Carcinogenesis vol.30 no.8 pp.1269–1280, 2009
doi:10.1093/carcin/bgp070
Advance Access publication March 25, 2009

REVIEW

Metabolic transformation in cancer

Daniel A.Tennant, Raúl V.Durán, Houda Boulahbel and Eyal Gottlieb*

Cancer Research UK, Beatson Institute for Cancer Research, Glasgow, G61 1BD, UK

*To whom correspondence should be addressed. Tel: +44 1413303981; Fax: +44 1419426521;
Email: e.gottlieb@beatson.gla.ac.uk

In 2000, Douglas Hanahan and Robert Weinberg published a review detailing the six hallmarks of cancer. These are six phenotypes that a tumour requires in order to become a fully fledged malignancy: persistent growth signals, evasion of apoptosis, insensitivity to anti-growth signals, unlimited replicative potential, angiogenesis and invasion and metastasis. However, it is becoming increasingly clear that these phenotypes do not portray the whole story and that other hallmarks are necessary: one of which is a shift in cellular metabolism. The tumour environment creates a unique collection of stresses to which cells must adapt in order to survive. This environment is formed by the uncontrolled proliferation of cells, which ignore the cues that would create normal tissue architecture. As a result, the cells forming the tumour are exposed to low oxygen and nutrient levels, as well as high levels of toxic cellular waste products, which is thought to propel cells towards a more transformed phenotype, resistant to cell death and pro-metastatic.

Introduction

In order to sustain the rapid proliferation and to counteract the hostile environment observed in tumours, cells must increase the rate of metabolic reactions to provide the adenosine triphosphate (ATP), lipids, nucleotides and amino acids necessary for daughter cell production (1). Cells that do not undergo these changes will not survive the tumour environment, resulting in the selection of those with a transformed metabolic phenotype. One seemingly necessary metabolic alteration is the increase in the rate of glycolysis, the conversion of glucose to pyruvate. In work beginning >80 years ago, Otto Warburg noted that tumour cells use glycolysis ('fermentation'), even in the presence of O_2 (2–4). This was termed 'aerobic glycolysis' and since then has been considered as a universal phenotype of tumours. In normal cells, an interplay exists between mitochondrial respiration and glycolysis in which mitochondrial respiration inhibits glycolytic flux—a phenomenon originally described in yeast by Pasteur in 1861 [the 'Pasteur Effect' (5)] and was expanded upon and extended to mammalian tissues by Crabtree (6). High rates of aerobic glycolysis is not a mechanism unique to tumours, as all energy-demanding cells utilize glycolysis as well as mitochondrial respiration for ATP production. However, the phenotype that is unique to cancer is the high levels of lactate that are produced from the increased rate of aerobic glycolysis. Forcing proliferating cells into a resting, differentiated phenotype can decrease glycolytic rate and promote oxidative phosphorylation (OXPHOS) as the major ATP generating process (7), indicating that, at least in the case of normal cells, this loss of the mitochondrial inhibition of glycolysis is reversible. Glycolysis produces only 2 mol of ATP per mole glucose, an inefficient bioenergetic process when compared with OXPHOS (up to 36 mol of ATP per mole glucose); so in order to maintain normal ATP levels in the tumour, the rate of glycolysis must be much greater than that observed in most normal tissues (exceptions include the heart and kidney). This hunger of tumours for glucose is utilized in current clinical practice, as primary and distant metastatic sites of tumours can be imaged in patients using their uptake of a radiolabelled glucose derivative (^{18}fluoro-2-deoxyglucose) (8). The change in metabolism cannot be purely attributed to alterations in allosteric and product/substrate regulation of the metabolic enzymes. A concerted 'energy response' also occurs involving factors such as mammalian target of rapamycin (mTOR), Myc and the hypoxia-inducible factors (HIFs), which is vital for the long term metabolic transformation of tumours.

Hypoxia and HIFs

Other than increased aerobic glycolysis, cancer cells also utilize glucose under anaerobic conditions to compensate for the reduced mitochondrial ATP generation (1). Hypoxia (low oxygen) and anoxia (complete lack of oxygen) are both present in most, if not all solid tumours. Hypoxia specifically is thought to be an important factor in supporting and directing tumour progression. However, contrary to being under constant hypoxia, one important facet of the tumour environment is that the hypoxia experienced by the cell is thought to be variable, even cycling between normal oxygen tension and acute hypoxia (<10 mm Hg O_2) (9,10). Acute hypoxia results in the stabilization of a group of transcription factors thought to be responsible for the majority of the transcriptional responses under hypoxia, known as HIFs. HIF1 and HIF2 consist of two subunits, α and β, both of which are members of the basic helix-loop-helix period circadian protein, aryl hydrocarbon receptor nuclear translocator, single-minded protein family (11,12). HIF1α and 2α can both dimerize with HIF1β (also known as the aryl hydrocarbon receptor nuclear translocator) and can up-regulate gene expression via hypoxia-responsive elements (see Figure 1). HIF3α is currently not well characterized but is thought to be a negative regulator of the other HIFs (13). Although HIF1β is constitutively expressed, the α subunit is highly labile under normoxia, being constitutively expressed and degraded (11). The majority of HIFα degradation is controlled by the hydroxylation of two prolyl residues in the oxygen-dependent degradation domain by HIF prolyl hydroxylases (PHDs) (14–17). These enzymes are members of a family of α-ketoglutarate-dependent dioxygenases, and use O_2 and α-ketoglutarate to convert a prolyl residue to hydroxy-prolyl, producing succinate and CO_2 (18). Once hydroxylated, the prolyl residues are bound by the von-Hippel Lindau protein (pVHL)-containing E3 ubiquitin ligase complex, allowing HIFα to be ubiquitylated and degraded by the proteasome (19,20). Under conditions of limiting O_2, HIFα subunits are stable, can dimerize with the β subunit, and activate its transcriptional targets. Another α-ketoglutarate-dependent dioxygenase, factor-inhibiting HIF (FIH), can act on HIFα by hydroxylating a C-terminal asparagine (Asp803 in HIF1α) (21,22). This disrupts the interaction with p300, cAMP responsive element binding (CREB)-binding protein and other transcription co-factors, inhibiting the transactivation of HIF target genes (23).

As mentioned, the oxygen sensors for the HIFα pathway are the PHD enzymes (PHD1–3) (24,25). Characterization of PHD targets has been slow thus far, but due to the universality of the hypoxic

Abbreviations: ATP, adenosine triphosphate; ASCT2, alanine serine cysteine transporter 2; COX, cytochrome c oxidase; ETC, electron transport chain; FH, fumarate hydratase; G6P, glucose 6-phosphate; HIF, hypoxia-inducible factor; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; NF-κB, nuclear factor-kappaB; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase 1; PFK2/FBPase, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PGM, phosphoglycerate mutase; PHD, prolyl hydroxylase; PK, pyruvate kinase; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TCA, tricarboxylic acid; TIGAR, TP53-induced glycolysis and apoptosis regulator; TSC1/2, tuberosclerosis complex 1/2.

© The Author 2009. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

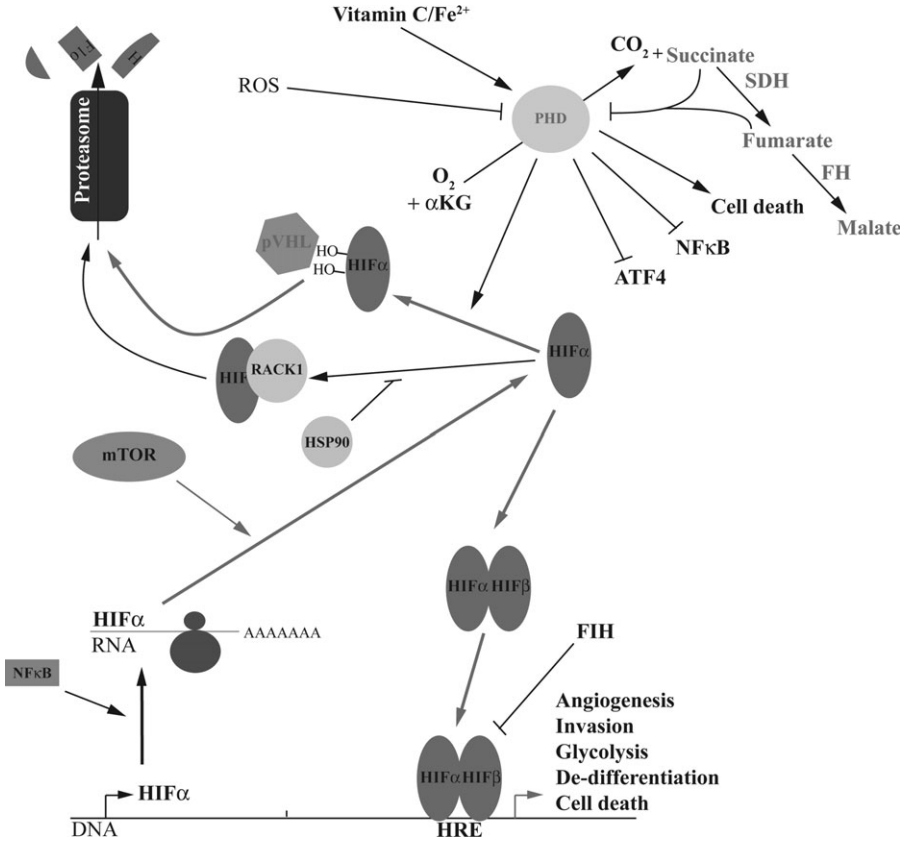

Fig. 1. Synthesis, degradation and regulation of HIFα. αKG, α-ketoglutarate; ATF4, activating transcription factor 4; FIH, factor-inhibiting HIF; FH, fumarate hydratase; HIF, hypoxia-inducible factor; HRE, hypoxia-responsive element; HSP90, heat shock protein 90; mTOR, mammalian target of rapamycin; NFκB, nuclear factor kappa B; PHD, HIF prolyl hydroxylase; pVHL, von-Hippel Lindau protein; RACK1, receptor of activated protein kinase C 1; ROS, reactive oxygen species; SDH, succinate dehydrogenase.

phenotype in solid tumours, PHDs are functionally inactivated in areas of most if not all tumour types. This leads to the hypothesis that the functional repression of PHDs could be advantageous to tumours, and not solely due to the resultant activation of HIFα subunits. Importantly, a recent paper has found a germline mutation in PHD2 and loss of heterozygosity of the second allele in a patient with paraganglioma, providing the first evidence for the PHDs as tumour suppressors (26). Results published by Lee *et al.* and Schlisio *et al.* (27,28) also support this as they have identified PHD3 (the gene product of *EGLN3*) as a necessary effector for normal growth factor withdrawal induced apoptosis in pheochromocytoma. PHD3 inactivation has also been found to stabilize another transcription factor, ATF4, via an oxygen-dependent mechanism, which may also have a role in tumorigenesis (29). Other transcription factors that are important in tumorigenesis and are modulated by hypoxia include nuclear factor-kappaB (NF-κB) and specificity protein 1 (SP1) (30–33). A recent paper by Couvelard *et al.* (34) has shown a correlation between tumour aggressiveness and the expression level of all three PHDs. As much of the tumour mass is not adequately oxygenated, this suggests roles for these proteins in addition to their oxygen-sensing enzymatic activity.

