Metabolic interactions in cancer: Cellular metabolism at the interface between the microenvironment, the cancer cell phenotype and the epigenetic landscape

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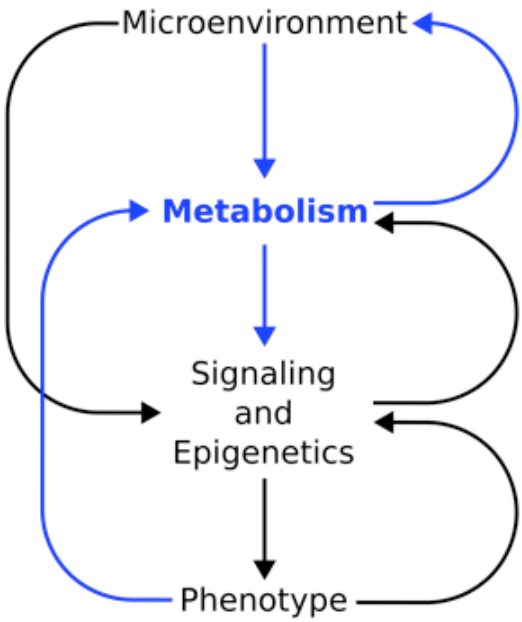

In brief: Understanding complex metabolic interactions in cancer is important for the design of novel therapeutic strategies.

Metabolic interactions in cancer: Cellular metabolism at the interface between the microenvironment, the cancer cell phenotype and the epigenetic landscape

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Abstract

Metabolism is tied into complex interactions with cell intrinsic and extrinsic processes that go beyond the conversion of nutrients into energy and biomass. Indeed, metabolism is a central cellular hub that that interconnects and influences the microenvironment, the cellular phenotype, cell signaling, and the (epi)genetic landscape. While these interactions evolved to support survival and function of normal cells, they are hijacked by cancer cells to enable cancer maintenance and progression. Thus, a mechanistic and functional understanding of complex metabolic interactions provides a basis for the discovery of novel metabolic vulnerabilities in cancer. In this review, we will summarize and provide context for the to-date discovered complex metabolic interactions by discussing how the microenvironment as well as the cellular phenotype define cancer metabolism, and how metabolism shapes the epigenetic state of cancer cells. Many of the studies investigating the crosstalk of metabolism with cell intrinsic and extrinsic processes have used integrative data analysis approaches at the interface between computational and experimental cancer research, and we will highlight those throughout the review. In conclusion, identifying and understanding complex metabolic interactions is a basis for deciphering novel metabolic vulnerabilities of cancer cells.

Introduction

Metabolism is a biochemical reaction network that converts nutrients into metabolites, which in turn are needed to sustain cell survival and proliferation. To date we have extensive knowledge on the metabolic architecture consisting of metabolites, reactions, and pathways that are present in cells, allowing the reconstruction of a global (human) metabolic map[1,2]. This resource knowledge provides the basis for investigating metabolism.

One vibrant area of metabolism research is focused on cancer, and metabolic enzymes have proven to be promising therapeutic targets against cancer[3–6]. To fully exploit this exciting potential of metabolic drug targets in cancer treatment, an integrated understanding of metabolism that accounts for complex metabolic interactions with cell signaling, epigenetics, the cellular phenotype, and the microenvironment is needed[7–9]. In this context, integrative approaches based on computational models have proven to be powerful tools to extrapolate and to extract functional information from "omics" data sets. Here, we will review the current discoveries on complex metabolic interactions and relate them to cancer maintenance and progression. In particular, we will review the impact of the nutrient microenvironment on defining cellular metabolism during cancer maintenance and progression; we will discuss the importance of metabolism in enabling changes in the cellular phenotype observed during matrix detachment; and we will conclude by exploring the mechanistic interconnection between metabolite concentrations and the epigenetic state of cancer cells.

The functional knowledge provided by the understanding of complex metabolic interactions can be the basis for an extended resource on metabolism that not only describes the metabolic architecture, but also the interconnection of metabolism with other cellular processes and the microenvironment. Such an extended resource has the potential to further advance metabolism-based drug discovery including cancer treatment.

The nutrient microenvironment defines metabolism

In order to fuel uncontrolled proliferation and increased survival, cancer cells rewire their metabolism to cope with the need for energy, redox co-factors and biomass[5,10]. Most of our current understanding on this metabolic rewiring in cancer cells derives from *in vitro* studies. Extensive research on the metabolic requirements of cancer cells *in vitro* pinpoints glucose and glutamine as the primary nutrients supporting energy, redox co-factor, and biomass production in proliferating cancer cells[9,11–13]. Accordingly, it has been established that glucose uptake is a hallmark of cellular transformation in many cancer cells (Figure 1)[3,14,15]. Increased flux of glucose through glycolysis grants fast energy production in form of ATP, and at the same time allows the diversion of glycolytic intermediates into branching pathways for biomass and redox cofactor production[12,14,16]. For instance, shunting of glucose-6-phosphate into the pentose phosphate pathway (PPP) leads to production of dihydronicotinamide-adenine dinucleotide phosphate (NADPH) (which is an essential co-factor for redox homeostasis) and ribose-5-phosphate (which is a precursor for *de novo* nucleotide synthesis)[10,17,18]. Moreover, fructose-6-phosphate can be diverted into the hexosamine biosynthetic pathway, providing substrates for glycosylation of proteins and lipids, which is important for protein folding as well as stability and cell-cell adhesion[12,19,20]. Glucose also contributes to

