**REVIEW**

**Metabolic transformation in cancer**

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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 O2 (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 and later termed 'the Pasteur Effect' (5). This and other more recent observations have been extended to 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. 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. In tumours, this interplay is thought to be a hallmark of cancer, as it allows cells to sustain rapid proliferation in the presence of limited oxygen and nutrients.

**Abbreviations:** ATP, adenosine triphosphate; ASC2T, alanine serine cysteine transporter 2; COX, cytochrome c oxidase; ETC, electron transport chain; FH, fumarate hydratase; G6P, glucose 6-phosphate; HIF, hypoxia-inducible factor; mTORC, mammalian target of rapamycin complex; NF-κB, nuclear factor-kappaB; OXPHOS, oxidative phosphorylation; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; 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; TIDAR, TP53-induced glycolysis and apoptosis regulator; TSC1/2, tuberous sclerosis complex 1/2.

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

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 is because 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 (3H-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 O2) (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 HIF1α 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 α-α-ketoglutarate-dependent dioxygenases, and use O2 and α-ketoglutarate to convert a prolyl residue to hydroxy-prolyl, producing succinate and CO2 (18). Once hydroxylated, these 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 O2, 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 to due to the universality of the hypoxic
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 HIF 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 O2 (37), which allows for continued HIF stabilization. However, fluctuating levels of O2 can also cause an increase in intracellular levels of reactive oxygen species (ROS). These are formed by the single electron reduction of molecular O2 forming O2−, a highly reactive and damaging free radical. The reaction of O2− with water can form H2O2 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 O2 consumption in mitochondria, increasing the availability of O2 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
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 α activity also up-regulated proteins necessary to remove this waste product. The monocarboxylic acid transporter-4 is a HIF1 α 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 HCO₃⁻, producing CO₂. 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 CO₂ 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 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 the forkhead box O4 and forkhead box O3α, which can both reduce HIF1 α levels and inhibit its activity (68,69). There is further reciprocal control on PHDs and HIF1α via the NF-κB-signalling pathway. Although PHD1 inhibition can lead to NF-κB activation by de-repression of IkB 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 expression, 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’ (CAGCGT 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 HIF1α 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 isoymes, 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...
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 phosphoglucone isomerase (PGI, Figure 2). This protein is 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 variety 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 elaborated 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. Importantl, 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 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, PGL, glyceraldehyde 3-phosphate dehydrogenase (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 oncogenes such as pp60src (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 phosphorytosing binding is therefore the inhibition of PK activity and build-up of phospho-sugar metabolites that can be used in anabolic reactions. Interestingly, when Christofo et al. measured PK-M2 activity after binding phosphorytosing-containing proteins, phosphopeptides derived from lactate dehydrogenase and enolase (other glycolytic enzymes) were among the most inhibitory. pp60src 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-bind-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 on 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 is associated with PDH and regulate the phosphorylation of the E1z 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 up-regulated 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 a 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 (NAD\(^{+}\)) to NADPH (from the
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

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.
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, 6P 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 novo synthesis of lipids for membrane assembly and energy. The TCA cycle enzyme, aconitase, is also frequently inactivated in cancer cells due to a change in gene expression (e.g. SDH mutation). The TCA cycle enzyme, aconitase, is also frequently inactivated in cancer cells due to a change in gene expression (e.g. SDH mutation). The TCA cycle enzyme, aconitase, is also frequently inactivated in cancer cells due to a change in gene expression (e.g. SDH mutation). The TCA cycle enzyme, aconitase, is also frequently inactivated in cancer cells due to a change in gene expression (e.g. SDH mutation).

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 (FADH2) 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 and C and D, which are embedded in the mitochondrial inner membrane and C and D, which are embedded in the mitochondrial inner membrane and C and D, which are embedded in the mitochondrial inner membrane and C and D, which are embedded.

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 O2, 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 FADH2 (complex II) ultimately to O2, 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, O2: it is estimated that ~1 to 2% of the O2 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 O2 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 genes 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
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-somatic 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 mTORC1 (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 glutamine transport. L-amino acid dehydrogenase is an enzyme responsible for the deamination of glutamate to generate α-ketoglutarate. A number of studies of disease states such as hyperinsulinaemia and hyperammonaemia 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 tumorigenesis, 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:
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
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.

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