Although strongly and rapidly up-regulated under short periods of hypoxia, during chronic hypoxia, HIF levels are decreased (35). Only the areas furthest from functional blood vessels experience this effect, and in the absence of an angiogenic response, they are thought to form the necrotic areas in a tumour. The down-regulation of HIFα in these circumstances is thought to help protect against the necrosis of cells, but this may only be for a limited time period (36). Most of the hypoxic regions found in tumours are exposed to fluctuating levels of O_2 (37), which allows for continued HIF stabilization. However, fluctuating levels of O_2 can also cause an increase in intracellular levels of reactive oxygen species (ROS). These are formed by the single electron reduction of molecular O_2 forming O_2^-, a highly reactive and damaging free radical. The reaction of O_2^- with water can form H_2O_2 and OH^-, the former of which is a comparatively long-lived molecule, and is thought to also have a role as a signalling molecule. It has been observed that ROS can be produced under hypoxia due to inefficient electron transport chain (ETC) activity, and the resultant leakage of electrons, mainly from complexes I and III of the ETC (38–42). The effect of hypoxic ROS production on HIFα expression is controversial. HIF1α has been shown to be stabilized by high ROS levels that can be observed under hypoxia, possibly by the deactivation of the PHD catalytic core (40). This would, in essence, put mitochondria in the central role of oxygen sensor, putting both PHD inactivation and HIFα stabilization downstream of this. The production of ROS in this model is at complex III and therefore it follows that it requires a functional ETC (up to complex III) (40,43). However, other studies have not been able to detect increased levels of ROS production under hypoxia (44). Another model of mitochondrial-mediated HIFα inhibition involves nitric oxide, an endogenous inhibitor of respiration. It has been proposed by Moncada *et al.* (45) that nitric oxide binding to cytochrome c oxidase (COX) inhibits O_2 consumption in mitochondria, increasing the availability of O_2 for PHDs and therefore their reactivation under hypoxia.

Over 100 genes are known to be up-regulated by HIF transcription factors, whose functions range from neovascularization, survival, intracellular pH regulation, cell migration, tumour growth and energy metabolism (46). HIF1 appears to be the major player in the hypoxic alterations of cellular metabolism, up-regulating a plethora of transporters and enzymes involved in glycolysis that act in parallel to increase the rate and channelling of metabolic intermediates through the pathway. As well as the concerted increase in glycolytic rate, HIF1 activity results in the up-regulation of lactate dehydrogenase A and

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

pyruvate dehydrogenase kinase (PDK) 1, which channel pyruvate away from the mitochondrion (47,48). This results in both an increase in lactate synthesis and a reduction of acetyl-CoA entering the tricarboxylic acid (TCA) cycle in the mitochondria (see Figure 3A, and further discussion below). The production of lactate would reduce the intracellular pH unless HIF1 activity also up-regulated proteins necessary to remove this waste product. The monocarboxylic acid transporter-4 is a HIF1 target, and is used for the export of lactate, symported with hydrogen ions (49). Intracellular H^+ is also excreted using an antiporter—the Na^+/H^+ exchanger (NHE1, also a HIF target) (50). The extracellular H^+ is buffered with HCO_3, producing CO_2. Another strongly up-regulated HIF target protein is a member of the family of carbonic anhydrases (CA)-IX (51). This plasma membrane-bound enzyme converts CO_2 to carbonic acid, and with the increase in lactate, lowers extracellular pH. This acidic microenvironment causes a remodelling response in the tumoural stroma, allowing the infiltration of new blood vessels initiated by tumour-derived angiogenic factors, and subsequent normalization of the pH. It has also been suggested that lactate alone can result in blood vessel growth into a region with low extracellular pH as well as tumour cell invasion away from the primary site, although a mechanism for these effects has not yet been fully elucidated (52–54). Finally, lactate has also been shown to inhibit the immune response against tumours by interfering with the activation and proliferation of cytotoxic T lymphocytes (55).

HIF expression in tumours—whether due to hypoxia, TCA cycle enzyme mutation (see below), mitochondrial dysfunction or aberrant growth factor stimulation—is known to be vitally important for their progression. Studies in xenografts have shown that decreasing HIF expression in tumours inhibits growth (56–60), and data from patient samples have shown a correlation between HIF, HIF target gene expression and disease progression and patient survival (61–63) (and reviewed in ref. 64). This positions HIFα firmly as a therapeutic target, and a number of antitumour therapies have been designed to interfere with HIF and its target genes.

Master regulators of metabolic transformation under normoxia

HIFα expression can also be modulated by a number of mechanisms other than hypoxia, providing crosstalk between different signalling pathways (Figure 1). Loss of tumour suppressors, pVHL or PTEN, lead to HIF activation via stabilization or AKT and glycogen synthase kinase 3β, respectively (65–67). AKT activity also inhibits forkhead box O4 and forkhead box O3a, which can both reduce HIF1α levels and inhibit its activity (68,69). There is further reciprocal control on PHDs and HIF1a via the NF-κB-signalling pathway. Although PHD1 inhibition can lead to NF-κB activation by de-repression of IκB and HIF1α can activate NF-κB (31,70), NF-κB has also recently been shown to promote HIF1α transcription, both *in vitro* and *in vivo* (71,72). Finally, HIFα subunit expression can be controlled by mTOR at the translational level (73). Tumours with alterations in mTOR pathway activity, such as those with hyperactivated AKT or loss of function of the tuberosclerosis complex 1/2 (TSC1/2), have increased HIFα translation, with no concurrent increase in degradation, leading to normoxic expression of HIFα (74). Although mTOR is inactivated under hypoxia, reactivation through the loss of TSC1/2 or loss of function of the promyelocytic leukaemia tumour suppressor can lead to further enhanced hypoxic HIFα expression levels (74,75).

Alongside HIF, Myc is another major transcription factor involved in the metabolic shift necessary for tumorigenesis. Myc is overexpressed in ∼70% of tumours in which it induces cellular proliferation, but also a concurrent increase in cell death (76,77). It acts via the activation or repression of genes containing 'E boxes' (CACGTG consensus). When bound with Max, the heterodimer activates gene expression, and when with Mad1 or Mxi it represses. In addition, it is now known that HIF1α or 2α can bind the Myc heterodimer and either potentiate or dampen the gene expression. HIF cooperates with Myc to create a more sustainable metabolically transformed phenotype (78). It has been proposed that the relative activity of HIF1 and 2 are important in determining the tumour phenotype. HIF2α expression can produce a more aggressive tumour than HIF1α, as HIF2α allows the Myc-mediated proliferative response, whereas HIF1α inhibits it. For a more in-depth review of HIF and Myc interactions see (79–81).

Glycolysis

In order to undergo glycolysis, glucose enters the cell via a facilitative glucose transporter (Figure 2). A number of glucose transporters are up-regulated in tumours, Glut1 being particularly important in the tumour response to hypoxia (82). Up-regulation of this transporter immediately increases the intracellular availability of glucose for metabolic reactions, most of which are initiated by its phosphorylation by hexokinase to give glucose 6-phosphate (G6P, see Figure 2). Hexokinase II, one of the four hexokinase isozymes, is a target of many transcription factors important in tumorigenesis, including HIF1 and Myc (through the 'carbohydrate response element') (83). Hexokinase is also thought to have a role in protecting the cell against

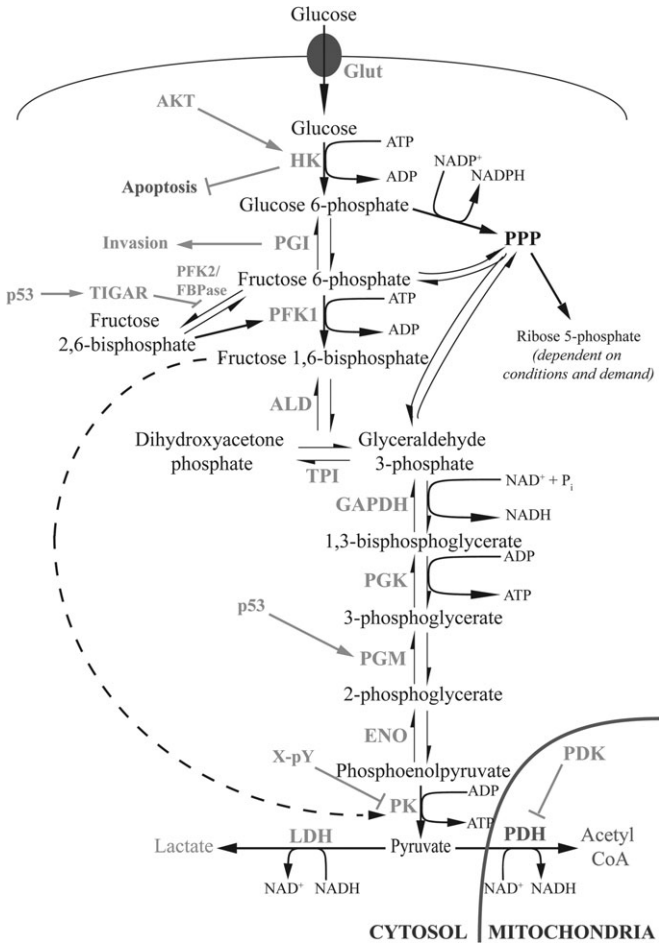

Fig. 2. Glycolysis and cancer. Green text—enhanced/activated in cancer. Red text—reduced/inhibited in cancer. ADP, adenosine diphosphate; ATP, adenosine triphosphate; ALD, aldolase; CoA, coenzyme A; ENO, enolase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Glut, glucose transporter; HK, hexokinase; LDH, lactate dehydrogenase; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; FBPase, fructose bisphosphatase; PGK, phosphoglycerate kinase; PGI, phosphoglucose isomerase; PGM, phosphoglycerate mutase; P_i, inorganic phosphate; PK, pyruvate kinase; PPP, pentose phosphate pathway; TIGAR, TP53-induced glycolysis and apoptosis regulator; TPI; triose phosphate isomerase; X-pY, phosphotyrosine-containing proteins.

