How cancer metabolism is tuned for proliferation and vulnerable to disruption

Almut Schulze¹ & Adrian L. Harris²

Cancer metabolism has received a substantial amount of interest over the past decade. The advances in analytical tools have, along with the rapid progress of cancer genomics, generated an increasingly complex understanding of metabolic reprogramming in cancer. As numerous connections between oncogenic signalling pathways and metabolic activities emerge, the importance of metabolic reprogramming in cancer is being increasingly recognized. The identification of metabolic weaknesses of cancer cells has been used to create strategies for treating cancer, but there are still challenges to be faced in bringing the drugs that target cancer metabolism to the clinic.

By the mid-twentieth century, cancer cells were known to show characteristic alterations in their metabolic activity. These early studies resulted in the hypothesis that irreversible inactivation of respiration is causally involved in the development of tumours¹. Later, increased rates of glutaminolysis and lipid synthesis were found in tumour tissue, and the close association between cancer-cell metabolism and hypoxia was established (reviewed in ref. 2).

Over the past decade, a more complex picture of cancer-cell metabolism has emerged. Many cancers show increased glucose uptake and enhanced glycolytic rates, suggesting that metabolic alteration provides a growth advantage for tumour cells². Some of these changes are similar to the metabolic response of non-transformed cells to growth-promoting signals, so it is not entirely clear whether these metabolic alterations are specific to cancer or just reflect the increased proliferation of tumour cells. However, different oncogenic signalling pathways target distinct components of the metabolic network. Moreover, tumours with the same genetic lesions have different metabolic profiles depending on the tissue they arise in³, suggesting that the tissue environment strongly affects the metabolic activity of cancer cells. Altered metabolic activity is crucial for supporting uncontrolled proliferation, evasion of growth-inhibitory signals, cell migration and the dissemination of metastatic cells into distant tissues. However, metabolic reprogramming also renders cancer cells more susceptible to perturbations within the metabolic network. Identifying these metabolic dependencies could open a window of opportunity for therapeutic intervention.

Oncogenic signalling drives metabolic reprogramming

Cancer cells need to generate large amounts of precursors for macromolecule biosynthesis to allow the accumulation of biomass during cell growth and proliferation (Fig. 1). Enhanced uptake of glucose supports the production of intermediates for the synthesis of lipids, proteins and nucleic acids⁴. In addition, cancer cells have increased glutamine uptake and glutaminolysis, which replenish intermediates of the tricarboxylic acid (TCA) cycle that are redirected into biosynthetic reactions — a process known as anaerolysis⁵.

Oncogenic signalling drives many of the same pathways that are responsible for the metabolic response of normal cells to growth-promoting signals. Activation of AKT by phosphatidylinositol-3-OH kinase (PI(3)K) results in increased glucose uptake, enhanced activity and mitochondrial localization of hexokinase and increased glycolytic flux. The mammalian target of rapamycin complex 1 (mTORC1) and hypoxia-inducible factor (HIF) (discussed in more detail later) also contribute to the increased expression and activity of glycolytic enzymes⁶. Oncogenic levels of MYC have been linked to increased glutaminolysis through a coordinated transcriptional program that results in glutamine addiction of MYC-transformed cells⁷. MYC also promotes the alternative splicing of the pyruvate kinase gene PKM, resulting in enhanced expression of the embryonic isoform PKM2 (ref. 8). PKM2 is highly expressed in rapidly proliferating tissues, and many cancer cells exclusively express this isoform. In contrast to other isoforms, PKM2 can switch from a tetrameric to a dimeric form with lower activity. This switch can be induced in response to tyrosine kinase signalling⁸ and allows the accumulation of glycolytic intermediates for biosynthetic processes.

Tumour suppressor pathways also affect metabolism. For example, TP53 maintains mitochondrial activity through the expression of cytochrome c oxidase 2, and loss of this gene recapitulates the metabolic consequences of the Warburg effect⁹. TP53 regulates glycolysis by inducing the expression of the TP53-induced glycolysis and apoptosis regulator (TIGAR), an enzyme with homology to fructose-2,6-bisphosphatase (ref. 11). Increased expression of this regulator inhibits glycolytic activity and increases the availability of glucose-6-phosphate (G6P) for entry into the oxidative arm of the pentose phosphate pathway (PPP) (Fig. 1), thereby supporting the production of riboses and NADPH for nucleotide biosynthesis as part of the DNA-damage response. However, p53 can also reduce the production of NADPH by inhibiting G6P dehydrogenase, the rate-limiting enzyme of this pathway¹². Recent evidence suggests that the metabolic functions of p53 may be essential for its role as a tumour suppressor, whereas other functions — including induction of cell-cycle arrest and apoptosis — are dispensable¹³.

