Regulation of cancer cell metabolism

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Abstract | Interest in the topic of tumour metabolism has waxed and waned over the past century of cancer research. The early observations of Warburg and his contemporaries established that there are fundamental differences in the central metabolic pathways operating in malignant tissue. However, the initial hypotheses that were based on these observations proved inadequate to explain tumorigenesis, and the oncogene revolution pushed tumour metabolism to the margins of cancer research. In recent years, interest has been renewed as it has become clear that many of the signalling pathways that are affected by genetic mutations and the tumour microenvironment have a profound effect on core metabolism, making this topic once again one of the most intense areas of research in cancer biology.

Redox status
Balance of the reduced state versus the oxidized state of a biochemical system. This balance is influenced by the level of reactive oxygen and nitrogen species (ROS and RNS) relative to the capacity of antioxidant systems to eliminate ROS and RNS.

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Over the past 25 years, the oncogene revolution has stimulated research, revealing that the crucial phenotypes that are characteristic of tumour cells result from a host of mutational events that combine to alter multiple signalling pathways. Moreover, high-throughput sequencing data suggest that the mutations leading to tumorigenesis are even more numerous and heterogeneous than previously thought[1,2]. It is now clear that there are thousands of point mutations, translocations, amplifications and deletions that may contribute to cancer development, and that the mutational range can differ even among histopathologically identical tumours. Detailed bioinformatic analyses have suggested that cancer-related driver mutations affect a dozen or more core signalling pathways and processes responsible for tumorigenesis[3]. These findings have led to questions about the usefulness of targeting individual signalling molecules as a practical therapeutic strategy. However, it is becoming clear that many key oncogenic signalling pathways converge to adapt tumour cell metabolism in order to support their growth and survival. Furthermore, some of these metabolic alterations seem to be absolutely required for malignant transformation. In view of these fundamental discoveries, we propose that alterations to cellular metabolism should be considered a crucial hallmark of cancer.

Multiple molecular mechanisms, both intrinsic and extrinsic, converge to alter core cellular metabolism and provide support for the three basic needs of dividing cells: rapid ATP generation to maintain energy status; increased biosynthesis of macromolecules; and tightened maintenance of appropriate cellular redox status (FIG. 1). To meet these needs, cancer cells acquire alterations to the metabolism of all four major classes of macromolecules: carbohydrates, proteins, lipids and nucleic acids. Many similar alterations are also observed in rapidly proliferating normal cells, in which they represent appropriate responses to physiological growth signals as opposed to constitutive cell autonomous adaptations[4,5]. In the case of cancer cells, these adaptations must be implemented in the stressful and dynamic microenvironment of the solid tumour, where concentrations of crucial nutrients such as glucose, glutamine and oxygen are spatially and temporally heterogeneous[6]. The nature and importance of metabolic restriction in cancer has often been masked owing to the use of tissue culture conditions in which both oxygen and nutrients are always in excess.

The link between cancer and altered metabolism is not new, as many observations made during the early period of cancer biology research identified metabolic changes as a common feature of cancerous tissues (such as the Warburg effect; discussed below)[7]. As much of the work in the field to date has focused on rapidly proliferating tumour models and cells *in vitro*, it is unclear to what extent these metabolic changes are important in low-grade slow growing tumours in which metabolic demands are not as extreme. Future clinical data describing the metabolic profiles of human tumours will be required to determine which metabolic alterations are most prevalent in specific tumour types. However, despite the lack of comprehensive clinical data, there has been substantial recent progress in understanding the molecular events that regulate some of these metabolic phenotypes.

The Warburg effect

In addition to the ATP that is required to maintain normal cellular processes, proliferating tumour cells must

At a glance

- Multiple molecular mechanisms, both intrinsic and extrinsic, converge to alter core cellular metabolism and provide support for the three basic needs of dividing cells: rapid ATP generation to maintain energy status; increased biosynthesis of macromolecules; and tightened maintenance of appropriate cellular redox status. Metabolic changes are a common feature of cancerous tissues, although it is unclear to what extent these metabolic changes are important in low-grade slow growing tumours.
- The best characterized metabolic phenotype observed in tumour cells is the Warburg effect, which is a shift from ATP generation through oxidative phosphorylation to ATP generation through glycolysis, even under normal oxygen concentrations. This effect is regulated by the PI3K, hypoxia-indicible factor (HIF), p53, MYC and AMP-activated protein kinase (AMPK)–liver kinase B1 (LKB1) pathways.
- Metabolic adaptation in tumours extends beyond the Warburg effect. It is becoming clear that alterations to metabolism balance the need of the cell for energy with its equally important need for macromolecular building blocks and maintenance of redox balance. To this end, a key molecule produced as a result of altered cancer metabolism is reduced nicotinamide adenine dinucleotide phosphate (NADPH), which functions as a cofactor and provides reducing power in many enzymatic reactions that are crucial for macromolecular biosynthesis. NADPH is also an antioxidant and forms part of the defence against reactive oxygen species (ROS) that are produced during rapid proliferation.
- High levels of ROS can cause damage to macromolecules, which can induce senescence and apoptosis. Cells counteract the detrimental effects of ROS by producing antioxidant molecules, such as reduced glutathione (GSH) and thioredoxin (TRX). Several of these antioxidant systems, including GSH and TRX, rely on the reducing power of NADPH to maintain their activities.
- In addition to the genetic changes that alter tumour cell metabolism, the abnormal tumour microenvironment — such as hypoxia, pH and low glucose concentrations — have a major role in determining the metabolic phenotype of tumour cells.
- Mutations in oncogenes and tumour suppressor genes cause alterations to multiple intracellular signalling pathways that affect tumour cell metabolism and re-engineer it to allow enhanced survival and growth.

also generate the energy that is required to support rapid cell division. Furthermore, tumour cells must evade the checkpoint controls that would normally block proliferation under the stressful metabolic conditions that are characteristic of the abnormal tumour microenvironment. Tumour cells reprogramme their metabolic pathways to meet these needs during the process of tumour progression. The best characterized metabolic phenotype observed in tumour cells is the Warburg effect (FIG. 2), which is a shift from ATP generation through oxidative phosphorylation to ATP generation through glycolysis, even under normal oxygen concentrations[7]. As a result, unlike most normal cells, many transformed cells derive a substantial amount of their energy from aerobic glycolysis, converting most incoming glucose to lactate rather than metabolizing it in the mitochondria through oxidative phosphorylation[7,8]. Although ATP production by glycolysis can be more rapid than by oxidative phosphorylation, it is far less efficient in terms of ATP generated per unit of glucose consumed. This shift therefore demands that tumour cells implement an abnormally high rate of glucose uptake to meet their increased energy, biosynthesis and redox needs.