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

apoptosis. It has been shown that hexokinases I and II are associated with mitochondria, binding the voltage-dependent anion channel on the mitochondrial outer membrane. Hexokinase binding to voltage-dependent anion channel is thought to be dependent on both glycolytic flux and AKT activity, although the former is both necessary and sufficient for its anti-apoptotic activity (84,85). This effect may well be mediated through the pro-apoptotic family members, Bax and Bak, which are unable to bind voltage-dependent anion channel when hexokinase is present (86). The binding and insertion of these proteins into the mitochondrial outer membrane leads to cytochrome c release and apoptotic cell death. Therefore, high flux through the glycolytic pathway, as observed in tumours, retains hexokinase on mitochondria and inhibits apoptosis.

The next step in glycolysis is the isomerization of G6P to fructose 6-phosphate (F6P) by phosphoglucose isomerase (PGI, Figure 2). This protein was independently identified as a secreted factor that positively regulates the motility of tumour cells and was therefore named autocrine motility factor (87). The expression of phosphoglucoisomerase/autocrine motility factor has been reported to be increased under hypoxia, and it has been found over-expressed alongside its extracellular receptor, autocrine motility factor receptor, in a wide varieties of cancers and associated with malignant progression (87–93). Its expression in 3T3 fibroblasts is associated with the resistance of cells to growth factor and nutrient deprivation and increased motility (94).

A major regulatory protein in glycolysis, which is also a HIF1 and Myc target is phosphofructokinase 1 (PFK1, Figure 2). This enzyme is under elaborate control, allowing metabolic intermediates to be diverted into pathways other than glycolysis [e.g. the pentose phosphate pathway (PPP)], as well as increasing or decreasing the rate of glycolysis depending on the energy status of the cell. Importantly, despite being a substrate for PFK1, ATP is a potent inhibitor of its activity. This is probably the most important mechanism by which OXPHOS regulates glycolysis (Pasteur Effect). A potent allosteric activator of PFK1 is fructose 2,6-bisphosphate. This metabolite is produced and held at steady-state levels by the action of a bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK2/FBPase, Figure 2). The form of PFK2/FBPase that is highly expressed in tumours, PFKBP3, has a net kinase activity, and therefore favours synthesis of fructose 2,6-bisphosphate, hence increased glycolytic flux. The increased level of PFKBP3 in tumours has been suggested as a contributory mechanism for aerobic glycolysis (95,96). This form is strongly induced by HIF1 (96), and its activity enhanced by both adenosine monophosphate-activated kinase in response to energetic stress and by the oncogene Ras (97–99). Furthermore, a p53-responsive gene product, TP53-induced glycolysis and apoptosis regulator (TIGAR) can also indirectly influence glycolysis and modulate p53-mediated cellular responses (100). TIGAR was found to have similarities with the bisphosphatase2 domain of PFK2/FBPase and its expression was found to inhibit glycolysis, presumably through the reduction of fructose 2,6-bisphosphate levels (Figure 2). It is also interesting to note that, with the prevalence of p53 inactivation in tumours, TIGAR was also found to be expressed in a p53-independent manner (100), and therefore may prove to have a role more generally in the survival and proliferation of transformed cells.

Another glycolytic enzyme whose levels can be altered by p53 expression is phosphoglycerate mutase (PGM) (101). In cells with high p53 expression, PGM expression is reduced, but loss of function or low levels of p53 allows increased PGM and hence glycolysis. PGM catalyses the conversion of 3-phosphoglycerate to 2-phosphoglycerate (Figure 2) and constitutes the only glycolytic enzyme whose levels are not affected by HIF1 or Myc (79,102). Interestingly, over-expression of PGM can immortalize mouse embryonic fibroblast (MEFs): a phenotype that is dependent upon its catalytic activity. The correlation between the rate of glycolysis and immortalization was strengthened by two further strands of evidence: that inhibition of a number of glycolytic enzymes [PGM, PGI, glyceraldehyde 3-phosphate dehtdrogenase (GAPDH) and phosphoglycerate kinase (PGK)] can trigger MEF senescence and that spontaneously immortalized MEFs also increase their glycolytic rate (101). The link between cellular lifespan and glycolytic rate requires much work.

The final enzyme in glycolysis is pyruvate kinase (PK). This enzyme is also under complex control, allowing the cell to sense the levels of anabolic precursors as well as the energy status of the cell (Figure 2). In addition to this, there is a further layer of control of PK at the level of isoform usage by tumours. PK has four isoforms expressed in mammalian tissues: L and R, which are found in liver and blood cells; M1, which is found in most other adult tissues and M2, expressed in foetal tissues and tumours (103). M2 is a splice variant of M1 that is necessary for the tumourigenicity of tumour cell lines and xenografts (104). M2 is found in a low activity dimeric form and a highly active tetrameric form. In tumours, it is the low activity form that is prevalent (105) and is induced by phosphorylation downstream of oncoproteins such as $pp60^{v\text{-}src}$ (106). More recently, PK-M2 has also been shown to be a phosphotyrosine-binding protein (107), the site of binding being the same as that bound by the allosteric activator, fructose 1,6-bisphosphate (Figure 2). The result of phosphotyrosine binding is therefore the inhibition of PK activity and build-up of phopho-sugar metabolites that can be used in anabolic reactions. Interestingly, when Christofk *et al.* measured PK-M2 activity after binding phosphotyrosine-containing proteins, phosphopeptides derived from lactate dehydrogenase and enolase (other glycolytic enzymes) were among the most inhibitory. $pp60^{v\text{-}src}$ has also been found capable of phosphorylating enolase and lactate dehydrogenase (108,109), but this has not previously been shown to affect enzyme activity. Perhaps in light of the phosphotyrosine-binding ability of PK-M2, further study should be made to elucidate the regulation of pyruvate and lactate production by tyrosine phosphorylation.

Pyruvate, the end-product of glycolysis, can take one of several metabolic processes, the major two being its conversion to lactate or acetyl-CoA (Figures 2 and 3A). Pyruvate dehydrogenase (PDH) catalyses the conversion of pyruvate to acetyl-CoA in the mitochondrion. This is the enzymatic step linking glycolysis to the TCA cycle and ATP production by OXPHOS (Figure 3A). PDH sits in a complex of enzymes known as the PDH complex, all of which act on PDH to control its activity. One of four PDKs and one of two PDH phosphatases are associated with PDH and regulate the phosphorylation of the E1α subunit, dictating PDH activity. When phosphorylated, PDH is inactivated, resulting in channelling of pyruvate to lactate, whereas the non-phosphorylated form is active and converts pyruvate into mitochondrial acetyl-CoA. PDK1 is a direct target of HIF1, and therefore hypoxia and/or activation of various oncogenes can act to inhibit the entry of pyruvate into the mitochondrion (48,110). The inhibition of PDK is increasingly becoming seen as a valid target for cancer therapy (111,112).

The conversion of pyruvate to lactate appears important for the maintenance of tumour cell viability. This is carried out by lactate dehydrogenase (Figures 2 and 3A), of which the A isoform is strongly upregulated in tumours. Lactate production is important for the recycling of cytosolic nicotinamide adenine dinucleotide (NAD^+) in the absence of functional mitochondrial-cytoplasmic NADH (the reduced form of NAD^+) shuttles due to decreased OXPHOS (Figure 3B). The regeneration of cytosolic NAD^+ is vital for efficient glycolysis. In studies carried out by Fantin *et al.* (113), lactate dehydrogenase A suppression not only pushed cells towards a more OXPHOS phenotype but also slowed their proliferation *in vitro*, and in an *in vivo* model of breast cancer almost tripled the survival of mice compared with an lactate dehydrogenase A expressing control.

The pentose phosphate pathway

In order to sustain the rapid proliferation characteristic of tumours, increased synthesis of both fatty acids and nucleotide precursors must occur. A mechanism used by cells to support this is the diversion of glycolytic intermediates into the PPP, either from G6P (using the oxidative arm) or from fructose 6-phosphate (using the non-oxidative arm). These intermediates can then be used to reduce nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH (from the