Mitochondrial metabolism

Contrary to previously held views, most cancer cells retain functional mitochondria. Mitochondria are essential for the synthesis of citrate by the TCA cycle for the production of cytoplasmic acetyl-coenzyme A (CoA), a central source of acetyl groups for lipid synthesis and protein acetylation. A large fraction of nuclear-encoded mitochondrial genes are part of the transcriptional program

¹Gene Expression Analysis Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln’s Inn Fields, London WC2A 3LY, UK. ²Cancer Research UK Growth Factor Group, The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK.

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induced by MYC (ref. 2). MYC-transformed cells have been shown to be highly dependent on AMP-activated protein kinase (AMPK)-related kinase 5 (ARK5) (also known as NUA1K), which limits the activity of mTORC1 and maintains the high respiratory capacity of these cells14. However, oxidative mitochondrial metabolism can be impaired in cancer cells as a result of mutations in components of the TCA cycle or electron transport chain. Moreover, tumour hypoxia inhibits the entry of pyruvate into the TCA cycle and prevents the synthesis of citrate through this route. Under these conditions, reductive carboxylation of glutamine-derived α-ketoglutarate by the NADPH-dependent isoforms of isocitrate dehydrogenase, IDH1 and IDH2, is used to generate citrate for lipid synthesis (Fig. 1). IDH2 is predominantly located within the mitochondria, so mitochondrial function contributes to macromolecule biosynthesis in cancer cells. Mitchellia could also be required to restore cytoplasmic pools of NAD+ through the malate–aspartate shuttle to support the high glycolytic flux of cancer cells. Therefore, mitochondria can no longer be viewed as inactive bystanders but should be recognized as important organelles, which are actively involved in the transformation process by maintaining the biosynthetic capacity of cancer cells.

Bioenergetics and redox balance
The increased biosynthetic activity of cancer cells requires not only enhanced uptake and conversion of nutrients, but also a corresponding increase in the production of NADPH as a reducing agent for anabolic reactions and to maintain cellular redox balance18. The reduced enzymatic activity of PKM2 may allow the accumulation of glycolytic intermediates and promote the entry of G6P into the oxidative arm of the PPP for the production of NADPH (Fig. 1). Indeed, inhibition of PKM2 by direct modification in response to oxidative stress increases the production of NADPH and reduced glutathione19. Furthermore, enhanced expression of PFKFB4, an isoform of the bifunctional enzyme phosphofructokinase 2 (PFK2), is essential to balance glycolytic activity and NADPH synthesis for the production of anti-oxidants in prostate cancer cells20. However, different cancers may depend on additional pathways for NADPH production as KRAS expression in a pancreatic cancer model has been found not to increase activity of the oxidative PPP21. Cytoplasmic NADPH can also be produced by the oxidative decarboxylation of malate to pyruvate by malic enzyme 1 (ME1) and the conversion of citrate into α-ketoglutarate by IDH1. Although the exact contribution of these enzymes to NADPH production in cancer is not

Figure 1 | Overview of metabolic activities in cancer cells. The main metabolic pathways that contribute to the production of macromolecules in mammalian cells are nucleotide synthesis, the pentose phosphate pathway, serine synthesis, glutaminolysis, cholesterol synthesis, fatty-acid synthesis and elongation desaturation. Glycogen synthesis and pH regulation contribute to cellular bioenergetics. The enzymes involved in these pathways are shown in bold, those induced in response to hypoxia are marked with an asterisk. Metabolic enzymes in the TCA cycle, fumarate hydratase (FH) and succinate dehydrogenase (SDH), can act as tumour suppressors. 2-hydroxyglutarate (2-HG) is produced from α-ketoglutarate (α-KG) by the mutant forms of isocitrate dehydrogenase 1 (IDH1) and IDH2 enzymes that are found in cancer (grey dashed arrow). Reductive carboxylation of α-KG by IDH1 and IDH2 produces citrate for lipid synthesis in hypoxic cells (black dashed arrow). ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ACO, aconitate; CA9, carbonic anhydrase 9; CoA, coenzyme A; CS, citrate synthase; F1,6BP, fructose-1,6-bisphosphate; F2,6BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; HK, hexokinase; PDK1, pyruvate dehydrogenase kinase 1; IDH1, isocitrate dehydrogenase 1; ME1, malic enzyme 1; NHE1, Na+/H+ exchange protein 1; NRF2, nuclear factor (erythroid-derived 2)-like 2; PDH, pyruvate dehydrogenase complex; PIM1, p38 mitogen-activated protein kinase; PFKFB3, fructose-2,6-bisphosphatase; PFKM2, phosphofructokinase 2; PFKM2, phosphofructokinase 2; PKM2, pyruvate kinase M2; PL, phospholipids; ROS, reactive oxygen species; SCD, stearoyl-CoA desaturase; TAG, triacylglycerides.
fully understood, they may present attractive targets for selectively killing tumour cells with high biosynthetic activity.