There is some debate about the most important selective advantage that glycolytic metabolism provides to proliferating tumour cells. Initial work focused on the concept that tumour cells develop defects in mitochondrial function, and that aerobic glycolysis is therefore a necessary adaptation to cope with a lack of ATP generation by oxidative phosphorylation. However, it was later appreciated that mitochondrial defects are rare[9] and that most tumours retain the capacity for oxidative phosphorylation and consume oxygen at rates similar to those observed in normal tissues[10]. In fact, mitochondrial function is crucial for transformation in some systems[11–13]. Other explanations include the concept that glycolysis has the capacity to generate ATP at a higher rate than oxidative phosphorylation and so would be advantageous as long as glucose supplies are not limited. Alternatively, it has been proposed that glycolytic metabolism arises as an adaptation to hypoxic conditions during the early avascular phase of tumour development, as it allows for ATP production in the absence of oxygen. Adaptation to the resulting acidic microenvironment that is caused by excess lactate production may further drive the evolution of the glycolytic phenotype[14,15]. Finally, most recently, it has been proposed that aerobic glycolysis provides a biosynthetic advantage for tumour cells, and that a high flux of substrate through glycolysis allows for effective shunting of carbon to key subsidiary biosynthetic pathways[4,5].

The reliance of cancer cells on increased glucose uptake has proved useful for tumour detection and monitoring, with this phenotype serving as the basis for clinical [^{18}F]fluorodeoxyglucose positron emission tomography (FDG–PET) imaging. FDG–PET uses a radioactive glucose analogue to detect regions of high glucose uptake, and has proved highly effective for the identification and monitoring of many tumour types. Accordingly, there is now a substantial body of useful clinical data regarding the importance of glucose as a fuel for malignancies[16–19]. Although there have been attempts to block aerobic glycolysis in tumour cells using compounds such as 2-deoxyglucose, effective therapeutic strategies have not yet been devised. Several new therapeutic approaches targeting numerous points in the glycolytic process are currently under evaluation, including the inhibition of lactate dehydrogenase and the inactivation of the monocarboxylate transporters that are responsible for conveying lactate across the plasma membrane[20,21].

The PI3K pathway. The PI3K pathway is one of the most commonly altered signalling pathways in human cancers. This pathway is activated by mutations in tumour suppressor genes, such as *PTEN*, mutations in the components of the PI3K complex itself or by aberrant signalling from receptor tyrosine kinases[22]. Once activated, the PI3K pathway not only provides strong growth and survival signals to tumour cells but also has profound effects on their metabolism. Indeed, it seems that the integration of growth and proliferation signals with alterations to central metabolism is crucial for the oncogenic effects of this signalling pathway[23].

The best-studied effector downstream of PI3K is AKT1 (also known as PKB). AKT1 is an important driver of the tumour glycolytic phenotype and stimulates ATP generation through multiple mechanisms, ensuring that cells have the bioenergetic capacity required to respond to growth signals[24,25]. AKT1 stimulates glycolysis

Oxidative phosphorylation
Oxygen-dependent process coupling the oxidation of macromolecules and the electron transport chain with ATP synthesis. In eukaryotic cells, it occurs within the mitochondria and is a source of ROS production.

Glycolysis
Oxygen-independent metabolism of glucose and other sugars into pyruvate to produce energy in the form of ATP and intermediate substrates for other metabolic pathways.

by increasing the expression and membrane translocation of glucose transporters and by phosphorylating key glycolytic enzymes, such as hexokinase and phosphofructokinase 2 (also known as PFKFB3)[24,26] (FIG. 2). The increased and prolonged AKT1 signalling that is associated with transformation inhibits forkhead box subfamily O (FOXO) transcription factors, resulting in a host of complex transcriptional changes that increase glycolytic capacity[27]. AKT1 also activates ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5), an enzyme that supports increased protein glycosylation in the endoplasmic reticulum and indirectly increases glycolysis by creating an ATP hydrolysis cycle[28]. Finally, AKT1 strongly stimulates signalling through the kinase mTOR by phosphorylating and inhibiting its negative regulator tuberous sclerosis 2 (TSC2; also known as tuberin)[26]. mTOR functions as a key metabolic integration point, coupling growth signals to nutrient availability. Activated mTOR stimulates protein and lipid biosynthesis and cell growth in response to sufficient nutrient and energy conditions and is often constitutively activated during tumorigenesis[29]. At the molecular level, mTOR directly stimulates mRNA translation and ribosome biogenesis, and indirectly causes other metabolic changes by activating transcription factors such as hypoxia-inducible factor 1 (HIF1) even under normoxic conditions. The subsequent HIF1-dependent metabolic changes are a major determinant of the glycolytic phenotype downstream of PI3K, AKT1 and mTOR (FIG. 2).

HIF1 and MYC. The HIF1 and HIF2 complexes are the major transcription factors that are responsible for gene expression changes during the cellular response to low oxygen conditions. They are heterodimers that are composed of the constitutively expressed HIF1β (also known as ARNT) subunit, and either the HIF1α or the HIF2α (also known as EPAS1) subunits, which are rapidly stabilized on exposure to hypoxia[30]. Under normoxic conditions, the HIFα subunits undergo oxygen-dependent hydroxylation by prolyl hydroxylase enzymes, which results in their recognition by von Hippel–Lindau tumour suppressor (VHL), an E3 ubiquitin ligase, and subsequent degradation. HIF1α is ubiquitously expressed, whereas the expression of HIF2α is restricted to a more limited subset of cell types[30]. Although these two transcription factors transactivate an overlapping set of genes, the effects on central metabolism have been better characterized for HIF1, and therefore our discussion is limited to HIF1 specifically.

In addition to its stabilization under hypoxic conditions, HIF1 can also be activated under normoxic conditions by oncogenic signalling pathways, including PI3K[23,31], and by mutations in tumour suppressor proteins such as VHL[32,33], succinate dehydrogenase (SDH)[34] and fumarate hydratase (FH)[35]. Once activated, HIF1 amplifies the transcription of genes encoding glucose transporters and most glycolytic enzymes, increasing the capacity of the cell to carry out glycolysis[36]. In addition, HIF1 activates the pyruvate dehydrogenase kinases (PDKs), which inactivate the mitochondrial pyruvate dehydrogenase complex and thereby reduce the flow of glucose-derived pyruvate into the tricarboxylic acid (TCA) cycle[37–39] (FIG. 2). This reduction in pyruvate flux into the TCA cycle decreases the rate of oxidative phosphorylation and oxygen consumption, reinforcing the glycolytic phenotype and sparing oxygen under hypoxic conditions.

Inhibitors of HIF1 or the PDKs could potentially reverse some of the metabolic effects of tumorigenic HIF1 signalling and several such candidates, including the PDK inhibitor dichloroacetic acid (DCA), are currently under evaluation for their therapeutic utility[40–43].

In addition to its well-described role in controlling cell growth and proliferation, the oncogenic transcription factor MYC also has several important effects on cell metabolism[44]. With respect to glycolysis, highly expressed oncogenic MYC has been shown to collaborate with HIF in the activation of several glucose transporters and glycolytic enzymes, as well as lactate dehydrogenase A (LDHA) and PDK1 (REFS 45,46). However, MYC also activates the transcription of targets that increase mitochondrial biogenesis and mitochondrial function, especially the metabolism of glutamine, which is discussed in further detail below[47].