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

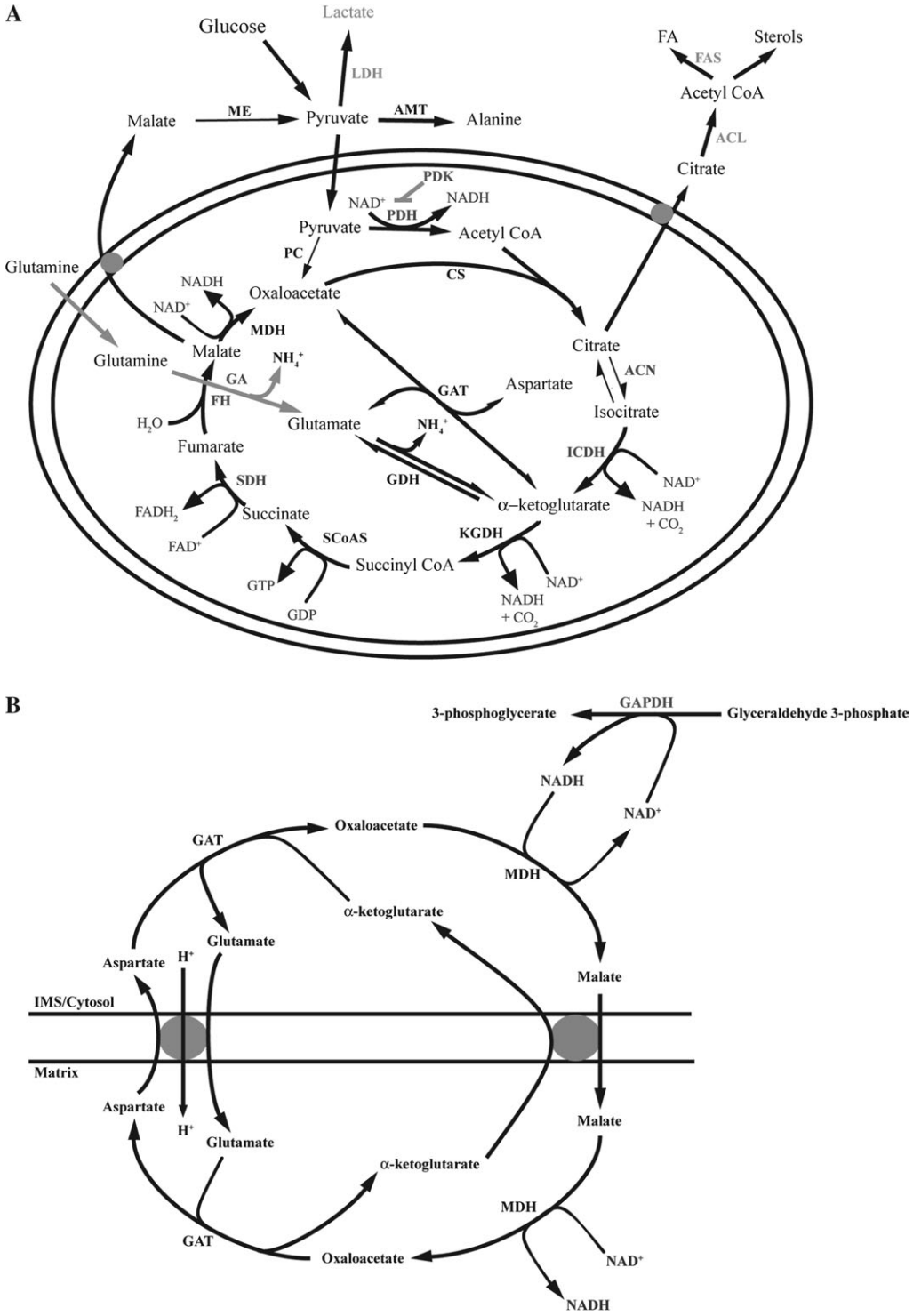

Fig. 3. (**A**) TCA cycle and cancer. (**B**) The malate/aspartate shuttle. This process is used to transfer electrons from the cytosolic NADH pool to the mitochondria to be oxidized by the ETC. Green text—enhanced/activated in cancer. Red text—reduced/inhibited in cancer. ACL, adenosine triphosphate citrate lyase; ACN, aconitase; AMT, aminomethyl transferase; CS, citrate synthase; FAD, flavin adenine dinucleotide; FAS, fatty acid synthase; GA, glutaminase; GAT; glutamate acetyltransferase; GDH, glutamate dehydrogenase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; ICDH, isocitrate dehydrogenase; IMS, intermembrane space; KGDH, α-ketoglutarate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; PC, pyruvate carboxylase; SCoAS; succinyl CoA synthase.

oxidative arm only) and synthesize ribose 5-phosphate (Figure 2). The control of glycolysis by PFK2/FBPase and TIGAR (as mentioned earlier) has the ability to divert substrates into the oxidative arm of the PPP. Increasing PFK2/FBPase phosphatase activity or inhibiting PFK1 by some other means (such as increase in ATP or citrate) will therefore increase PPP activity and support rapid cellular proliferation. The diversion of G6P into the PPP flow not only has the capacity to increase nucleotide biosynthesis but also increase the antioxidant capacity of the cell due to the generation of NADPH required for the reduction of oxidized glutathione. In this respect, the acceleration of the

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

PPP after DNA damage, or during tumorigenesis in general, may prove important, as it provides much of the necessary equipment with that to replicate and repair the DNA. NADPH generated by the oxidative PPP also supports fatty acid biosynthesis required for tumour growth (see below). Interestingly, the first two enzymes in this pathway, G6P dehydrogenase and 6-phosphogluconate dehydrogenase are also up-regulated in transformed cells (114).

Fatty acid synthesis

Proliferating cells in general and cancer cells in particular require *de novo* synthesis of lipids for membrane assembly. Under conditions where PDH is not inhibited, pyruvate is converted into acetyl-CoA and enters the TCA cycle by condensing with oxaloacetate to form citrate (Figure 3A). This intermediate is mostly further oxidized in the TCA cycle to produce reducing potential for the mitochondrial ETC, but can also be used for fatty acid synthesis in the cytosol. Cytosolic citrate is converted back into oxaloacetate and acetyl-CoA by the action of ATP citrate lyase. The reduction in levels or activity of any of the three enzymes involved in fatty acid synthesis has been shown to inhibit tumour growth and may therefore represent a target for tumour therapies (115,116). Interestingly, activation of AKT has been found to inhibit the β oxidation (degradation) of lipids by inhibiting the expression of carnitine palmitoyltransferase (CPT1A) (117). This further support the anabolic reprogramming observed in tumorigenesis and their push towards increased proliferation.

Glutaminolysis

There are two major sources of energy and carbon for cancer cells: glucose and glutamine (4,80,118,119). Cancer cells appear to use excessive amounts of both nutrients: more than they need for their function (120). One possible explanation is that high rates of flux through these metabolic pathways can affect the regulation of other metabolic branches, allowing high rates of proliferation (121). A consequence of this excess is the increased secretion of by-products of glucose and glutamine degradation, mainly lactate, alanine and ammonium (Figure 3A) (120). It has been recently proposed that in this context, glucose accounts mainly for lipid and nucleotide synthesis, whereas glutamine is responsible for anaplerotic re-feeding of the TCA cycle, for amino acid synthesis and for nitrogen incorporation into purine and pyrimidine for nucleotide synthesis (120). Glycolysis is capable of re-feeding the TCA cycle in the presence of functional pyruvate carboxylase (Figure 3A). In light of the rapid growth and proliferation of tumour cells, catabolic reactions are unlikely to be used to feed the TCA cycle, predicting that in order for cells to efficiently use glutamine for anabolic reactions, at least some pyruvate must enter the TCA cycle, instead of being converted to lactate.

Once in the cell, glutamine is initially deaminated to form glutamate, a process catalysed by the enzyme glutaminase (Figure 3A). Glutamate in turn can be converted into α-ketoglutarate either by a second deamination process catalysed by the enzyme glutamate dehydrogenase or through transamination. On entering the TCA cycle, α-ketoglutarate is metabolized to eventually generate oxaloacetate, an important anabolic precursor that will condense with the acetyl-CoA generated from glycolysis or glutaminolysis to produce citrate. The importance of glutaminolysis in cancer metabolism is evident from the considerable release of ammonium in the *de venous* effluent of cancer patients, and by the fact that, with time, the majority of patients develop glutamine depletion (122,123). In fact, glutaminase has been found to be over-expressed in a variety of tumour models and human malignancies, and the rate of glutaminase activity correlates with the rate of tumour growth (124). Unfortunately, despite promising signs in leukaemic mouse models, mammary tumours and colon carcinoma, therapeutic strategies designed to limit the availability of glutamine to cancer cells with inhibitors of glutaminase (6-diazo-5-oxo-L-norleucine or acividin) failed due to severe side effects during clinical trials (125,126). However, better knowledge of the biochemical and regulatory processes of glutamine uptake and degradation in normal and cancer cells could constitute a major goal in designing new strategies against cancer.

The TCA cycle and OXPHOS

Mitochondrial citrate not exported for anabolic use is used in the TCA cycle to produce reducing equivalents for the ETC (Figure 3A). Two of the enzymes in this pathway succinate dehydrogenase (SDH) and fumarate hydratase (FH) are of particular importance for cancer. SDH is also complex II of the ETC, where reduced flavine adenine dinucleotide ($FADH_2$) is generated and further oxidized. It consists of four subunits: A and B, which are associated with the inner leaflet of the mitochondrial inner membrane and C and D, which are embedded in the mitochondrial inner membrane. Although their function is vital for the normal working of the TCA cycle, mutations in either FH or SDHB, SDHC or SDHD are known causes of a number of familial and sporadic cancers, namely leiomyoma, leiomyosarcoma or renal cell carcinoma (FH), paraganglioma and pheochromocytoma (SDHB, SDHC and SDHD). For a review, please refer to Gottlieb *et al.* (127). Phenotypically, all of these mutations result in pseudohypoxia, referring to the normoxic induction of HIFα subunits (128,129). Mechanistically, it has been shown that the increase in succinate (SDH mutations) or fumarate (FH mutations) levels is responsible for inactivation of the PHDs even in the presence of O_2, leading to the normoxic stabilization of HIFα and up-regulation of its downstream effectors (Figure 1) (130–132). As discussed earlier, mitochondrial ROS can be produced from both complexes I and III of the ETC under hypoxia. However, it has been suggested that SDHB, SDHC and SDHD mutations can also result in normoxic ROS production and HIF activation (133), though the role of ROS in pseudohypoxia of SDH-deficient cells is debatable (134).

The role of mitochondrial ETC in tumorigenesis is not well understood. In normal cells, mitochondria use reducing potential generated by glycolysis and the TCA cycle to form a proton gradient across the mitochondrial inner membrane, which is then used to drive the synthesis of ATP. The ETC consists of four complexes, which are used to pass electrons from reduced NADH (complex I) or $FADH_2$ (complex II) ultimately to O_2, the terminal electron acceptor at complex IV (cytochrome c oxidase; COX). In doing so, complexes I, III and IV pump hydrogen ions against their concentration gradient, forming the mitochondrial membrane potential. In normal cells, the ETC is an extremely efficient system for passing electrons safely from reduced species through to the terminal acceptor, O_2: it is estimated that ∼1 to 2% of the O_2 consumed during respiration is used aberrantly in the formation of ROS (135). Indeed, it has been shown that in isolated mitochondria, the efficiency of ETC and low ROS production are retained (136). Interestingly, this was achieved regardless of the O_2 levels, arguing against a mitochondrial ROS-signalling mechanism under hypoxia (Discussed above). In whole cells, when mitochondrial ROS are produced, they can easily damage components of this organelle, especially mitochondrial lipids, mitochondrial DNA (mtDNA), enzymes of the TCA cycle and proteins within the mitochondrial inner and outer membranes. mtDNA in particular is a frequent target of ROS damage. The few gene products encoded by the mtDNA are all vital for mitochondrial ATP production, therefore ROS-mediated damage can severely affect the efficiency of OXPHOS in the cell and presumably contribute to the aerobic glycolysis phenotype. Damage of the mtDNA will also be passed down to progeny cells, so during tumorigenesis, progressive loss of mitochondrial ATP generation would lead to the loss of the ability of the tumour to use OXPHOS to generate ATP. However, the multiplicity of mtDNA within mitochondria, and the exchange of mitochondrial contents by fission and fusion events within a cell, ensure by complementation that OXPHOS will prevail despite redox stress. Therefore, progressive loss of mitochondrial function would impact on a cell only gradually, unless something global was to inhibit mitochondrial OXPHOS, such as a change in nuclear gene expression (e.g. SDH mutation). The TCA cycle enzyme, aconitase, is also frequently inactivated by ROS. This would result in the inhibition of the right hand side of the TCA cycle, allowing glutaminolytic, but not glycolytic products to be used for OXPHOS (Figure 3A). The result of this would be to make cells more dependent upon glutaminolysis for

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

mitochondrial ATP production (if the ETC was still properly functioning).