Lactate transport and pH regulation
Enhanced uptake of glucose and its conversion into lactate creates the problem of intracellular acidification and lactate accumulation. Maintaining an alkaline intracellular milieu is essential for cancer-cell survival, whereas acidification of the extracellular micro-environment may be important to facilitate cancer-cell invasion and metastasis formation (reviewed in ref. 22).

Three main acid-regulatory systems have been implicated in the pH regulation of cancer cells; these involve Na+/H+ exchangers, carboxy anhydrase 9 (CA9) and monocarboxylate transporters (Fig. 1). Regulation of intracellular pH by an isoform of an Na+/H+ exchanger protein (NHE1) is required for tumour growth, cell migration and metastasis formation23. CA9 is a target gene of HIF and prevents the acidification of cells under hypoxic conditions24. The catalytic domain of CA9 is located at the extracellular face of the plasma membrane and catalyses the conversion of membrane-permeant carbon dioxide into bicarbonate, thereby removing protons from the cell to maintain an alkaline intracellular milieu.

Lactate transport across the plasma membrane is facilitated by monocarboxylate transporters and is coupled to the symport of protons25. Some monocarboxylate transporters require association with an ancillary protein (CD147, also known as basigin) for membrane localization and activity, and it has been shown that silencing of CD147 in cancer cells prevents transformation and tumour formation because of the inhibition of MCT1 and MCT4 function26. Notably, MCT4 is highly overexpressed in renal cancer and depletion of MCT4 causes accumulation of lactate, acidification and cell death in renal cancer cells27. Renal cancer is associated with loss of the von Hippel-Lindau tumour suppressor protein (pVHL), resulting in stabilization and activation of the α-subunits of HIF (HIF1α and HIF2α). The dependency of renal cancer cells on MCT4 is likely to be caused by their high glycolytic activity owing to a pseudohypoxic state. However, the complete picture of lactate transport in cancer may be more complex. Tumour-derived lactate can be taken up and oxidized by stromal cells28, and there is evidence that cells within oxygenated areas of solid tumours may use lactate produced by hypoxic tumour cells29. This use increases glucose availability and supports the survival of cells within the hypoxic part of the tumour. Nevertheless, monocarboxylate transporters are attractive targets for cancer therapy, and inhibitors of MCT1, such as AZD-3965, are currently being tested in clinical trials.

The role of lipid synthesis in cancer
In addition to glucose and glutamine metabolism, the increased biosynthesis of macromolecules — particularly lipids — has been recognized as a component of the metabolic reprogramming in cancer cells29. Although most cells in the adult body rely on lipids from the bloodstream, many cancer cells show a reactivation of de novo fatty-acid synthesis29 (Fig. 1). Expression of enzymes involved in the synthesis of cholesterol and fatty acids is controlled by the sterol regulatory element binding proteins (SREBPs). These proteins are activated by AKT in an mTOR1-dependent manner, and SREBP target genes represent one of the main components of the transcriptional program downstream of mTORC1 (refs 31, 32). Inhibition of SREBP function affects cell and organ size in fruitflies (Drosophila melanogaster) suggesting that lipid synthesis is essential for cell growth30. Furthermore, increased de novo fatty-acid synthesis, incorporation of newly synthesized lipids into phosphoglycerides and enhanced expression of SREBP1 have been shown to correlate with breast cancer progression31.

Although the exact role of lipid synthesis in cancer is not fully understood, it is likely that de novo lipogenesis contributes to the generation of structural lipids, such as sterols and phosphoglycerides that are required for the generation of biological membranes. Triacylglycerides are stored in lipid droplets and can be used to generate energy, whereas some lipids act as second messengers and could contribute to signalling processes in cancer cells. Indeed, monocacyl-glycerol lipase, an enzyme that is overexpressed in aggressive cancers in humans, induces a specific lipid signature that could trigger signalling events that are involved in cell migration and invasion32.

Enhanced expression of enzymes within the cholesterol biosynthesis (mevalonate) pathway has been shown to induce breast epithelial cells to form three-dimensional structures that may represent early stages of cancer33. P53 was found to associate with SREBPs to drive the expression of these genes34. The cholesterol biosynthesis pathway also provides intermediates for protein isoprenylation and loss of retinoblastoma protein results in enhanced prenylation of NRAS through activation of SREBP35.

The rate-limiting enzyme of this pathway, 3-hydroxy-3-methylglutaryl-CoA synthase (HMGR), is the molecular target of statins — a group of widely used cholesterol-lowering drugs. HMGR promotes cancer-cell proliferation and can cooperate with RAS to transform mouse embryo fibroblasts, suggesting that this pathway is crucial for cancer development36. A recent meta-analysis37 failed to establish any beneficial effects of statin use on cancer incidence. However, altered study design, improved pharmacokinetics or the combination of statins with other chemotherapeutic agents may yet demonstrate their potential value for cancer therapy.