AMP-activated protein kinase. AMP-activated protein kinase (AMPK) is a crucial sensor of energy status and has an important pleiotropic role in cellular responses to metabolic stress. The AMPK pathway couples energy status to growth signals; biochemically, AMPK opposes the effects of AKT1 and functions as a potent inhibitor of mTOR (FIG. 2). The AMPK complex thus functions as a metabolic checkpoint, regulating the cellular response to energy availability. During periods of energetic stress, AMPK becomes activated in response to an increased AMP/ATP ratio, and is responsible for shifting cells to an oxidative metabolic phenotype and inhibiting cell proliferation[48–50]. Tumour cells must overcome this checkpoint in order to proliferate in response to activated growth

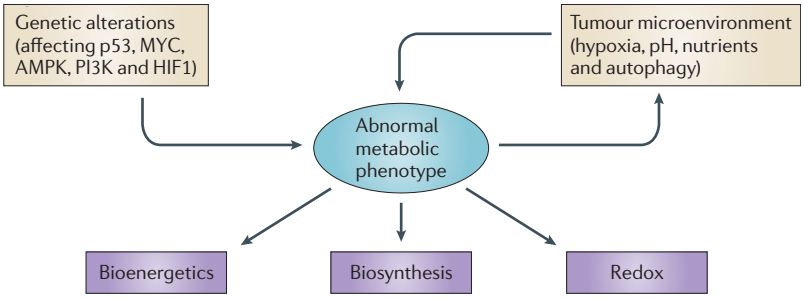

Figure 1 | **Determinants of the tumour metabolic phenotype.** The metabolic phenotype of tumour cells is controlled by intrinsic genetic mutations and external responses to the tumour microenvironment. Oncogenic signalling pathways controlling growth and survival are often activated by the loss of tumour suppressors (such as p53) or the activation of oncoproteins (such as PI3K). The resulting altered signalling modifies cellular metabolism to match the requirements of cell division. Abnormal microenvironmental conditions such as hypoxia, low pH and/or nutrient deprivation elicit responses from tumour cells, including autophagy, which further affect metabolic activity. These adaptations optimize tumour cell metabolism for proliferation by providing appropriate levels of energy in the form of ATP, biosynthetic capacity and the maintenance of balanced redox status. AMPK, AMP-activated protein kinase; HIF1, hypoxia-inducible factor 1.

signalling pathways, even in a less than ideal microenvironment[49]. Several oncogenic mutations and signalling pathways can suppress AMPK signalling[49], which uncouples fuel signals from growth signals, allowing tumour cells to divide under abnormal nutrient conditions. This uncoupling permits tumour cells to respond to inappropriate growth signalling pathways that are activated by oncogenes and the loss of tumour suppressors. Accordingly, many cancer cells exhibit a loss of appropriate AMPK signalling: an event that may also contribute to their glycolytic phenotype.

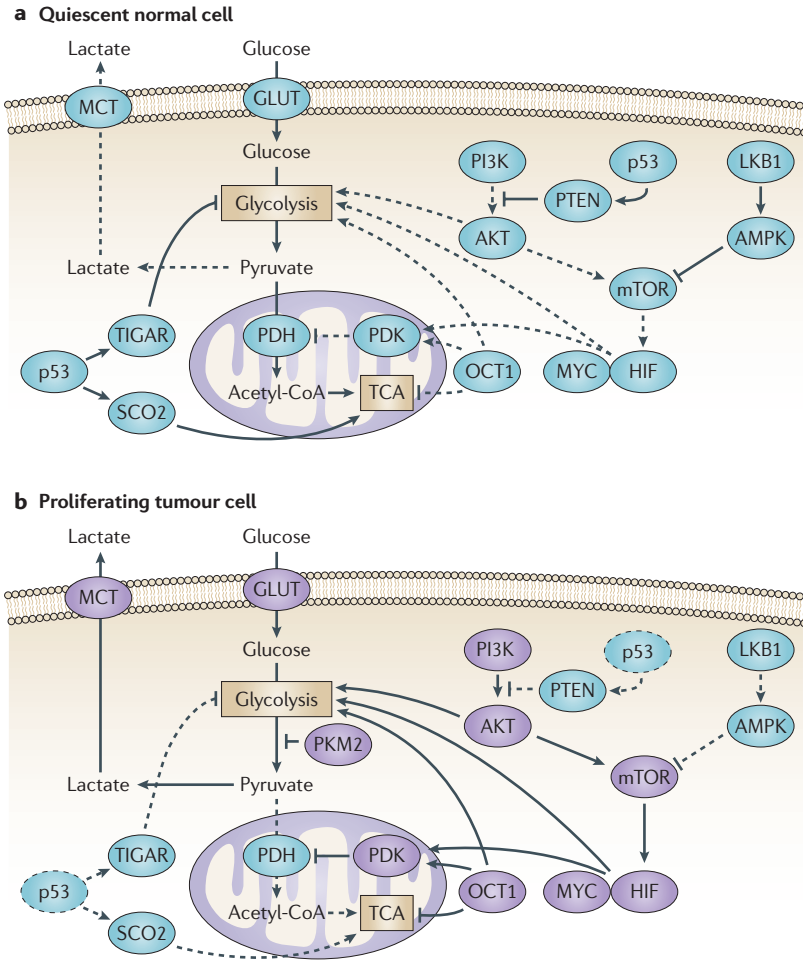

Figure 2 | **Molecular mechanisms driving the Warburg effect.** Relative to normal cells (part **a**) the shift to aerobic glycolysis in tumour cells (part **b**) is driven by multiple oncogenic signalling pathways. PI3K activates AKT, which stimulates glycolysis by directly regulating glycolytic enzymes and by activating mTOR. The liver kinase B1 (LKB1) tumour suppressor, through AMP-activated protein kinase (AMPK) activation, opposes the glycolytic phenotype by inhibiting mTOR. mTOR alters metabolism in a variety of ways, but it has an effect on the glycolytic phenotype by enhancing hypoxia-inducible factor 1 (HIF1) activity, which engages a hypoxia-adaptive transcriptional programme. HIF1 increases the expression of glucose transporters (GLUT), glycolytic enzymes and pyruvate dehydrogenase kinase, isozyme 1 (PDK1), which blocks the entry of pyruvate into the tricarboxylic acid (TCA) cycle. MYC cooperates with HIF in activating several genes that encode glycolytic proteins, but also increases mitochondrial metabolism. The tumour suppressor p53 opposes the glycolytic phenotype by suppressing glycolysis through TP53-induced glycolysis and apoptosis regulator (TIGAR), increasing mitochondrial metabolism via SCO2 and supporting expression of PTEN. OCT1 (also known as POU2F1) acts in an opposing manner to activate the transcription of genes that drive glycolysis and suppress oxidative phosphorylation. The switch to the pyruvate kinase M2 (PKM2) isoform affects glycolysis by slowing the pyruvate kinase reaction and diverting substrates into alternative biosynthetic and reduced nicotinamide adenine dinucleotide phosphate (NADPH)-generating pathways. MCT, monocarboxylate transporter; PDH, pyruvate dehydrogenase. The dashed lines indicate loss of p53 function.