Recent work has indicated a role for p53 in the regulation of mitochondrial function during tumorigenesis. The gene-encoding synthesis of cytochrome c oxidase 2 is induced in a p53-dependent manner, and this along with synthesis of cytochrome c oxidase 1 is necessary for assembly of functional COX (137). Hence in tumours with non-functional p53 (majority of tumours), the ETC is compromised, and glycolysis is the more efficient mechanism for generation of ATP. A further level of regulation of COX is also carried out in a HIF1-dependent manner (138). Under normoxic conditions, COX4-1 subunit is expressed constitutively. Activation of the HIF1 transcription factor results in the up-regulation of the mitochondrial protease, LON, and the COX4-2 subunit. LON (the mammalian homologue of a bacterial ATP-dependent protease), acts by degrading COX4-1, leaving COX4-2 to replace it. This work also suggests that when these subunits were aberrantly expressed an increase in ROS levels and in cell death-activating proteins was observed.

Amino acids and their transporters

To sustain high proliferation rates, cancer cells are extremely dependent on extra-energy and nutrient supply. Therefore, nutrient uptake and metabolism are frequently altered and enhanced in tumour cells (139). Amino acids are the primary source of cellular nitrogen. In addition to being the building blocks for protein synthesis, they are used for nucleotide and glutathione synthesis, and the carbon backbone can also be used for ATP synthesis. Moreover, amino acids have an important role in regulating signalling pathways that govern cell growth and survival. Many human tumour cells express high levels of amino acid transporters, and this correlates with disease progression (140). A notable example is the alanine serine cysteine transporter 2 (ASCT2) transporter, a non-specific neutral amino acid transporter that functions as the major transporter of glutamine in numerous cell lines. Given that glutamine has a key role in tumour cell metabolism (discussed above) and that glutamine transport is increased in tumour cells (141), it is not surprising that ASCT2 expression is also enhanced during tumour development. ASCT2 expression is enhanced in breast, liver and brain tumours, and inhibition of ASCT2-dependent glutamine transport inhibited the growth of colon carcinoma cell lines (reviewed in ref. 140). Moreover, silencing of the ASCT2 messenger RNA transcript causes dramatic apoptosis in hepatoma cells and this appears to occur in parallel with its role in glutamine uptake (142,143). Enhanced expression of L-type amino acid transporter (LAT1), another amino acid transporter with high affinity for several essential amino acids including leucine, tryptophan and methionine, has been reported in high-grade astrocytomas and correlates with poor survival. LAT1 inhibition has been shown to block glioma cell growth in both *in vitro* and *in vivo* models (144). These findings highlight the growth advantage conferred to tumour cells by increased amino acid transporter expression and point to a potential role for amino acid transporter inhibition as a therapeutic strategy.

Mammalian target of rapamycin

As previously mentioned, cells strictly depend on nutrient availability and growth stimuli to sustain growth and proliferation. The regulation of these stimuli is integrated by target of rapamycin (TOR), a highly evolutionarily conserved mechanism, present from unicellular eukaryotes to mammals. In mammals, mTOR is involved in four different sensing mechanisms: growth factor signalling; nutrient availability; oxygen availability and internal energetic status. All four factors are especially important for tumour development. Solid tumours can become limited for nutrient, oxygen and growth factors, a situation potentially leading to energetic limitations inside the cancer cell. Therefore, de-regulation of the molecular process that controls these mechanisms could be critical for tumour development.

mTOR has been described to function in two different complexes (Figure 4). The first complex, named mTOR complex 1 (mTORC1), controls protein synthesis and cell cycle progression. The core of this complex is formed by mTOR, Raptor (regulatory associated protein of TOR), mLST8 (mammalian Lethal with Sec-13) and PRAS40 (proline-rich AKT substrate 40). A compound originally isolated from *Streptomyces hygroscopicus* called rapamycin can inhibit mTORC1 by binding to FKBP12 (FK506-binding protein 12) resulting in the dissociation of Raptor from the complex (145). Upon this core, other proteins can act as effectors to regulate the kinase activity of the mTORC1; Rheb (Ras homologue enriched in brain) and more recently, Rag (Ras-related guanosine triphosphate-binding protein) being the most significant ones (146–148). In response to nutrients and growth signalling, mTORC1 phosphorylates and activates S6K and 4E-BP1, both regulators of messenger RNA translation (149).

The second complex, mTORC2 is insensitive to rapamycin and consists of mTOR, mLST8, Rictor (rapamycin-insensitive companion of mTOR) and mSIN1. mTORC2 has so far been less well characterized, but current data point to possible roles in proliferation and regulation of the cytoskeleton and AKT phosphorylation and activation (150,151). Although mTORC2 is described as rapamycin insensitive, it has been shown that prolonged rapamycin exposure can result in AKT down-regulation due to the inhibition of mTORC2 (152). This feeds back to mTORC1 which acts downstream of AKT (Figure 4).

In contrast to the lack of knowledge of mTORC2 regulation, the mechanisms whereby growth factors regulate mTORC1 activity have received much attention during the last decade. Upstream of mTORC1 is TSC1/2, an inhibitor of mTORC1 kinase activity. TSC1/2 could be considered a 'reception centre' for the different stimuli which are transduced to mTORC1, including growth factor signalling through AKT and extracellular signal-regulated kinase pathways, hypoxia through the HIF1 target, REDD1 and energy status through adenosine monophosphate-activated kinase (153). Some amino acids and their transporters can also regulate mTOR activity (147,148,154). Suppression of the ASCT2 amino acid transporter in hepatoma cells inhibits mTOR signalling and represses cell growth (155). A further role that mTOR appears to have is in the trafficking and maintenance of amino acid transporters on the plasma membrane (156,157).

Interestingly, despite the importance of glutamine from an energetic point of view, leucine is the amino acid that seems to be necessary and sufficient for mTORC1 activation. Unlike glutamine, leucine is an essential amino acid and only found in low levels in the plasma. Interestingly however, the connection between these two amino acids could rise from the positive regulation that leucine exerts upon glutamate dehydrogenase, the enzyme responsible for the deamination of glutamate to generate α-ketoglutarate. A number of studies of disease states such as hyperinsulinaemia and hyperamonaemia have found that leucine could be an important regulator of glutaminolysis (158–161), and therefore as a regulator of the anaplerotic re-feeding of α-ketoglutarate into the TCA cycle, thus explaining the importance of leucine in activating mTORC1.

mTOR is believed to play a critical role in tumorigenesis. mTORC1 activation by Rheb over-expression or the loss of the TSC1/2 complex is able to drive tumorigenesis, modulating apoptosis, cellular senescence and response to treatment (162–164), providing a possible explanation for the finding that Rheb is over-expressed in some human lymphomas and possibly other cancers (165). mTOR also acts downstream of the PI3K/AKT signal transduction pathway, and by inhibiting TSC1/2, AKT also controls mTOR activity (Figure 4) (166). This is considered the main link between mTOR and tumourigenesis, as rapamycin treatment can alleviate tumour phenotype in some activated AKT models (167). PTEN-deficient cancer cells exhibit constitutive activation of AKT and mTORC1 and present an aggressive phenotype with poor prognosis (168). However, no mutations of mTOR have been described in cancer thus far, and it is still controversial whether mTOR on its own, in the absence of AKT activation, can promote tumorigenesis.

As described previously, activated mTORC1 promotes phosphorylation and activation of messenger RNA translation regulators S6K and 4E-BP1 (Figure 4). This obvious benefit for cancer development is not the only proposed connection between mTOR and cancer. Downstream of mTOR is a potential major target of cancer therapy:

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

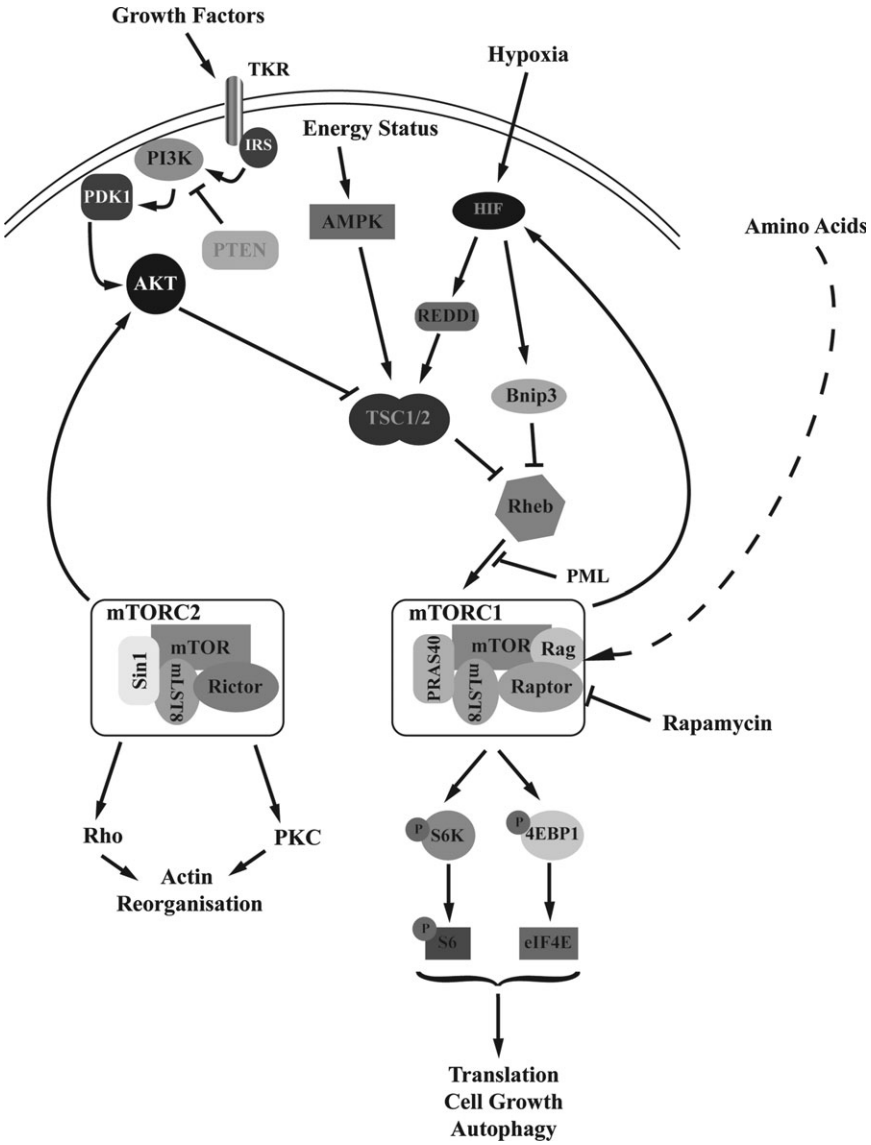

Fig. 4. Pathways involved in the regulation of mTOR. 4EBP1, 4E-binding protein 1; AMPK, AMP kinase; Bnip3, Bcl-2/E1B 19 kDa-interacting protein; eIF4E, elongation and initiation factor 4E; mLST8, mammalian lethal with Sec-13; mTOR; mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; PKC, protein kinase C; PDK1, phosphoinositol-dependent kinase; PI-3K, phosphatidylinositol-3 kinase; PML, promyeloid leukaemia; PRAS40, proline-rich AKT substrate of 40 kDa; PTEN, phosphatase and tensin homologue; Rag, Ras-related GTP-binding protein; Raptor, regulatory associated protein of mTOR; REDD1, regulated in development and DNA damage 1; Rheb, Ras homologue enriched in brain; Rho, Ras homologue; Rictor, rapamycin-insensitive companion of mTOR; S6K, S6 kinase; Sin1, stress-activated protein kinase interacting protein 1; TSC, tuberosclerosis complex.

HIF1, the transcriptional factor responsible for metabolic and angiogenic changes mediated by hypoxia in tumours. Expression of HIF1α has been proposed to be dependent on mTOR activity, probably through the activation of S6K. As a consequence, inhibition of mTOR by rapamycin also leads to an inhibition of HIF expression (167,169). Additionally, the link between mTORC1 and amino acid transporter regulation could also contribute a growth advantage by enhancing the nutrient uptake and availability for metabolism (155). Despite the clear connection between AKT/PTEN and mTOR in tumorigenesis, clinical trials using rapamycin and its analogues on PTEN-deficient cancer produced only modest results. This may be due to the negative feedback between S6K and AKT (170). Activation of S6K by mTOR leads to the phosphorylation and inhibition of the insulin receptor substrate and therefore, inhibition of AKT (171). mTORC1 inhibition with rapamycin treatment would therefore cause AKT reactivation due to the loss of S6K activity, which may account for poor results in clinical trials (172). Given the ability of mTORC2 to activate AKT, it could be hypothesized that specifically inhibiting mTORC2 or both mTOR complexes would generate a better outcome. At present, there are several rapamycin analogues in clinical trials.

Summary

The extent to which metabolism plays a role in tumorigenesis should not be underestimated, and drugs that can selectively target the metabolic phenotype of the tumour and its environment are likely to at least delay, if not halt tumour progression. The resistance of tumours to both radiotherapy and chemotherapy can often be attributed to its aberrant metabolism. It therefore follows that the reactivation of a more 'normal' metabolism could very well re-sensitize tumours to these agents. Cell metabolism is inextricably linked to its differentiated state: if we can reverse the metabolism of a de-differentiated, aggressive tumour to that of a more quiescent state it may become more amenable to other interventions. Therapies that target tumour

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

metabolism are already being tested in pre-clinical and clinical studies, but this field is very much in its infancy. It is anticipated that the next few years will provide more new therapeutic approaches that target metabolic transformation.

Funding

All authors were supported by Cancer Research, UK.

Acknowledgements

We would like to thank Mary Selak and Christian Frezza for their critical reading and constructive discussion of the manuscript.

Conflict of Interest Statement: None declared.

References

1. Frezza,C. *et al*. (2009) Mitochondria in cancer: not just innocent bystanders. *Semin. Cancer Biol.*, **19**, 4–11.
2. Warburg,O. (1923) Metabolism of tumours. *Biochem. Z.*, **142**, 317–333.
3. Warburg,O. (1956) On respiratory impairment in cancer cells. *Science*, **124**, 269–270.
4. Warburg,O. (1956) On the origin of cancer cells. *Science*, **123**, 309–314.
5. Pasteur,L. (1861) Expériences et vues nouvelles sur la nature des fermentations. *C. R. Acad. Sci.*, **52**, 344–347.
6. Crabtree,H. (1928) The carbohydrate metabolism of certain pathological growths. *Biochem. J.*, **22**, 1289–1298.
7. Kondoh,H. *et al*. (2007) A high glycolytic flux supports the proliferative potential of murine embryonic stem cells. *Antioxid. Redox Signal.*, **9**, 293–299.
8. Gatenby,R.A. *et al*. (2007) Glycolysis in cancer: a potential target for therapy. *Int. J. Biochem. Cell Biol.*, **39**, 1358–1366.
9. Dewhirst,M.W. (1998) Concepts of oxygen transport at the microcirculatory level. *Semin. Radiat. Oncol.*, **8**, 143–150.
10. Rofstad,E.K. *et al*. (2007) Fluctuating and diffusion-limited hypoxia in hypoxia-induced metastasis. *Clin. Cancer Res.*, **13**, 1971–1978.
11. Wang,G.L. *et al*. (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc. Natl Acad. Sci. USA*, **92**, 5510–5514.
12. Tian,H. *et al*. (1997) Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev.*, **11**, 72–82.
13. Maynard,M.A. *et al*. (2007) Dominant-negative HIF-3 alpha 4 suppresses VHL-null renal cell carcinoma progression. *Cell Cycle*, **6**, 2810–2816.
14. Bruick,R.K. *et al*. (2001) A conserved family of prolyl-4-hydroxylases that modify HIF. *Science*, **294**, 1337–1340.
15. Hirsila,M. *et al*. (2003) Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. *J. Biol. Chem.*, **278**, 30772–30780.
16. Epstein,A.C. *et al*. (2001) *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell*, **107**, 43–54.
17. Huang,J. *et al*. (2002) Sequence determinants in hypoxia-inducible factor-1alpha for hydroxylation by the prolyl hydroxylases PHD1, PHD2, and PHD3. *J. Biol. Chem.*, **277**, 39792–39800.
18. Hewitson,K.S. *et al*. (2003) The role of iron and 2-oxoglutarate oxygenases in signalling. *Biochem. Soc. Trans.*, **31**, 510–515.
19. Maxwell,P.H. *et al*. (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*, **399**, 271–275.
20. Cockman,M.E. *et al*. (2000) Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J. Biol. Chem.*, **275**, 25733–25741.
21. Lando,D. *et al*. (2002) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev.*, **16**, 1466–1471.
22. McNeill,L.A. *et al*. (2002) Hypoxia-inducible factor asparaginyl hydroxylase (FIH-1) catalyses hydroxylation at the beta-carbon of asparagine-803. *Biochem. J.*, **367**, 571–575.
23. Lando,D. *et al*. (2002) Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science*, **295**, 858–861.
24. Berra,E. *et al*. (2003) HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. *EMBO J.*, **22**, 4082–4090.
25. Appelhoff,R.J. *et al*. (2004) Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J. Biol. Chem.*, **279**, 38458–38465.
26. Ladroue,C. *et al*. (2008) PHD2 mutation and congenital erythrocytosis with paraganglioma. *N. Engl. J. Med.*, **359**, 2685–2692.
27. Lee,S. *et al*. (2005) Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. *Cancer Cell*, **8**, 155–167.
28. Schlisio,S. *et al*. (2008) The kinesin KIF1Bbeta acts downstream from EglN3 to induce apoptosis and is a potential 1p36 tumor suppressor. *Genes Dev.*, **22**, 884–893.
29. Koditz,J. *et al*. (2007) Oxygen-dependent ATF-4 stability is mediated by the PHD3 oxygen sensor. *Blood*, **110**, 3610–3617.
30. Koong,A.C. *et al*. (1994) Hypoxia causes the activation of nuclear factor kappa B through the phosphorylation of I kappa B alpha on tyrosine residues. *Cancer Res.*, **54**, 1425–1430.
31. Cummins,E.P. *et al*. (2006) Prolyl hydroxylase-1 negatively regulates IkappaB kinase-beta, giving insight into hypoxia-induced NFkappaB activity. *Proc. Natl Acad. Sci. USA*, **103**, 18154–18159.
32. Discher,D.J. *et al*. (1998) Hypoxia regulates beta-enolase and pyruvate kinase-M promoters by modulating Sp1/Sp3 binding to a conserved GC element. *J. Biol. Chem.*, **273**, 26087–26093.
33. Kaluz,S. *et al*. (2003) Expression of the hypoxia marker carbonic anhydrase IX is critically dependent on SP1 activity. Identification of a novel type of hypoxia-responsive enhancer. *Cancer Res.*, **63**, 917–922.
34. Couvelard,A. *et al*. (2008) Overexpression of the oxygen sensors PHD-1, PHD-2, PHD-3, and FIH Is associated with tumor aggressiveness in pancreatic endocrine tumors. *Clin. Cancer Res.*, **14**, 6634–6639.
35. Chavez,J.C. *et al*. (2000) Expression of hypoxia-inducible factor-1alpha in the brain of rats during chronic hypoxia. *J. Appl. Physiol.*, **89**, 1937–1942.
36. Ginouves,A. *et al*. (2008) PHDs overactivation during chronic hypoxia ''desensitizes'' HIFalpha and protects cells from necrosis. *Proc. Natl Acad. Sci. USA*, **105**, 4745–4750.
37. Dewhirst,M.W. (2007) Intermittent hypoxia furthers the rationale for hypoxia-inducible factor-1 targeting. *Cancer Res.*, **67**, 854–855.
38. Brunelle,J.K. *et al*. (2005) Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. *Cell Metab.*, **1**, 409–414.
39. Chandel,N.S. *et al*. (1998) Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc. Natl Acad. Sci. USA*, **95**, 11715–11720.
40. Chandel,N.S. *et al*. (2000) Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. *J. Biol. Chem.*, **275**, 25130–25138.
41. Guzy,R.D. *et al*. (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metab.*, **1**, 401–408.
42. Mansfield,K.D. *et al*. (2005) Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation. *Cell Metab.*, **1**, 393–399.
43. DeHaan,C. *et al*. (2004) Mutation in mitochondrial complex I ND6 subunit is associated with defective response to hypoxia in human glioma cells. *Mol. Cancer*, **3**, 19.
44. Wang,W. *et al*. (2008) Superoxide flashes in single mitochondria. *Cell*, **134**, 279–90.
45. Hagen,T. *et al*. (2003) Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha. *Science*, **302**, 1975–1978.
46. Semenza,G.L. (2003) Targeting HIF-1 for cancer therapy. *Nat. Rev. Cancer*, **3**, 721–732.
47. Semenza,G.L. *et al*. (1996) Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J. Biol. Chem.*, **271**, 32529–32537.
48. Kim,J.W. *et al*. (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.*, **3**, 177–185.
49. Ullah,M.S. *et al*. (2006) The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. *J. Biol. Chem.*, **281**, 9030–9037.
50. Shimoda,L.A. *et al*. (2006) HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **291**, L941–L949.
51. Wykoff,C.C. *et al*. (2000) Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res.*, **60**, 7075–7083.
52. Hunt,T.K. *et al*. (2007) Aerobically derived lactate stimulates revascularization and tissue repair via redox mechanisms. *Antioxid. Redox Signal.*, **9**, 1115–1124.