the *de novo* biosynthesis of fatty acids, triglycerides and phospholipids, by yielding both cytosolic acetyl-CoA (AcCoA) via oxidation to citrate, and glycerol-3-phosphate via glyceraldehyde-3-phosphate[10,12]. Another glycolytic intermediate, 3-phosphoglycerate, can be redirected toward the serine biosynthesis pathway, allowing *de novo* serine and glycine production and shunting glucose carbons into the one-carbon metabolism and the folate pool[21–23]. Likewise, glutamine is the most abundant amino acid and the second most consumed nutrient in cell culture (Figure 1)[24]. Glutamine anaplerosis is the prime mean for replenishing the TCA cycle in cultured cells[25–27]. In turn, several non-essential amino acids, such as aspartate, can be synthetized from TCA cycle intermediates[28,29]. Glutamine is also the primary source of nitrogen for proliferating cells *in vitro*, and it is therefore critical for *de novo* nucleotide and amino acid synthesis[25]. Moreover, glutamine can contribute in multiple ways to fatty acid synthesis, depending on the cellular context and on the microenvironment. Both glutaminolysis (conversion of glutamine to pyruvate that re-enters the TCA cycle in form of AcCoA) and reductive glutamine metabolism can contribute to fatty acid synthesis[30–34]. Despite glucose being the main contributor to AcCoA for fatty acid synthesis in normoxia, in hypoxia the contribution of glucose to AcCoA production decreases and reductive glutamine metabolism as well as other non-glucose carbon sources, such as acetate, supply 2-carbon units for *de novo* fatty acid synthesis[30,31,33,35]. Thus, *in vitro* many cancer cells display a segregation between glucose and glutamine fueled metabolism.

In vitro studies have an undeniable credit in defining the backbone of cancer metabolism. Nonetheless, cell culture conditions have intrinsic limitations. The actual process of establishing cell lines from primary tumors selects for the fastest-growing clones, to the detriment of slowly-proliferating or quiescent cell[12]. Notably, these negatively selected characteristics are found *in vivo* in subpopulations with stem cell-like and mesenchymal phenotypes, which play a key role in cancer maintenance, metastasis formation and resistance to therapy. Cell culture conditions hence select for a relatively homogeneous population of cancer cells, offering clean and easily controllable models, yet not fully representative of the original tumor. Furthermore, standard cell culture media contain nutrients often in large excess and in different ratios compared to plasma and bodily fluids[12]. Moreover, in many cell culture media the diversity of nutrients is less than what is found *in vivo*, with many components not represented[36–38]. In addition, cell-extrinsic drivers such as altered perfusion from abnormal tumor vasculature[39], interaction with stromal and immune cells[40–42], and nutrient availability, also affect the metabolism of cancer cells. Hence, simplistic models based upon cultured cells may only partially represent the metabolic phenotype of cancer cells *in vivo*. Thus, a comprehensive understanding of cancer pathophysiology is subject to the complex interaction of metabolism with the microenvironment.

In recent years, the combined application of stable isotope tracers and computational methods (already widely used to investigate cellular metabolism in cell culture[43,44]), applied to *in vivo* and *ex vivo* studies, led to findings that added a layer of complexity to the simplistic view of a functionally segregated glucose and glutamine metabolism in cancer cells. It emerged that glutamine- and glucose-dependent anaplerosis are most likely both of importance for cancer metabolism *in vivo* (Figure 1). Yet, their relative importance seems to vary depending on the cell origin, the oncogenic drivers, and the nutrient microenvironment[45,46]. For instance, in cultured glioma cells glutamine anaplerosis was shown to be activated, whereas pyruvate carboxylase (PC) activity, accountable for glucose-dependent anaplerosis, was absent[25]. Still, glioma cells can exploit PC to compensate for suppression of glutaminase (GLS, an enzyme important for glutamine anaplerosis) or glutamine restriction[47]. Indeed, whereas GLS grants glioma cells maximal growth in culture and in xenografts models, even glutamine-addicted cells can compensate GLS loss by rerouting carbons from glucose into metabolite pools normally supplied by glutamine, as predicted by computational