Lipid synthesis may also have a non-cell-autonomous role in cancer development. Adipocytes promote growth and metastasis formation of ovarian cancer cells and provide them with lipids for energy generation38. It is also possible that lipogenesis in cancer cells could support the growth of cells located within nutrient-limited areas, thereby contributing to symbiotic relationships within tumours.

Surviving the cancer environment
The micro-environment of many solid tumours is characterized by limited oxygen availability as a result of the distance to the vasculature. HIF drives metabolic adaptation to hypoxic conditions by inducing a distinct transcriptional program39. HIF induces the expression of glucose transporters and glycolytic enzymes, including glucose transporter 1 and 3 (GLUT1 and GLUT3), hexokinase 2 and some isoforms of PKF2 (ref. 40). HIF also prevents the entry of pyruvate into the TCA cycle by inducing pyruvate dehydrogenase kinase 1 (PDHK1) (ref. 41, 42) and lowers cellular respiration by regulating cytochrome c oxidase isofrom expression and inhibiting mitochondrial biogenesis43.

Other metabolic constraints imposed by the in vivo tumour micro-environment are less well studied. Glucose starvation can select for oncogenic mutations in KRA5 and thus promote cell transformation44. In vivo distribution of other metabolites, including lipids and lipoproteins, could also significantly affect tumour-cell survival and cancer development. Metabolite analysis by magnetic resonance spectroscopy (MRS) and the use of labelled metabolites as tracers for positron emission tomography (PET) are required to establish a more complete picture of the metabolic profile of cancer cells within a solid tumour (Box 1). For example, stable isotope labelling, coupled with nuclear magnetic resonance analysis, has demonstrated that orthotopically implanted human glioblastoma cells use glucose — rather than glutamine — to produce TCA-cycle intermediates45. Analysis of cells in the context of intact tissues is clearly required to fully understand the effect of the tumour micro-environment on the metabolic activity of cancer cells.

Oxidative stress and transformation
Metabolic processes have an essential role in the regulation of cellular redox balance. The mitochondrial respiratory chain is the main source of free radicals, mainly through the production of superoxide radicals by complex I and III. Although superoxide produced
Metabolic oncogenes and tumour suppressors

In addition to the role metabolic alterations have in facilitating the growth-promoting response to oncogene activation, they can also actively drive the transformation process. This revised view of cancer metabolism emerged from the recognition that metabolic enzymes are themselves subject to genetic alterations in cancer.

Mitochondrial tumour suppressors

The discovery that inherited mutations in genes that encode succinate dehydrogenase (SDHB, SDHC and SDHD) or fumarate hydratase (FH) are associated with familial cancer syndromes raised interest in the role of these genes as tumour suppressors (reviewed in ref. 50). The enzymes encoded by these genes are components of the TCA-cycle.

Inhibition of succinate dehydrogenase leads to the inhibition of prolyl hydroxylases by the accumulation of the TCA cycle intermediate succinate. Prolyl hydroxylases are responsible for the hydroxylation of HIF on two proline residues, which labels the protein for VHL-dependent ubiquitylation and subsequent degradation in tissues with a normal oxygen concentration. Prolyl hydroxylases use the oxidative decarboxylation of α-ketoglutarate to transfer a hydroxyl group onto their substrates. This reaction is inhibited in the presence of succinate or fumarate and can result in the accumulation of prolyl hydroxylase substrates, including HIF56. Loss of succinate dehydrogenase or fumarate hydratase is associated with certain forms of hereditary renal cancer, and accumulation of succinate as a result of feedback inhibition and stabilization of HIF could lead to cancer development. Recent evidence suggests that renal cyst formation after Ph1 deletion is independent of HIF, but involves activation of the NRF2 pathway by fumarate, and that activation of NRF2 may contribute to the development of fumarate-hydration-deficient cancers57. However, altered epigenetic regulation owing to the inhibition of other α-ketoglutarate-dependent dioxygenases (including histone and DNA demethylases, discussed in more detail later) may also contribute to tumorigenesis in these syndromes52.

Oncogenic mutation of metabolic enzymes

Somatic mutations in IDH1 and IDH2 have been found at high frequency in secondary glioblastoma52,53 and in acute myeloid leukaemia (AML)54. These mutations always cause a single amino-acid change in one of the two alleles of either gene (arginine 132 in IDH1 or arginine 172 in IDH2). Initially, mutation of IDH1 in glioma was thought to lead to dominant-negative inhibition of the wild-type protein and cause activation of HIF through the decreased availability of α-ketoglutarate55. However, mutant IDH proteins have been subsequently shown to acquire a neomorphic enzymatic