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

53. Gatenby,R.A. *et al*. (2001) Mathematical models of tumour invasion mediated by transformation-induced alteration of microenvironmental pH. *Novartis Found. Symp.*, **240**, 85–96(discussion 96–99).
54. Gatenby,R.A. *et al*. (2003) The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models. *Cancer Res.*, **63**, 3847–3854.
55. Fischer,K. *et al*. (2007) Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood*, **109**, 3812–3819.
56. Liao,D. *et al*. (2007) Hypoxia-inducible factor-1alpha is a key regulator of metastasis in a transgenic model of cancer initiation and progression. *Cancer Res.*, **67**, 563–572.
57. Maxwell,P.H. *et al*. (1997) Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc. Natl Acad. Sci. USA*, **94**, 8104–8109.
58. Raval,R.R. *et al*. (2005) Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol. Cell. Biol.*, **25**, 5675–5686.
59. Ryan,H.E. *et al*. (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO J.*, **17**, 3005–3015.
60. Ryan,H.E. *et al*. (2000) Hypoxia-inducible factor-1alpha is a positive factor in solid tumor growth. *Cancer Res.*, **60**, 4010–4015.
61. Birner,P. *et al*. (2000) Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. *Cancer Res.*, **60**, 4693–4696.
62. Schindl,M. *et al*. (2002) Overexpression of hypoxia-inducible factor 1alpha is associated with an unfavorable prognosis in lymph node-positive breast cancer. *Clin. Cancer Res.*, **8**, 1831–1837.
63. Takahashi,R. *et al*. (2003) Hypoxia-inducible factor-1alpha expression and angiogenesis in gastrointestinal stromal tumor of the stomach. *Oncol. Rep.*, **10**, 797–802.
64. Semenza,G.L. (2002) Involvement of hypoxia-inducible factor 1 in human cancer. *Intern. Med.*, **41**, 79–83.
65. Kaelin,W.G. (2007) Von Hippel-Lindau disease. *Annu. Rev. Pathol.*, **2**, 145–173.
66. Zundel,W. *et al*. (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev.*, **14**, 391–396.
67. Flugel,D. *et al*. (2007) Glycogen synthase kinase 3 phosphorylates hypoxia-inducible factor 1alpha and mediates its destabilization in a VHL-independent manner. *Mol. Cell. Biol.*, **27**, 3253–3265.
68. Emerling,B.M. *et al*. (2008) PTEN regulates p300-dependent hypoxia-inducible factor 1 transcriptional activity through Forkhead transcription factor 3a (FOXO3a). *Proc. Natl Acad. Sci. USA*, **105**, 2622–2627.
69. Tang,T.T. *et al*. (2003) The forkhead transcription factor FOXO4 induces the down-regulation of hypoxia-inducible factor 1 alpha by a von Hippel-Lindau protein-independent mechanism. *J. Biol. Chem.*, **278**, 30125–30135.
70. Walmsley,S.R. *et al*. (2005) Hypoxia-induced neutrophil survival is mediated by HIF-1alpha-dependent NF-kappaB activity. *J. Exp. Med.*, **201**, 105–115.
71. Belaiba,R.S. *et al*. (2007) Hypoxia up-regulates hypoxia-inducible factor-1alpha transcription by involving phosphatidylinositol 3-kinase and nuclear factor kappaB in pulmonary artery smooth muscle cells. *Mol. Biol. Cell*, **18**, 4691–4697.
72. Rius,J. *et al*. (2008) NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature*, **453**, 807–811.
73. Hudson,C.C. *et al*. (2002) Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol. Cell. Biol.*, **22**, 7004–7014.
74. Hebert,C. *et al*. (2006) Hypoxia-inducible factor-1alpha polymorphisms and TSC1/2 mutations are complementary in head and neck cancers. *Mol. Cancer*, **5**, 3.
75. Bernardi,R. *et al*. (2006) PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. *Nature*, **442**, 779–785.
76. Dang,C.V. (1999) c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol. Cell. Biol.*, **19**, 1–11.
77. Adhikary,S. *et al*. (2005) Transcriptional regulation and transformation by Myc proteins. *Nat. Rev. Mol. Cell Biol.*, **6**, 635–645.
78. Kim,J.W. *et al*. (2007) Hypoxia-inducible factor 1 and dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. *Mol. Cell. Biol.*, **27**, 7381–7393.
79. Yeung,S.J. *et al*. (2008) Roles of p53, Myc and HIF-1 in regulating glycolysis—the seventh hallmark of cancer. *Cell. Mol. Life Sci.*, **65**, 3981–3999.
80. DeBerardinis,R.J. *et al*. (2008) The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab.*, **7**, 11–20.
81. Zhang,H. *et al*. (2007) HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. *Cancer Cell*, **11**, 407–420.
82. Macheda,M.L. *et al*. (2005) Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J. Cell. Physiol.*, **202**, 654–662.
83. Dang,C.V. *et al*. (1997) Oncogenes in tumor metabolism, tumorigenesis, and apoptosis. *J. Bioenerg. Biomembr.*, **29**, 345–354.
84. Gottlob,K. *et al*. (2001) Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. *Genes Dev.*, **15**, 1406–1418.
85. Majewski,N. *et al*. (2004) Hexokinase-mitochondria interaction mediated by Akt is required to inhibit apoptosis in the presence or absence of Bax and Bak. *Mol. Cell*, **16**, 819–830.
86. Pastorino,J.G. *et al*. (2002) Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome c release and apoptosis. *J. Biol. Chem.*, **277**, 7610–7618.
87. Yanagawa,T. *et al*. (2004) Novel roles of the autocrine motility factor/phosphoglucose isomerase in tumor malignancy. *Endocr. Relat. Cancer*, **11**, 749–759.
88. Maruyama,K. *et al*. (1995) Expression of autocrine motility factor receptor in human esophageal squamous cell carcinoma. *Int. J. Cancer*, **64**, 316–321.
89. Nakamori,S. *et al*. (1994) Expression of autocrine motility factor receptor in colorectal cancer as a predictor for disease recurrence. *Cancer*, **74**, 1855–1862.
90. Niizeki,H. *et al*. (2002) Hypoxia enhances the expression of autocrine motility factor and the motility of human pancreatic cancer cells. *Br. J. Cancer*, **86**, 1914–1919.
91. Takanami,I. *et al*. (1998) Autocrine motility factor in pulmonary adenocarcinomas: results of an immunohistochemical study. *Tumour Biol.*, **19**, 384–389.
92. Taniguchi,K. *et al*. (1998) The relation between the growth patterns of gastric carcinoma and the expression of hepatocyte growth factor receptor (c-met), autocrine motility factor receptor, and urokinase-type plasminogen activator receptor. *Cancer*, **82**, 2112–2122.
93. Yoon,D.Y. *et al*. (2001) Identification of genes differentially induced by hypoxia in pancreatic cancer cells. *Biochem. Biophys. Res. Commun.*, **288**, 882–886.
94. Tsutsumi,S. *et al*. (2003) Overexpression of the autocrine motility factor/phosphoglucose isomerase induces transformation and survival of NIH-3T3 fibroblasts. *Cancer Res.*, **63**, 242–249.
95. Atsumi,T. *et al*. (2002) High expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (iPFK-2; PFKFB3) in human cancers. *Cancer Res.*, **62**, 5881–5887.
96. Minchenko,A. *et al*. (2002) Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect. *J. Biol. Chem.*, **277**, 6183–6187.
97. Almeida,A. *et al*. (2004) Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. *Nat. Cell Biol.*, **6**, 45–51.
98. Kole,H.K. *et al*. (1991) Regulation of 6-phosphofructo-1-kinase activity in ras-transformed rat-1 fibroblasts. *Arch. Biochem. Biophys.*, **286**, 586–590.
99. Telang,S. *et al*. (2006) Ras transformation requires metabolic control by 6-phosphofructo-2-kinase. *Oncogene*, **25**, 7225–7234.
100. Bensaad,K. *et al*. (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell*, **126**, 107–120.
101. Kondoh,H. *et al*. (2005) Glycolytic enzymes can modulate cellular life span. *Cancer Res.*, **65**, 177–185.
102. Iyer,N.V. *et al*. (1998) Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev.*, **12**, 149–162.
103. Yamada,K. *et al*. (1999) Nutrient and hormonal regulation of pyruvate kinase gene expression. *Biochem. J.*, **337**, 1–11.
104. Christofk,H.R. *et al*. (2008) The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature*, **452**, 230–233.
105. Eigenbrodt,E. *et al*. (1992) Double role for pyruvate kinase type M2 in the expansion of phosphometabolite pools found in tumor cells. *Crit. Rev. Oncog.*, **3**, 91–115.
106. Presek,P. *et al*. (1988) Pyruvate kinase type M2 is phosphorylated at tyrosine residues in cells transformed by Rous sarcoma virus. *FEBS Lett.*, **242**, 194–198.
107. Christofk,H.R. *et al*. (2008) Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature*, **452**, 181–186.
108. Eigenbrodt,E. *et al*. (1983) Influence of transformation by Rous sarcoma virus on the amount, phosphorylation and enzyme kinetic properties of enolase. *EMBO J.*, **2**, 1565–1570.