Given the role of AMPK, it is not surprising that *STK11*, which encodes liver kinase B1 (LKB1) — the upstream kinase necessary for AMPK activation — has been identified as a tumour suppressor gene and is mutated in Peutz–Jeghers syndrome[51]. This syndrome is characterized by the development of benign gastrointestinal and oral lesions and an increased risk of developing a broad range of malignancies. LKB1 is also frequently mutated in sporadic cases of non-small-cell lung cancer[52] and cervical carcinoma[53]. Recent evidence suggests that LKB1 mutations are tumorigenic owing to the resulting decrease in AMPK signalling and loss of mTOR inhibition[49]. The loss of AMPK signalling allows the activation of mTOR and HIF1, and therefore might also support the shift towards glycolytic metabolism. Clinically, there is currently considerable interest in evaluating whether AMPK agonists can be used to re-couple fuel and growth signals in tumour cells and to shut down cell growth. Two such agonists are the commonly used antidiabetic drugs metformin and phenformin[49,54–56]. It remains to be seen whether these agents represent a useful class of metabolic modifiers with antitumour activity.

p53 and OCT1. Although the transcription factor and tumour suppressor p53 is best known for its functions in the DNA damage response (DDR) and apoptosis, it is becoming clear that p53 is also an important regulator of metabolism[57]. p53 activates the expression of hexokinase 2 (HK2), which converts glucose to glucose-6-phosphate (G6P)[58]. G6P then either enters glycolysis to produce ATP, or enters the pentose phosphate pathway (PPP), which supports macromolecular biosynthesis by producing reducing potential in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and/or ribose, the building blocks for nucleotide synthesis. However, p53 inhibits the glycolytic pathway by upregulating the expression of TP53-induced glycolysis and apoptosis regulator (TIGAR), an enzyme that decreases the levels of the glycolytic activator fructose-2,6-bisphosphate[59] (FIG. 2). Wild-type p53 also supports the expression of PTEN, which inhibits the PI3K pathway, thereby suppressing glycolysis (as discussed above)[60]. Furthermore, p53 promotes oxidative phosphorylation by activating the expression of SCO2, which is required for the assembly of the cytochrome *c* oxidase complex of the electron transport chain[61]. Thus, the loss of p53 might also be a major force behind the acquisition of the glycolytic phenotype.

OCT1 (also known as POU2F1) is a transcription factor, the expression of which is increased in several human cancers, and it may cooperate with p53 in regulating the balance between oxidative and glycolytic metabolism[62–64]. The transcriptional programme that is initiated by OCT1 supports resistance to oxidative stress and this may cooperate with the loss of p53 during transformation[64]. Data from studies of knockout mice and human cancer cell lines show that OCT1 regulates a set

of genes that increase glucose metabolism and reduce mitochondrial respiration. One of these genes encodes an isoform of PDK (PDK4) that has the same function as the PDK enzymes that are activated by HIF1 (REF. 64) (FIG. 2). Although the mechanisms by which OCT1 is upregulated in tumour cells are poorly understood, its downstream effectors may be potential targets for therapeutic intervention.

Beyond the Warburg effect

Metabolic adaptation in tumours extends beyond the Warburg effect. It is becoming clear that alterations to metabolism balance the need of the cell for energy with its equally important need for macromolecular building blocks and maintenance of redox balance.

Pyruvate kinase (PK). As previously discussed, the generation of energy in the form of ATP through aerobic glycolysis is required for unrestricted cancer cell proliferation[7]. However, studies of the M2 isoform of PK (PKM2) have shown that ATP generation by aerobic glycolysis is not the sole metabolic requirement of a cancer cell, and that alterations to metabolism not only bolster ATP resources but also stimulate macromolecular biosynthesis and redox control.

PK catalyses the rate-limiting, ATP-generating step of glycolysis in which phosphoenolpyruvate (PEP) is converted to pyruvate[65]. Multiple isoenzymes of PK exist in mammals: type L, which is found in the liver and kidneys; type R, which is expressed in erythrocytes; type M1, which is found in tissues such as muscle and brain; and type M2, which is present in self-renewing cells such as embryonic and adult stem cells[65]. Intriguingly, PKM2 is also expressed by many tumour cells. Furthermore, it was discovered that although PKM1 could efficiently promote glycolysis and rapid energy generation, PKM2 is characteristically found in an inactive state and is ineffective at promoting glycolysis[66–68].

This observation was ignored by the scientific community for several years owing to its shear counterintuitive nature: a tumour-specific glycolytic enzyme that inhibits ATP generation and antagonizes the Warburg effect. Only on closer examination of the full metabolic requirements of a cancer cell was the advantage of PKM2 expression revealed. A cancer cell, like any normal cell, must obtain the building blocks that are required for the synthesis of lipids, nucleotides and amino acids. Without sufficient precursors available for this purpose, rapid cell proliferation will halt, no matter how vast a supply of ATP is present. PKM2 provides an advantage to cancer cells because, by slowing glycolysis, this isozyme allows carbohydrate metabolites to enter other subsidiary pathways, including the hexosamine pathway, uridine diphosphate (UDP)–glucose synthesis, glycerol synthesis and the PPP, which generate macromolecule precursors, that are necessary to support cell proliferation, and reducing equivalents such as NADPH[4,28,69] (FIG. 3). Subsequent studies have confirmed that PKM2 expression by lung cancer cells confers a tumorigenic advantage over cells expressing the PKM1 isoform[70]. Interestingly, the classical oncoprotein MYC has been found to promote preferential expression of PKM2 over PKM1 by modulating exon splicing. The inclusion of exon 9 in the *PK* mRNA leads to translation of the PKM1 isoform, whereas inclusion of exon 10 produces PKM2 (REF. 71). MYC upregulates the expression of heterogeneous nuclear ribonucleoproteins (hnRNPs) that bind to exon 9 of the *PK* mRNA and lead to the preferential inclusion of exon 10 and thus to the predominant production of PKM2. By promoting PKM2 expression, MYC promotes the production of NADPH in order to match the increased ATP production and to satisfy the auxiliary needs required for increased proliferation.