within the mitochondrial matrix can be detoxified through the actions of superoxide dismutase and catalase, free radicals released into the intermembrane space can contribute to the generation of cytoplastic reactive oxygen species (ROS). Mitochondrial ROS production is required for RAS-dependent cell transformation56 and contributes to hypoxic stabilisation of HIF56. Inhibition of MYC-dependent expression of mitochondrial genes by FOXO3a modulates ROS metabolism and prevents HIF stabilisation in hypoxic cells57. However, expression of physiological levels of oncogenic KRAS (KRAS<sup>G12D</sup>) in mouse embryo fibroblasts actually lowers ROS levels by activating the nuclear factor (erythroid-derived 2)-like 2 (NRF2) pathway by fumarate, and that activation of the NRF2 pathway is a pro-oxidant response to oncogene activation55. The discovery that inherited mutations in genes that encode succinate dehydrogenase (SDHB, SDHC and SDHD) or fumarate hydratase (FH) are associated with familial cancer syndromes raised interest in the role of these genes as tumour suppressors (reviewed in ref. 50). The enzymes encoded by these genes are components of the TCA-cycle.

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2-Hydroxyglutarate produced by mutant IDH consists exclusively of the (R)-enantiomer (one of two possible stereoisomers). (R)-2-Hydroxyglutarate was found to stimulate the activity of the prolyl hydroxylase EGLN1 in human astrocytes (Fig. 2). This resulted in the degradation of HIF and the promotion of cell transformation. Although this result was initially surprising, it was supported by the observation that IDH-mutant gliomas express lower levels of HIF target genes, suggesting that HIF has a tumour suppressive role in this subset of tumours.

Many cellular processes are highly dependent on the availability of metabolites, so metabolic reprogramming may have even more of a marked effect in cancer cells. Post-translational modification of proteins — particularly histones — through acetylation requires acetyl-CoA, and inhibition of acetyl-CoA production can affect histone acetylation in response to growth-factor stimulation. One example of this effect is the sirtuins, a class of NAD⁺-dependent protein deacetylases, which are important regulators of energy metabolism and stress resistance. Changes in the availability of acetyl-CoA or NAD⁺ caused by metabolic reprogramming in cancer may thus affect numerous cellular processes, including gene expression.

The complex role of 2-hydroxyglutarate suggests that altered metabolic activity can have extensive effects on transcriptional and epigenetic regulation in cancer cells. The analysis of complete cancer metabolomes will probably uncover additional oncometabolites with similarly complex functions.

**Getting to the clinic**

Metabolic reprogramming in cancer is now widely recognized as important in offering opportunities for cancer treatment; however, strategies that target the enhanced glycolytic activity in cancer have, so far, not been very successful as treatments (reviewed in refs 68 and 69). The early antimetabolite 2-deoxyglucose had only limited clinical success as a single-agent treatment, mainly because of a lack of efficacy. One mechanism of resistance to this compound is the induction of autophagy, a mechanism of self-degradation of cellular components. In addition, dose-limiting toxicities that affect brain and cardiac function have been observed, reflecting drug uptake by normal tissue in these key organs.

There is a clear need to explore other metabolic pathways, and potential synthetic lethality, that may be more successful. The increased metabolic activity of cancer cells, which is often associated with hypoxia, should render them selectively sensitive to perturbations within the metabolic network.

**Finding new targets**

Metabolic reprogramming may render cancer cells highly dependent on specific metabolic enzymes or processes that could be exploited for cancer therapy. However, the search for suitable targets may be complicated by the high plasticity of the metabolic network that can induce compensatory biosynthetic routes to generate the limiting metabolites, as well as the exchange of metabolites between cancer cells and the surrounding tissues. Several studies have used RNA interference screening tools to identify metabolic weaknesses in cancer cells. One study identified phosphoglycerate dehydrogenase, which catalyses the first committed step within the serine biosynthesis pathway, as essential for the *in vivo* growth of breast cancer cells. The gene encoding this enzyme, *PGHDH*, lies in a region of frequent copy-number gain in breast cancer and melanomas, suggesting that this enzyme has an important role in the development of these cancers. An independent study found that phosphoglycerate dehydrogenase is responsible for the enhanced diversion of carbon into the serine biosynthesis pathway in cancer cells. Increased serine biosynthesis could provide additional α-ketoglutarate for anaplerosis in cancer cells or be involved in the generation of glycine for nucleotide biosynthesis and cysteine for the production of glutathione. Enhanced flux of glycolytic intermediates into the serine