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

109. Kamps,M.P. *et al*. (1986) Rous sarcoma virus transforming protein lacking myristic acid phosphorylates known polypeptide substrates without inducing transformation. *Cell*, **45**, 105–112.
110. Papandreou,I. *et al*. (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab.*, **3**, 187–197.
111. Bonnet,S. *et al*. (2007) A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell*, **11**, 37–51.
112. Pearson,H. (2007) Cancer patients opt for unapproved drug. *Nature*, **446**, 474–475.
113. Fantin,V.R. *et al*. (2006) Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell*, **9**, 425–434.
114. Board,M. *et al*. (1990) Maximum activities of key enzymes of glycolysis, glutaminolysis, pentose phosphate pathway and tricarboxylic acid cycle in normal, neoplastic and suppressed cells. *Biochem J.*, **265**, 503–509.
115. Hatzivassiliou,G. *et al*. (2005) ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell*, **8**, 311–321.
116. Pizer,E.S. *et al*. (1996) Inhibition of fatty acid synthesis delays disease progression in a xenograft model of ovarian cancer. *Cancer Res.*, **56**, 1189–1193.
117. Deberardinis,R.J. *et al*. (2006) Phosphatidylinositol 3-kinase-dependent modulation of carnitine palmitoyltransferase 1A expression regulates lipid metabolism during hematopoietic cell growth. *J. Biol. Chem.*, **281**, 37372–37380.
118. Curi,R. *et al*. (2005) Molecular mechanisms of glutamine action. *J. Cell. Physiol.*, **204**, 392–401.
119. Eagle,H. (1955) Nutrition needs of mammalian cells in tissue culture. *Science*, **122**, 501–514.
120. DeBerardinis,R.J. *et al*. (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc. Natl Acad. Sci. USA*, **104**, 19345–19350.
121. Crabtree,B. *et al*. (1985) A quantitative approach to metabolic control. *Curr. Top. Cell. Regul.*, **25**, 21–76.
122. Fischer,J.E. *et al*. (1990) Total parenteral nutrition, glutamine, and tumor growth. *JPEN J. Parenter. Enteral. Nutr.*, **14**, 86S–89S.
123. Souba,W.W. (1993) Glutamine and cancer. *Ann. Surg.*, **218**, 715–728.
124. Perez-Gomez,C. *et al*. (2005) Co-expression of glutaminase K and L isoenzymes in human tumour cells. *Biochem. J.*, **386**, 535–542.
125. Chance,W.T. *et al*. (1988) Insulin and acivicin improve host nutrition and prevent tumor growth during total parenteral nutrition. *Ann. Surg.*, **208**, 524–531.
126. Ovejera,A.A. *et al*. (1979) Efficacy of 6-diazo-5-oxo-L-norleucine and N-[N-gamma-glutamyl-6-diazo-5-oxo-norleucinyl]-6-diazo-5-oxo-norleucine against experimental tumors in conventional and nude mice. *Cancer Res.*, **39**, 3220–3224.
127. Gottlieb,E. *et al*. (2005) Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat. Rev. Cancer*, **5**, 857–866.
128. Gimenez-Roqueplo,A.P. *et al*. (2001) The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. *Am. J. Hum. Genet.*, **69**, 1186–1197.
129. Pollard,P.J. *et al*. (2005) Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. *Hum. Mol. Genet.*, **14**, 2231–2239.
130. Isaacs,J.S. *et al*. (2005) HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell*, **8**, 143–153.
131. Koivunen,P. *et al*. (2007) Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF. *J. Biol. Chem.*, **282**, 4524–4532.
132. Selak,M.A. *et al*. (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell*, **7**, 77–85.
133. Guzy,R.D. *et al*. (2008) Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol. Cell. Biol.*, **28**, 718–731.
134. Selak,M.A. *et al*. (2006) Redox stress is not essential for the pseudohypoxic phenotype of succinate dehydrogenase deficient cells. *Biochim. Biophys. Acta*, **1757**, 567–572.
135. Murphy,M.P. (2009) How mitochondria produce reactive oxygen species. *Biochem. J.*, **417**, 1–13.
136. Hoffman,D.L. *et al*. (2007) Response of mitochondrial reactive oxygen species generation to steady-state oxygen tension: implications for hypoxic cell signaling. *Am. J. Physiol. Heart Circ. Physiol.*, **292**, H101–H8.
137. Matoba,S. *et al*. (2006) p53 regulates mitochondrial respiration. *Science*, **312**, 1650–1653.
138. Fukuda,R. *et al*. (2007) HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell*, **129**, 111–122.
139. Baggetto,L.G. (1992) Deviant energetic metabolism of glycolytic cancer cells. *Biochimie*, **74**, 959–974.
140. Fuchs,B.C. *et al*. (2005) Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? *Semin. Cancer Biol.*, **15**, 254–266.
141. Bode,B.P. *et al*. (2002) Molecular and functional analysis of glutamine uptake in human hepatoma and liver-derived cells. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **283**, G1062–G1073.
142. Fuchs,B.C. *et al*. (2004) Inducible antisense RNA targeting amino acid transporter ATB0/ASCT2 elicits apoptosis in human hepatoma cells. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **286**, G467–G478.
143. McGivan,J.D. *et al*. (2007) The transport of glutamine into mammalian cells. *Front. Biosci.*, **12**, 874–882.
144. Nawashiro,H. *et al*. (2006) L-type amino acid transporter 1 as a potential molecular target in human astrocytic tumors. *Int. J. Cancer*, **119**, 484–492.
145. Oshiro,N. *et al*. (2004) Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes Cells*, **9**, 359–366.
146. Avruch,J. *et al*. (2006) Insulin and amino-acid regulation of mTOR signaling and kinase activity through the Rheb GTPase. *Oncogene*, **25**, 6361–6372.
147. Kim,E. *et al*. (2008) Regulation of TORC1 by Rag GTPases in nutrient response. *Nat. Cell Biol.*, **10**, 935–945.
148. Sancak,Y. *et al*. (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science*, **320**, 1496–1501.
149. Sarbassov,D.D. *et al*. (2005) Growing roles for the mTOR pathway. *Curr. Opin. Cell Biol.*, **17**, 596–603.
150. Jacinto,E. *et al*. (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.*, **6**, 1122–1128.
151. Hresko,R.C. *et al*. (2005) mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. *J. Biol. Chem.*, **280**, 40406–40416.
152. Sarbassov,D.D. *et al*. (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol. Cell*, **22**, 159–168.
153. Wullschleger,S. *et al*. (2006) TOR signaling in growth and metabolism. *Cell*, **124**, 471–484.
154. Long,X. *et al*. (2005) Rheb binding to mammalian target of rapamycin (mTOR) is regulated by amino acid sufficiency. *J. Biol. Chem.*, **280**, 23433–23436.
155. Fuchs,B.C. *et al*. (2007) ASCT2 silencing regulates mammalian target-of-rapamycin growth and survival signaling in human hepatoma cells. *Am. J. Physiol. Cell Physiol.*, **293**, C55–C63.
156. Edinger,A.L. *et al*. (2003) Differential effects of rapamycin on mammalian target of rapamycin signaling functions in mammalian cells. *Cancer Res.*, **63**, 8451–8460.
157. Edinger,A.L. *et al*. (2002) Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. *Mol. Biol. Cell*, **13**, 2276–2288.
158. Li,C. *et al*. (2003) Regulation of leucine-stimulated insulin secretion and glutamine metabolism in isolated rat islets. *J. Biol. Chem.*, **278**, 2853–2858.
159. Li,C. *et al*. (2006) Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. *J. Biol. Chem.*, **281**, 15064–15072.
160. Stanley,C.A. *et al*. (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N. Engl. J. Med.*, **338**, 1352–1357.
161. Meissner,T. *et al*. (2004) Urinary alpha-ketoglutarate is elevated in patients with hyperinsulinism-hyperammonemia syndrome. *Clin. Chim. Acta*, **341**, 23–26.
162. Cantley,L.C. *et al*. (1999) New insights into tumor suppression: pTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc. Natl Acad. Sci. USA*, **96**, 4240–4245.
163. Tee,A.R. *et al*. (2002) Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *Proc. Natl Acad. Sci. USA*, **99**, 13571–13576.
164. Mavrakis,K.J. *et al*. (2008) Tumorigenic activity and therapeutic inhibition of Rheb GTPase. *Genes Dev.*, **22**, 2178–2188.
165. Lassman,A.B. *et al*. (2005) Molecular study of malignant gliomas treated with epidermal growth factor receptor inhibitors: tissue analysis from

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022

North American Brain Tumor Consortium Trials 01-03 and 00-01. *Clin. Cancer Res.*, **11**, 7841–7850.
166. LoPiccolo,J. *et al*. (2008) Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. *Drug Resist. Updat.*, **11**, 32–50.
167. Majumder,P.K. *et al*. (2004) mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat. Med.*, **10**, 594–601.
168. Saal,L.H. *et al*. (2007) Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. *Proc. Natl Acad. Sci. USA*, **104**, 7564–7569.
169. Thomas,G.V. *et al*. (2006) Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat. Med.*, **12**, 122–127.
170. Guertin,D.A. *et al*. (2007) Defining the role of mTOR in cancer. *Cancer Cell*, **12**, 9–22.
171. Um,S.H. *et al*. (2004) Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature*, **431**, 200–205.
172. Abraham,R.T. *et al*. (2008) Mammalian target of rapamycin as a therapeutic target in oncology. *Expert Opin. Ther. Targets*, **12**, 209–222.

Received January 12, 2009; revised March 3, 2009; accepted March 18, 2009

Downloaded from https://academic.oup.com/carcin/article/30/8/1269/2476788 by guest on 20 August 2022