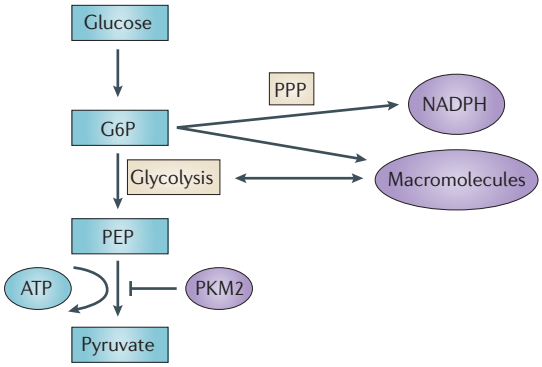

Figure 3 | **PKM2 and its effect on glycolysis and the pentose phosphate pathway.** Pyruvate kinase isoform M2 (PKM2) is present in very few types of proliferating normal cells but is present at high levels in cancer cells. PKM2 catalyses the rate-limiting step of glycolysis, controlling the conversion of phosphoenolpyruvate (PEP) to pyruvate, and thus ATP generation. Although counterintuitive, PKM2 opposes the Warburg effect by inhibiting glycolysis and the generation of ATP in tumours. Although such an effect might at first seem to be detrimental to tumour growth, the opposite is true. By slowing the passage of metabolites through glycolysis, PKM2 promotes the shuttling of these substrates through the pentose phosphate pathway (PPP) and other alternative pathways so that large quantities of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and other macromolecules are produced. These molecules are required for macromolecule biosynthesis and the maintenance of redox balance that is needed to support the rapid cell division that occurs within a tumour. G6P, glucose-6-phosphate.

At the clinical level, increased PKM2 expression has been documented in patient samples of various cancer types, leading to the proposal that PKM2 might be a useful biomarker for the early detection of tumours[65,72–74]. However, further study of the prevalence of PKM2 in cancers and the effect of PKM2 on tumorigenesis is still required.

NADPH. A key molecule produced as a result of the promotion of the oxidative PPP by PKM2 is NADPH (FIG. 4). NADPH functions as a cofactor and provides reducing power in many enzymatic reactions that are crucial for macromolecular biosynthesis. Although other metabolites are produced as a result of increased PPP activity, including ribose, which can be converted

Pentose phosphate pathway
PPP. Biochemical pathway converting glucose into substrates for nucleotide biosynthesis and redox control, such as ribose and NADPH. Owing to multiple connections to the glycolytic pathway, the PPP can operate in various modes to allow the production of NADPH and/or ribose as required.

Macromolecular biosynthesis
Biochemical synthesis of the carbohydrates, nucleotides, proteins and lipids that make up cells and tissues. These pathways require energy, reducing power and appropriate substrates.

Reduced nicotinamide adenine dinucleotide phosphate
NADPH. Cofactor that drives anabolic biochemical reactions and provides reducing capacity to combat oxidative stress.

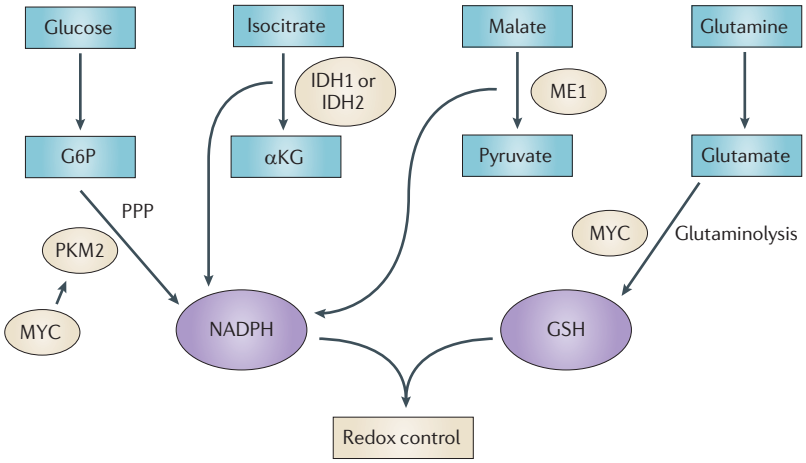

Figure 4 | **Mechanisms of redox control and their alterations in cancer.** The production of two of the most abundant antioxidants, reduced nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH), has been shown to be modulated in cancers. Pyruvate kinase isoform M2 (PKM2), which is overexpressed in many cancer cells, can divert metabolic precursors away from glycolysis and into the pentose phosphate pathway (PPP) to produce NADPH. NADP-dependent isocitrate dehydrogenase 1 (IDH1), IDH2 and malic enzyme 1 (ME1) also contribute to NADPH production. MYC increases glutamine uptake and glutaminolysis, driving the *de novo* synthesis of GSH. Additionally, MYC contributes to NADPH production by promoting the expression of PKM2. Together, NADPH and GSH control increased levels of reactive oxygen species (ROS) driven by increased cancer cell proliferation. αKG, α-ketoglutarate; G6P, glucose-6-phosphate.

into nucleotides, the supply of these building blocks may not be as important as the production of NADPH. Not only does NADPH fuel macromolecular biosynthesis, but it is also a crucial antioxidant, quenching the reactive oxygen species (ROS) produced during rapid cell proliferation. In particular, NADPH provides the reducing power for both the glutathione (GSH) and thioredoxin (TRX) systems that scavenge ROS and repair ROS-induced damage[75]. The double-pronged importance of NADPH in cancer cell metabolism has prompted proposals of clinical intervention by inhibiting NADPH production. Attenuation of the PPP would theoretically dampen NADPH production in cancer cells, slowing macromolecular biosynthesis and rendering the transformed cells vulnerable to free radical-mediated damage. In this way, the advantage conferred by PKM2 expression would be eliminated. In preclinical studies, drugs such as 6-amino-nicotinamide (6-AN), which inhibits G6P dehydrogenase (G6PD; the enzyme that initiates the PPP) have demonstrated anti-tumorigenic effects in leukaemia, glioblastoma and lung cancer cell lines[76]. However, additional basic research and complete clinical trials will be required to properly assess their therapeutic potential.

The discovery and subsequent investigation of the effects of PKM2 expression has shown that we must construct a post-Warburg model of cancer metabolism, in which ATP generation is not the sole metabolic requirement of tumour cells. This turning point has led to the realization that the metabolic alterations present in cancer cells promote not only ATP resources, but also macromolecular biosynthesis and redox control (FIG. 1).

2-hydroxyglutarate
2-HG. A dicarboxylic acid metabolite produced from αKG by the NADPH-dependent reaction of the mutated forms of IDH1 and IDH2. It is also produced at low levels by other enzymes.

Isocitrate dehydrogenases. Another mechanism by which NADPH is produced in mammalian cells is the reaction converting isocitrate to α-ketoglutarate (αKG), which is catalysed by NADP-dependent isocitrate dehydrogenase 1 (IDH1) and IDH2. IDH1 and IDH2 are homodimeric enzymes that act in the cytoplasm and mitochondria, respectively, to produce NADPH by this reaction. IDH1 and IDH2 are highly homologous and structurally and functionally distinct from the NAD-dependent enzyme IDH3, which functions in the TCA cycle to produce the NADH that is required for oxidative phosphorylation.