Figure 2 | Epigenetic regulation by 2-hydroxyglutarate. Mutant forms of isocitrate dehydrogenase (IDH) 1 or 2 found in cancer produce the oncometabolite 2-hydroxyglutarate (2-HG) through a neomorphic enzymatic activity. 2-Hydroxyglutarate inhibits DNA demethylases, including TET2, inhibiting haematopoietic differentiation. Inhibition of DNA demethylases by 2-deoxyglucose is also associated with increased genome-wide DNA methylation and a CpG island methylator phenotype in glioma. 2-Hydroxyglutarate inhibits histone demethylases, including lysine-specific demethylase 4C (KDM4C), lysine-specific demethylase 7A (KDM7A) and lysine-specific demethylase 4A (KDM4A). This causes increased histone methylation in glioma cells and leads to the inhibition of expression of lineage-specific differentiation genes in primary astrocytes. The (R)-enantiomer of 2-hydroxyglutarate stimulates the activity of the prolylhydroxylase EGLN1, which results in enhanced degradation of HIF1α and promotes transformation in human astrocytes. α-KG, α-ketoglutarate.
biosynthesis pathway, owing to the reduced pyruvate kinase activity in PKM2-expressing cancer cells, can also be important to maintain mTORC1 activity under conditions of serine depletion. A study using a screening approach identified an isoform of phosphofructokinase 2, called PFKFB4, as an important enzyme for the survival of prostate cancer cells.

An additional challenge is to identify those metabolic processes that specifically support the survival of cancer cells that carry defined oncogenic drivers. For example, AMP-activated kinase family member 5 (ARK5) was found to be essential for the viability of cells that expressed oncogenic levels of MYC (ref. 14). ARK5 was required to limit mTORC1-dependent protein synthesis and maintain mitochondrial activity and glutamine metabolism. Another study used a chemical synthetic-lethal screen to show that inhibition of GLUT1 selectively kills VHL-deficient renal cancer cells.

Metabolic weaknesses in cancer can also be predicted using an in silico approach. This uses stoichiometric models of metabolic networks coupled to metabolic flux balance analysis and constraint-based modelling to generate models of cancer metabolism and to predict which metabolic genes are essential for cancer-cell survival. This method was used to identify metabolic pathways that are essential to support the viability of FH1-mutant cancer cells (synthetic lethal). A highly parallel metabolomics approach established cellular consumption and release profiles of more than 200 metabolites across the NCI-60 panel (the National Cancer Institute's collection of 60 human cancer cell lines). This study reported that glucose consumption correlates with cellular proliferation rate, and suggests that targeting glycine metabolism could selectively compromise nucleotide biosynthesis in rapidly proliferating cancer cells. These approaches have the potential to differentiate between common events that are essential for the metabolic reprogramming of most cancer types and specific metabolic alterations that apply only to cancers from a specific tissue or genetic background. Improved analytical capacity and high-throughput screening will continue to provide insight into the complexity of cancer metabolism.

Patient selection
Selecting the patients who are most likely to benefit from therapies that target cancer metabolism is an additional challenge. One possibility is to stratify patients on the basis of genetic drivers. Metabolic sensitivities in cancer can depend on the activation state of specific oncogenes. The genetic complexity of cancer is, therefore, also likely to be reflected in the cells’ specific metabolic requirements. Understanding this complexity is essential for identifying patients who are most likely to benefit from a specific treatment. For example, loss of p53 sensitizes cells to metformin, an inhibitor of mitochondrial ATP production and an activator of AMPK. Metformin enhances the use of fatty acids for energy production and triggers autophagy. Both processes can be used to provide energy and promote cell survival when nutrients are scarce, but they rely on functional p53.

Another oncogenic driver that is frequently activated in human cancer is KRAS. Expression of oncogenic KRAS was found to be essential for tumour maintenance in a genetic model of pancreatic ductal adenocarcinoma. Inhibition of KRAS expression was accompanied by specific metabolic alterations that demonstrated the role of KRAS in glycosylation by the induction of the hexosamine biosynthesis pathway and nucleotide biosynthesis through the non-oxidative arm of the PPP. Treatments that target these pathways may only be effective when this oncogenic driver is present and could be offered to patients who carry this mutation. Other oncogenic lesions with a strong metabolic signal include activation of PI3K (also known as PIK3CA), AKT and MYC and loss of VHL. Alterations in these genes should be considered when assessing patient responses to targeted treatment.

The metabolic profile of tumours depends not only on the type of genetic lesion but also on the tissue in which the mutation arises. This is further complicated by the increasingly recognized genetic intratumour heterogeneity of solid tumours. However, because the metabolic state of cancer cells is strongly affected by the tumour micro-environment, biomarkers for tumour hypoxia could be used for patient selection. Several hypoxia gene signatures have been published, which include many of the genes that are involved in glycolytic metabolism. These have been useful in classifying patients who are likely to benefit from radiotherapy in combination with the hypoxic radiosensitizer nimorazole. Similarly, tumours that are already hypoxic may be more sensitive to further deprivation of oxygen through anti-angiogenic therapy. Importantly, in vivo assessment of tumour hypoxia by fluorodeoxyglucose-PET imaging of 18F-labeled FAZA or misonidazole scans could be vital to select those tumours that may be particularly sensitive to these therapies and to monitor treatment response.

