is a fructose-2,6-bisphosphatase. This results in a decrease in the levels of fructose-2,6-bisphosphate, a potent allosteric activator of the glycolytic enzyme 6-phosphofructo-1-kinase (PFK1), resulting in inhibition of glycolytic flux and increased flux into the pentose phosphate pathway. The latter provides reducing power, in the form of NADPH, which acts to reduce the levels of damaging reactive oxygen species (ROS), which are increased following cellular insults, and also provides ribose-5-phosphate for DNA synthesis and repair.

Recent work has shown that PKM2 also appears to be involved in the cell's metabolic response to oxidative stress ⁹. Cysteine 358 in PKM2 is oxidized by hydrogen peroxide (H₂O₂), which decreases PKM2 activity, decreasing pyruvate formation and diverting flux from glycolysis into the pentose phosphate pathway and the production of NADPH. In yeast increased levels of PEP have been shown to inhibit triosephosphate isomerase and increase flux into the PPP ¹⁰.

Phosphoglycerate mutase 1 (PGAM1) also controls flux into the pentose phosphate pathway (PPP). PGAM1, whose expression is negatively regulated by p53, regulates biosynthesis by controlling levels of 3-phosphoglycerate (3-PG) and 2-phosphoglycerate (2-PG). 3-PG binds to and inhibits 6-phosphogluconate dehydrogenase in the PPP, while 2-PG activates 3-phosphoglycerate dehydrogenase to provide feedback control of 3-PG levels by increasing flux into the pathway of serine biosynthesis ¹¹.

Metabolic imaging

The aberrant metabolism displayed by tumor cells offers numerous opportunities for tumor detection and treatment response monitoring using metabolic imaging ¹². Positron emission tomography (PET) measurements of the uptake and trapping of the ¹⁸F-labelled glucose analog, fluorodeoxyglucose (FDG), have long been used for whole body imaging to detect tumors and decreases in its uptake have been used to detect treatment response in some tumor types. PET measurements of the uptake and trapping of ¹¹C acetate, due to increased FASN expression, has been used to detect prostate metastases ¹³ and ¹H MRS has been used to detect increased tumor lactate concentrations and decreases in response to treatment ¹⁴. ¹³C MRSI measurements of the conversion of hyperpolarized [1-¹³C]pyruvate into lactate have been used to image tumor LDH activity and decreases in this activity due to chemotherapy-dependent decreases in NAD(H) concentration and decreases in enzyme concentration due to inhibition of the PI3K-Akt pathway ^{15,16}. It is becoming increasingly clear that tumors face significant oxidative stress and that their

metabolism has become adapted to cope with this¹⁷. In this regard the introduction of hyperpolarized [1-¹³C]dehydroascorbic acid, the oxidized form of Vitamin C, as an *in vivo* sensor of oxidative stress promises new insights into how tumor cells deal with ROS^{18,19}. There is recent evidence that cytoplasmic lipid droplets, which have long been known to be detectable by ¹H MRS *in vivo*²⁰, can provide a metabolic signature of metabolic stress²¹. Mitochondrial ROS reversibly oxidize sulfhydryl groups in the enzymes of fatty acid oxidation, inhibiting the activities of these enzymes, and thus fatty acid oxidation, and resulting in the diversion of these fatty acids into triacylglycerol synthesis, which accumulates as cytoplasmic lipid droplets. Mammalian cells express >60 dioxygenases that utilize α -ketoglutarate (α -KG), an intermediate in the TCA cycle, as a co-substrate, including the prolyl hydroxylases that control the stability of HIF1, and the histone demethylases and the TET family of 5-methylcytosine hydroxylases. Many of these α -KG-dependent dioxygenases have a Km for α -KG near physiological concentrations suggesting that its concentration may influence HIF1 stability and gene expression^{22,23}. We have shown, using hyperpolarized [1-¹³C]glutamate, that we can detect α -KG in a tumor *in vivo*²⁴, raising the possibility that we may be able to interrogate the potential role of α -KG in controlling gene expression and HIF1 stability in a tumor.

A better understanding of the altered metabolism of tumor cells should stimulate the further development of new metabolic imaging methods for detecting tumors, determining prognosis and monitoring treatment response.

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