It has recently been found that specific mutations in IDH1 and IDH2 are linked to tumorigenesis. Two independent cancer genome sequencing projects identified driver mutations in IDH1 in glioblastoma and acute myeloid leukaemia (AML)[3,77]. Subsequent studies revealed that IDH1 or IDH2 is mutated in approximately 80% of adult grade II and grade III gliomas and secondary glioblastomas, and in approximately 30% of cytogenetically normal cases of AML[78–80]. The IDH1 and IDH2 mutations associated with the development of glioma and AML are restricted to crucial arginine residues required for isocitrate binding in the active site of the protein: R132 in IDH1, and R172 and R140 in IDH2 (REFS 3,77,79,80). Affected patients are heterozygous for these mutations, suggesting that these alterations may cause an oncogenic gain-of-function. The range of mutations differs in the two diseases, with the IDH1 R132H mutation predominating in gliomas (>90%), whereas a more diverse collection of mutations in both IDH1 and IDH2 are found in AML[4,78–80].

It was initially proposed that these mutations might act through dominant-negative inhibition of IDH1 and IDH2 activity, which could lead to a reduction in cytoplasmic αKG concentration, inhibition of prolyl hydroxylase activity and stabilization of HIF1 (REF. 81). However, it has recently been shown that these mutations cause IDH1 and IDH2 to acquire a novel enzymatic activity that converts αKG to 2-hydroxyglutarate (2-HG) in a NADPH-dependent manner[79,80,82] (FIG. 5). In fact, this change causes the mutated IDH1 and IDH2 enzymes to switch from NADPH production to NADPH consumption, with potentially important consequences for the cellular redox balance. The product of the novel reaction, 2-HG, is a poorly understood metabolite. 2-HG is present at low concentrations in normal cells and tissues. However, in patients with somatic *IDH1* or *IDH2* mutations, 2-HG builds up to high levels in glioma tissues, and in the leukaemic cells and sera of patients with AML[79,80,82]. It remains to be determined whether these high concentrations of 2-HG are mechanistically responsible for the ability of IDH1 and IDH2 mutations to drive tumorigenesis. Importantly, levels of αKG, isocitrate and several other TCA metabolites are not altered in cell lines or tissues expressing IDH1 mutations, suggesting that other metabolic pathways can adjust and maintain normal levels of these essential metabolites[79,82].

Studies of IDH1 and IDH2 have established a new paradigm in oncogenesis: a driver mutation that confers a new metabolic enzymatic activity that produces a

potential oncometabolite. The molecular mechanisms by which IDH1 and IDH2 mutations contribute to tumorigenesis are still under investigation, as is the possibility that these mutant enzymes may be useful targets for therapy. Curiously, although IDH1 and IDH2 mutations are clearly powerful drivers of glioma and AML, they seem to be rare or absent in other tumour types[78,83,84]. This observation highlights the importance of the specific cellular context in understanding metabolic perturbations in cancer cells.

Metabolic alterations supporting redox status

ROS are a diverse class of radical species that are produced in all cells as a normal byproduct of metabolic processes. ROS are heterogeneous in their properties and have a plethora of downstream effects, depending on the concentrations at which they are present.

At low levels, ROS increase cell proliferation and survival through the post-translational modification of kinases and phosphatases[85–87]. The production of this low level of ROS can be driven by NADPH and NADPH oxidase (NOX) and is required for homeostatic signalling events. At moderate levels, ROS induce the expression of stress-responsive genes such as *HIF1A*, which in turn trigger the expression of proteins providing pro-survival signals, such as the glucose transporter GLUT1 (also known as SLC2A1) and vascular endothelial growth factor (VEGF)[88,89]. However, at high levels, ROS can cause damage to macromolecules, including DNA; induce the activation of protein kinase Cδ (PKCδ), triggering senescence[90,91]; and/or cause permeabilization of the mitochondria, leading to the release of cytochrome *c* and apoptosis[92,93]. Cells counteract the detrimental effects of ROS by producing antioxidant molecules, such as reduced GSH and TRX. These molecules reduce excessive levels of ROS to prevent irreversible cellular damage[94]. Importantly, several of these antioxidant systems, including GSH and TRX, rely on the reducing power of NADPH to maintain their activities. In highly proliferative cancer cells, ROS regulation is crucial owing to the presence of oncogenic mutations that promote aberrant metabolism and protein translation, resulting in increased rates of ROS production. Transformed cells counteract this accumulation of ROS by further upregulating antioxidant systems, seemingly creating a paradox of high ROS production in the presence of high antioxidant levels[95–98] (FIG. 6).

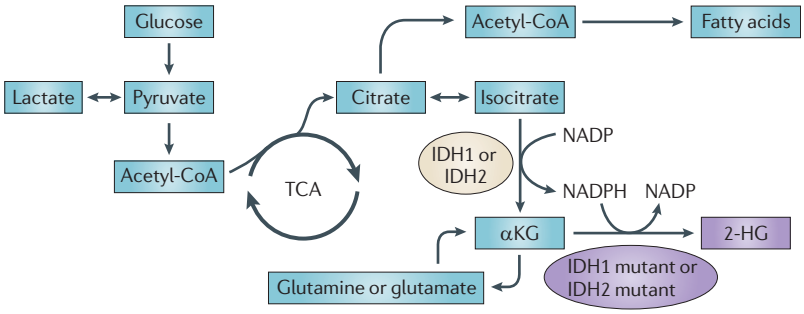

Figure 5 | **IDH1 and IDH2 mutations cause an oncometabolic gain of function.** Certain somatic mutations at crucial arginine residues in isocitrate dehydrogenase 1 (IDH1, which is cytoplasmic) and IDH2 (which is mitochondrial) are common early driver mutations in glioma and acute myeloid leukaemia (AML). These mutations are unusual because they cause the gain of a novel enzymatic activity. Instead of isocitrate being converted to α-ketoglutarate (αKG) with the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH), αKG is converted to 2-hydroxyglutarate (2-HG) with the consumption of NADPH. 2-HG builds up to high levels in tumour cells and tissues of affected patients and supports tumour progression by a mechanism that is yet to be determined. TCA, tricarboxylic acid cycle.

RB, PTEN and p53. There is currently a scientific consensus that cancer cells alter their metabolic pathways and regulatory mechanisms so that ROS and antioxidants are tightly controlled and maintained at higher levels than in normal cells. However, during the process of tumorigenesis, loss of tumour suppressors may cause cells to become overloaded with the products of aberrant metabolism and lose control of redox balance. For example, when the tumour suppressor *TSC2* is deleted, mTOR becomes hyperactivated[99]. Hyperactivated mTOR leads to an upregulation of translation and increased ROS production[100]. In a cancer cell that has additionally lost function of the tumour suppressor retinoblastoma (RB), which normally participates in the antioxidant response, the increased ROS production is not countered and the cell will undergo apoptosis[99]. Similar results have been seen with loss of PTEN, and hyperactivation of AKT1 leads to FOXO inactivation and increased oxidative stress[101].