Targeting the tumour stroma
The cells of a tumour are strongly influenced by its stromal component. Evidence suggests that there is an exchange of metabolites between cancer and stromal cells to provide nutrients. Stromal cells can have an important role in ROS metabolism within the tumour compartment. This may be particularly important in maintaining the replicative potential of cancer stem cells. Although the metabolic requirements of cancer stem cells have not been investigated, early stem-cell development involves a metabolic switch to glycolysis that is reminiscent of the Warburg effect in cancer. Cancer stem cells require an environment low in ROS and may therefore be highly dependent on specific metabolic activities. Metabolic perturbations that selectively target cancer stem cells may be particularly effective for improving therapeutic response and preventing cancer recurrence. However, suitable molecular markers are required to identify different types of stroma and to predict treatment response.

Cancer diagnostics and dynamic monitoring of therapy
Metabolic reprogramming in cancer has already been exploited for cancer diagnosis and to monitor treatment response (Box 1). Metabolic processes that are highly active in cancer cells can produce specific by-products that can be detected not only in tumour biopsies but also in blood or urine samples. Enhanced glucose uptake forms the basis of tumour imaging by fluorodeoxyglucose-PET imaging that can be used as an early indicator of drug efficacy. However, not all tumours can be detected using FDG-PET imaging. The development of molecular tracers based on other metabolites, such as glutamine, acetate, thymidine or glycine, will offer the possibility of profiling the metabolic state of individual tumours and of monitoring the alterations to their metabolic state during treatment.

Nuclear magnetic resonance (NMR) spectroscopy offers a non-invasive method for the detection of selected metabolites in vivo and can offer insight into the body’s metabolic response to targeted therapy. Importantly, changes in metabolic activity may occur quite rapidly in response to therapy, providing the opportunity to revise therapeutic strategies or add agents. Imaging of carbon-13-labelled glucose by dynamic MRS has elucidated many aspects of human brain glucose and metabolism, and could be used to monitor rapid changes in response to therapy. These should be integrated with the use of drugs to target relevant metabolic pathways (for example, glutaminase inhibitors).

Sparing normal tissue and avoiding toxicity
Several compounds that specifically target cancer metabolism are already approved or under clinical trial, and many more are in preclinical development. One of the greatest challenges that is faced during the development of therapeutic strategies to target cancer metabolism is the possibility of toxic effects on non-cancerous tissues. Toxic side effects may be restricted to metabolic tissues, such as liver, but may also have marked effects on whole
body metabolism. For example, inhibitors of fatty-acid synthase decreased body weight by affecting the hormonal control of food intake in mice.\(^a\)

Whether metabolic reprogramming in cancer is intrinsically different from the metabolic response to proliferative stimuli in non-transformed cells is not clear. Indeed, the proliferative response to receptor ligation in T cells involves increased nutrient uptake and transformed cells is not clear. Indeed, the proliferative response to anti-angiogenic therapy (for example, bevacizumab) can lead to increased production of ROS and cell death. When pyruvate dehydrogenase (PDH) is inhibited, pyruvate is converted into lactate by lactate dehydrogenase A (LDHA). LDHA is also induced by hypoxia. Activators (such as TEPP-46) of the pyruvate kinase M2 (PKM2) increase glycolytic flux and reduce lactate production in cancer cells. This depletes glycolytic intermediates required for the serine biosynthesis pathway and reduces the availability of glucose-6-phosphate (G6P) for entry into the PPP. Metformin blocks mitochondrial ATP production, resulting in activation of AMP-activated protein kinase (AMPK) and increased glycolysis. Inhibition of purine metabolism by antimitobolites such as pemetrexed causes the accumulation of aminoimidazole carboxamide ribonucleotide (ZMP), a cell-intrinsic activator of AMPK. Combination of metformin or pemetrexed with the glycosylation inhibitor 2-deoxyglucose (2-DG) prevents the metabolic adaptation to AMPK activation. Activation of hypoxia-inducible factor (HIF) in response to anti-angiogenic drugs decreases mitochondrial oxidation of pyruvate through induction of PDHK1, increases the conversion of pyruvate to lactate by inducing expression of LDHA and regulates intracellular pH by inducing carbonic anhydrase 9 (CA9). Combining anti-angiogenic drugs with inhibitors of HIF or inhibitors of its downstream targets (LDHA and CA9) blocks the adaptive response to hypoxia.

Reactivating a suppressed pathway

Particularly relevant is the switch to glycolysis from oxidative phosphorylation by hypoxic induction of PDHK1. PDHK1 is inhibited by dichloroacetate. In patients with glioblastoma, this drug was shown to reactivate mitochondrial function and generate free radicals, which were toxic to tumour growth. A combination of this class of drug with drugs that are activated by hypoxia or those that induce hypoxia could result in synergistic effects (Fig. 3b). Of note was the anti-angiogenic effect after treatment with dichloroacetate. Some of the changes associated with metabolic reprogramming in cancer are also recapitulated in the tumour endothelium; this tissue may be targeted using similar therapeutic strategies. Another strategy
involving the reactivation of a suppressed pathway would be to reactivate PKM2 in cancer cells using small molecules (Fig. 3c). One of these activators, TEPP-46, decreased the growth of non-small-cell lung cancer cells in a xenograft model.24.