A comparable theory can be proposed for p53. p53 may promote oxidative stress while inducing apoptosis[102–104], but it also has an important role in reducing oxidative stress as a defence mechanism[105,106]. Glutaminase 2 (GLS2) is upregulated by p53 and drives *de novo* synthesis of GSH[107]. Furthermore, through the p53 target gene cyclin-dependent kinase inhibitor 1A (*CDKN1A*, which encodes p21), p53 promotes the stabilization of the transcription factor NRF2 (also known as NFE2L2)[108]. NRF2 is the master antioxidant transcription factor and upregulates the expression of several antioxidant and detoxifying molecules[108]. When ROS levels are low, NRF2 binds to kelch-like ECH-associated protein 1 (KEAP1), which triggers NRF2 degradation. Under oxidative stress, p53 is activated and stimulates expression of p21. p21 prevents the KEAP1–NRF2 interaction and preserves NRF2, driving antioxidant countermeasures[108]. Loss of p53 in a cancer cell inactivates this redox maintenance mechanism: because p21 is not activated, NRF2 continues to be degraded, antioxidant proteins are not expressed and the redox balance is lost. From a clinical point of view, it may be possible to exploit loss-of-function p53 mutations or other tumour suppressor genes by applying additional oxidative stress. In the absence of the redox maintenance pathway that is supported by these tumour suppressors, malignant cells might be selectively killed[109–111].

DJ1. Much of the research involving ROS and oxidative stress has emerged from work in the field of neurodegenerative diseases. Only recently has it been realized that similar mechanisms maintain appropriate redox status in both normal neurons and cancer cells. One protein involved in preventing neurodegeneration that

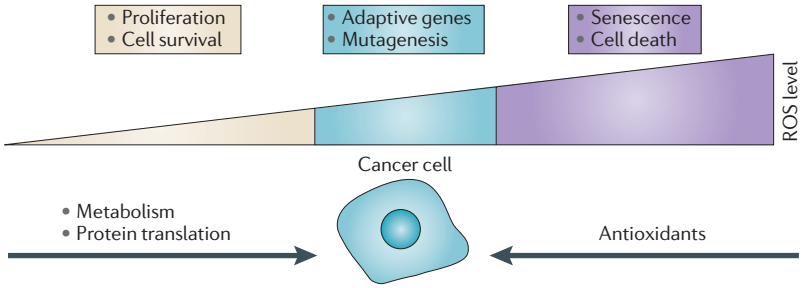

Figure 6 | **Relationship between the levels of ROS and cancer.** The effect of reactive oxygen species (ROS) on cell fate depends on the level at which ROS are present. Low levels of ROS (yellow) provide a beneficial effect, supporting cell proliferation and survival pathways. However, once levels of ROS become excessively high (purple), they cause detrimental oxidative stress that can lead to cell death. To counter such oxidative stress, a cell uses antioxidants that prevent ROS from accumulating at high levels. In a cancer cell, aberrant metabolism and protein translation generate abnormally high levels of ROS. Through additional mutations and adaptations, a cancer cell exerts tight regulation of ROS and antioxidants in such a way that the cell survives and the levels of ROS are reduced to moderate levels (blue). This extraordinary control of ROS and the mechanisms designed to counter it allow the cancer cell to avoid the detrimental effects of high levels of ROS, but also increase the chance that the cell will experience additional ROS-mediated mutagenic events and stress responses that promote tumorigenesis. Figure inspired by discussions with Navdeep Chandel, Northwestern University, Chicago, USA.

has also been investigated in the context of cancer is DJ1 (also known as PARK7). Similar to p21, DJ1 stabilizes NRF2 and thereby promotes antioxidant responses[112]. DJ1 is mutated and inactive in several neurodegenerative disorders, most notably Parkinson's disease[113]. In these disorders, it is believed that loss of DJ1 function leads to elevated oxidative stress in the brain and increased neuronal cell death[114]. In the context of cancer, *PARK7* has been described as an oncogene[115]. In patients with lung, ovarian and oesophageal cancers, high DJ1 expression in the tumour predicts a poor outcome[115–117]. At a mechanistic level, DJ1 stimulates AKT1 activity both *in vitro* and *in vivo* by regulating the function of the tumour suppressor PTEN[115]. Although this function seems to be a logical candidate for the mechanism underlying the tumorigenic role of DJ1, high DJ1 expression may also promote tumorigenesis by reducing the oxidative stress caused by aberrant cell proliferation and thereby prevent ROS-induced cell death.

Several other proteins that are inactivated in neurodegenerative disorders have antioxidant properties, including the enzyme superoxide dismutase 1 (SOD1). Mutations in SOD1 are responsible for 20% of familial cases of amyotrophic lateral sclerosis (ALS)[118]. However, it is still unknown whether SOD1 or other key antioxidant enzymes are hyperactivated in cancer cells and whether they have important roles in tumorigenesis. Supporting the notion that loss of DJ1 prevents appropriate redox control in cancers, an inverse correlation has been reported between cancer risk and Parkinson's disease. A recent meta-analysis of patients with Parkinson's disease determined that they have an approximately 30% lower risk of developing cancers compared with controls[119]. This lower risk was associated with several different cancer types, including lung, prostate and colorectal cancers. Additional investigation of the cancer risk of patients with other neurodegenerative disorders, such as ALS, may provide key insights into potential therapeutic exploitation of the heightened need to maintain redox balance in a cancer cell.

Glutamine and MYC. It has long been known that cell culture medium must be supplemented with high concentrations of glutamine to support robust cell proliferation[120–122]. However, it has recently been shown that transformation stimulates glutaminolysis and that many tumour cells are critically dependent on this amino acid[123,124]. After glutamine enters the cell, glutaminase enzymes convert it to glutamate, which has several fates. Glutamate can be converted directly into GSH by the enzyme glutathione cysteine ligase (GCL) (FIG. 4). Reduced GSH is one of the most abundant antioxidants found in mammalian cells and is vital to controlling the redox state of all subcellular compartments[97]. Glutamate can also be converted to αKG and enter the TCA cycle. This process of anapleurosis supplies the carbon input required for the TCA cycle to function as a biosynthetic 'hub' and permits the production of other amino acids and fatty acids. There is also recent evidence that some glutamine-derived carbon can exit the TCA cycle as malate and serve as a substrate for malic enzyme 1 (ME1), which produces NADPH[125]. The precise mechanisms regulating the fate of glutamine in tumour cells are not completely understood, and it is likely that genetic background and microenvironmental factors have a role.

One factor that is known to have a major role in regulating glutaminolysis is MYC, further supporting the concept that MYC promotes not only proliferation but also the production of accompanying macromolecules and antioxidants that are required for growth. MYC increases glutamine uptake by directly inducing the expression of the glutamine transporters SLC5A1 and SLC7A1 (also known as CAT1)[124]. Furthermore, MYC indirectly increases the level of glutaminase 1 (GLS1), the first enzyme of glutaminolysis, by repressing the expression of *microRNA-23A* and *microRNA-23B*, which inhibit *GLS1* (REF. 124). Thus, MYC may support antioxidant capacity by driving PPP-based NADPH production through promoting the expression of the PKM2 isoform, as described above, and also by increasing the synthesis of GSH through glutaminolysis (FIG. 4). A comprehensive and quantitative investigation of glutamine metabolism in patient samples has not yet been reported. However, new techniques for measuring glutamine and its metabolites have been developed and should soon permit the detailed examination of glutamine metabolism and MYC expression in patient tumours[126]. Furthermore, work is underway to determine whether other oncoproteins such as PI3K and SRC have a role in promoting glutaminolysis. Supporting this theory, it has been shown that cells with a hyperactive Ras oncogene require a stable flow of glutamine and GSH generation in order to balance redox demands[13,111]. It is also interesting to speculate that part of the mechanism responsible for the clinical efficacy of L-asparaginase in treating certain leukaemias may be related to this phenomenon, as L-asparaginase therapy reduces serum levels of both asparagine and

Parkinson's disease
A neurodegenerative disorder affecting the CNS, which is characterized by muscle rigidity and the onset of tremors.

Amyotrophic lateral sclerosis
ALS. Also known as Lou Gehrig's disease; it occurs owing to the degeneration of the CNS and leads to the inability to control muscles and eventual muscle atrophy.

Glutaminolysis
The catabolic metabolism of glutamine, which yields substrates that replenish the TCA cycle, produce GSH and supply building blocks for amino acid and nucleotide synthesis.

Anapleurosis
Category of reactions that serve to replenish the intermediate substrates of an anabolic biochemical pathway, especially important in the TCA cycle.

glutamine[127,128]. Nevertheless, several questions regarding the role of glutamine in tumorigenesis remain to be answered.

Metabolic adaptation to the microenvironment

In addition to the genetic changes that alter tumour cell metabolism, the abnormal tumour microenvironment has a major role in determining the metabolic phenotype of tumour cells. Tumour vasculature is structurally and functionally abnormal, and combined with intrinsically altered tumour cell metabolism, creates spatial and temporal heterogeneity in oxygenation, pH, and the concentrations of glucose and many other metabolites. These extreme conditions induce a collection of cellular stress responses that further contribute to the distorted metabolic phenotype of tumour cells and influence tumour progression[129].

Response to hypoxia. The response to hypoxia is the best studied of tumour cell stress responses owing to the well-known effects of hypoxia on tumour radioresistance and metastasis. Consequently, tumour hypoxia is a poor prognostic factor in a number of malignancies[6,129–131]. Several molecular pathways that influence cellular metabolism are altered under hypoxia. As described above, hypoxia alters transcription through the stabilization of HIF, which increases glycolytic capacity and decreases mitochondrial respiration[132]. In addition, and independently of HIF, hypoxia inhibits signalling through mTOR, which is a major regulator of multiple mechanisms contributing to the altered metabolic phenotype[133,134]. Specifically, the induction of autophagy may be of crucial importance[135]. Although mTOR inhibition would usually be considered tumour suppressive, there is evidence that in advanced malignancies such a response can increase the tolerance to hypoxia and promote tumour cell survival during metabolic stress. This finding supports the concept that, in certain microenvironmental or genetic contexts, as in the case of RB inactivation, tumour cells may benefit from retaining the ability to moderate mTOR signalling[99]. Finally, extreme hypoxia (<0.02% O_2) causes endoplasmic reticulum stress and activates the unfolded protein response, which provides a further adaptive mechanism that allows tumour cells to survive under adverse metabolic conditions[134,136–138].

Other metabolic stress conditions such as low pH and low glucose are also prevalent in solid tumours and are likely to be major determinants of the metabolic phenotype. The molecular pathways that are involved in responding to these conditions are currently under investigation, which will undoubtedly enhance our knowledge of the mechanistic determinants of tumour cell metabolism. Since it has been well established that microenvironmental factors affect sensitivity to radiation, traditional chemotherapy and targeted therapies, a better understanding of the diverse avenues of metabolic regulation in cancer cells may offer new opportunities to modify the tumour microenvironment for therapeutic gain[139].

It should be noted that the relationship between the tumour microenvironment and cancer cell metabolism is not one of simple cause and effect, in which biochemical conditions in the tumour influence cellular metabolism. Because metabolite concentrations are governed by both supply by the vasculature and demand by the tissue, changes in metabolism of both the tumour and normal stromal cells also have a profound effect on microenvironmental conditions (FIG. 1). The complex and dynamic relationship between tumour metabolism and the microenvironment emphasizes the importance of studying metabolic regulation *in vivo* using appropriate model systems, as well as the need for more sophisticated measurements of cell metabolism and relevant microenvironmental conditions in human tumours.

Metabolic flexibility. Although aerobic glycolysis (the Warburg effect) is the best documented metabolic phenotype of tumour cells, it is not a universal feature of all human cancers[140]. Moreover, even in glycolytic tumours, oxidative phosphorylation is not completely shut down. It is clear from both clinical FDG–PET data, as well as *in vitro* and *in vivo* experimental studies, that tumour cells are capable of using alternative fuel sources. In fact, up to 30% of tumours are considered FDG–PET-negative depending on the tumour type[16,17]. Amino acids, fatty acids and even lactate have been shown to function as fuels for tumour cells in certain genetic and microenvironmental contexts[125,141,142]. The carnitine palmitoyltransferase enzymes that regulate the β-oxidation of fatty acids may have a key role in determining some of these phenotypes. Furthermore, owing to the dynamic nature of the tumour microenvironment, it is likely that the metabolic phenotype of tumour cells changes to adapt to the prevailing local conditions. The regulation of this metabolic flexibility is poorly understood and will require a much greater degree of understanding if effective therapeutic strategies targeting metabolism are to be developed and effectively deployed.

Conclusion

Mutations in oncogenes and tumour suppressor genes cause alterations to multiple intracellular signalling pathways that affect tumour cell metabolism and re-engineer it to allow enhanced survival and growth. In fact, it is likely that metabolic alterations are required for tumour cells to be able to respond to the proliferative signals that are delivered by oncogenic signalling pathways. In addition, the unique biochemical microenvironment further influences the metabolic phenotype of tumour cells, and thus affects tumour progression, response to therapy and patient outcome. These metabolic adaptations must balance the three crucial requirements of tumour cells: increased energy production, sufficient macromolecular biosynthesis and maintenance of redox balance. Only by thoroughly dissecting these processes will we discover the Achilles heels of tumour metabolic pathways and be able to translate this knowledge to the development and implementation of novel classes of therapeutics. The ultimate goal is to design treatment strategies that slow tumour progression, improve the response to therapy and result in a positive clinical outcome.

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Competing interests statement
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