Blocking the escape route
Metformin is being widely investigated for prevention and treatment of cancer. The drug’s main action is through the non-reversible inhibition of complex I of the mitochondrial respiratory chain, leading to a reduction in the ATP:AMP ratio and activation of AMPK. In a preclinical study, a combination of metformin and 2-deoxyglucose was effective in a wide range of tumour types.25. Antimetabolites that inhibit purine metabolism, such as pemetrexed, result in accumulation of aminoimidazole carboxamide ribonucleotide, an endogenous analogue of AMP and activator of AMPK26, and provide the option of combining the inhibition of glycolysis and purine metabolism for synergistic effects (Fig. 3d).

Hypoxia is rapidly induced by anti-angiogenic therapy that targets vascular endothelial growth factor (VEGF) and by vascular targeting agents. Thus, further induction of many of these metabolic pathways will occur, potentially contributing to the survival of tumour cells and their resistance to therapy. Using induced hypoxia to synergize with other drugs, by targeting either HIF itself or the key downstream survival pathways, such as CA9, has proven effective in preclinical models (Fig. 3e). The early assessment of degree of hypoxia induction using PET imaging or CA9 detection could help to classify the responses for personalized intervention.

Synthetic lethality
The most likely way to produce anticancer effects is synthetic lethality, and this follows well-established principles in antibiotic therapy or their combination with chemotherapy. A clear example of synthetic lethal effects is the combination of lactate dehydrogenase A (LDHA) inhibitor with a drug that blocks the synthesis of NAD+ through the salvage pathway. NAD+ can be synthesised de novo or recycled from nicotinamide through the salvage pathway, involving the enzyme nicotinamide phosphoribosyltransferase (NAMPT). Inhibition of LDHA results in the depletion of NAD+ — which is crucial to maintain glycolytic flux — and inhibition of NAMPT enhances the effectiveness of LDHA inhibitors27 (Fig. 3f). NAMPT inhibitors may also be useful in combination with classical chemotherapeutic agents. Genotoxic damage caused by ionizing radiation or DNA-alkylating drugs leads to the activation of poly-ADP-ribose) polymerase (PARP) and can rapidly deplete cellular pools of cofactor NAD+. Inhibitors of NAMPT block the restoration of NAD+ pools and could increase the toxicity of DNA-damaging agents. Inhibition of NAMPT could also be combined with PARP inhibitors, which have been proven to be useful in the treatment of tumours that carry mutations in DNA-repair pathways, such as BRCA-mutant breast cancers. NAMPT inhibitors are currently being tested in phase II clinical trials.

Future research
Elucidating the complex interplay between oncogenic signalling pathways and cellular metabolic activity is an exciting challenge for future research. Metabolic reprogramming of cancer cells can clearly not simply be explained by a shift from oxidative phosphorylation to aerobic glycolysis. However, relatively little is known about the differences in metabolic dependencies of genetically diverse cancer cells or the complex metabolic interactions between tumour and stroma. Although the importance of ROS metabolism in cell transformation and tumour maintenance is becoming more evident, the relative contribution of different metabolic pathways to anti-oxidant production in cancer is not fully understood. Many metabolic pathways involved in the reprogramming of cancer cells are closely linked to the metabolic changes associated with hypoxia. Future research should address how cancer cells maintain the balance between enhanced biosynthetic activity and the need for antioxidative production. Disrupting this balance should selectively impair the viability of cancer cells and, together with appropriate biomarkers and dynamic cancer imaging, provide new strategies for the treatment of cancer.

4. Yuneva, M. O. et al. The metabolic profile of tumors depends on both the responsible genetic lesion and tissue type. Cell Metab. 15, 157–170 (2012).
5. This study found that metabolic alterations, which are associated with tumorigenesis, are dependent on the oncogenic driver and the tissue in which the tumour arises.
14. References 15 to 17 describe the reductive carboxylation of α-ketoglutarate for the production of citrate.
19. This study used stable isotope labelling and metabolic flux analysis to demonstrate that oncometabolic Kras induces the non-oxidative arm of the pentose phosphate pathway.
This study reports that tumour-associated mutant forms of p53 can bind to SREBP and induce the expression of the mevalonate pathway.


References 71 and 72 used different strategies to identify the role of centromere in cancer-cell growth.


References 77 and 78 describe the application of metabolic models to drug discovery and to identify synthetic-lethal metabolic processes in fumarate-hydratase-deficient tumours.


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