analysis of ^{13}C labeling patterns from glucose upon GLS suppression[47]. Other studies even point at PC, rather than GLS, as the major anaplerotic route in primary glioma xenografts[48]. Likewise, despite glutamine being an important source of TCA cycle carbons in non-small cell lung cancer (NSCLC) in culture, glutamine anaplerosis is minimal in normal lung and in Ras-induced lung tumors, with no significant difference in its contribution to the TCA cycle in normal and tumor tissue[49]. Conversely, glucose oxidation seems dispensable for lung cancer cell proliferation in culture, but glucose contribution to the TCA cycle is required for tumor growth *in vivo*[49]. Indeed, glucose is a major source of TCA cycle carbons in lung tumors. This occurs via increased pyruvate dehydrogenase (PDH) activity and PC-dependent anaplerosis. *PDH* or *PC* deletion minimally affects proliferation of NSCLC *in vitro*, but both enzymes are required for tumor initiation and growth *in vivo*[46,49]. Most interestingly, the mere shifting from the *in vitro* culture conditions to an *in vivo* lung microenvironment is sufficient to rewire the metabolism of lung cancer cells: despite a lack of preferential glutamine use by Ras-induced lung tumors *in vivo*, cells from such tumors rely on glutamine metabolism to proliferate when propagated *in vitro*, but transplantation back into the lung results in tumors with a metabolic phenotype similar to spontaneously arising Ras-induced lung cancers[49]. Thus, the shift from the *in vitro* to the *in vivo* microenvironment can alter glutamine metabolism.

Moreover, there is evidence that also more subtle changes in the microenvironment as they occur *in vivo* are sufficient to alter cancer metabolism. In particular the *in vivo* lung nutrient microenvironment seems to induce dependency on PC activity[49]. A possible mechanistic explanation for this finding is provided by Christen et al., who recently demonstrated that PC-dependent anaplerosis can be induced by pyruvate availability in the microenvironment. Applying *in vivo* ^{13}C tracer analysis, they showed that PC activity is increased in breast cancer-derived lung metastases compared to the primary tumor. By means of computational analysis of ^{13}C labeling patterns that allowed determining the distribution of pyruvate between the cytosol and the mitochondria, they found that pyruvate availability in the microenvironment leads to an increase in mitochondrial pyruvate concentrations, resulting in PC-dependent anaplerosis based on substrate driven enzyme kinetics (*i.e.* mitochondrial pyruvate concentrations were within the K_m range for PC)[50]. Across metabolism, absolute metabolite concentrations on average exceed the binding site affinity of the associated enzymes supporting enzymatic efficiency. An exception is represented by central carbon metabolism substrates, whose concentrations are generally close to the enzyme K_m[51]. The finding that mitochondrial pyruvate fluctuations span in the range of the K_m of PC[50] nicely fits in this model, and underlies the need for flexibility in adapting to the carbon sources provided by the microenvironment.

Not only changes in the inter-organ microenvironment, but also in the intra-tumor microenvironment impact cancer metabolism. The integration of ^{13}C-labeling data from glucose infusions with spatial data on tumor perfusion identified intra-tumor metabolic heterogeneity in human lung cancer patients[52]. Glucose and mainly non-glucose nutrients were found to fuel the TCA cycle in well-perfused tumor areas[52], whereas less perfused areas relied mainly on a glucose-fueled TCA cycle[41,53]. Thus, based on these data it emerged that the *in vivo* nutrient availability strongly influences the metabolism of cancer cells to an extent that it can be used for novel treatment ideas. A first evidence for such an approach is the finding that inhibition of fatty acid synthesis through acetyl-CoA synthetase 2 (ACSS2) or the inhibition of fatty acid desaturation through stearoyl-CoA desaturase (SCD) can specifically inhibit cancer cells that are in tumor areas with microenvironment-induced metabolic stress[54,55].

Additionally, also the cell origin (in conjunction with the microenvironment) impacts cancer metabolism. For instance, an increase of glutamine catabolism correlates with a decrease of glutamine synthetase (GS) expression in MYC-induced liver tumors, whereas in MYC-induced lung tumors both GLS and GS are active,

which seems to result in an accumulation of glutamine[56]. Moreover, normal brain metabolism features *de novo* glutamine biosynthesis from glutamate, a characteristic also found in glioblastoma[38]. The importance of the cell origin was also extended to other amino acids than glutamine. Branched chain amino acids (BCAA) are abundant in serum and can feed carbons into the TCA cycle via branched-chain amino acid transaminase 1 (BCAT1), which is required for progression of some tumor types[57,58]. Interestingly, pancreatic ductal adenocarcinoma (PDAC) and NSCLC show differential usage of BCAA as alternative carbon source, despite being driven by the same Ras mutation. NSCLC display enhanced uptake of BCAA, which are used for protein synthesis and as nitrogen source[59]. PDAC, on the other hand, rather increase plasma BCAA levels already at an early-stage of the disease due to increased protein breakdown[60]. Accordingly, macropinocytosis, a common amino acid supply route in Ras-transformed cells[27], is decreased in cells derived from mouse NSCLC, but seems to play a key role in human and mouse PDAC cells to enable the utilization of collagen-derived proline during nutrient limitation [59,61]. Thus, the cell origin together with the microenvironment can override the genetic driver as determinant of *in vivo* cancer metabolism.

Based on the studies discussed above it is evident that cancer metabolism is defined by the microenvironment. However, also the metabolism of cancer cells impacts the microenvironment in order to create permissive conditions for tumor maintenance and progression[42,62]. The adaptive response triggered by the tumor microenvironment in cancer cell metabolism in turn can reshape the microenvironment itself: in hypoxic conditions, Hypoxia-inducible factor (HIF)-induced upregulation of glycolysis ultimately leads to increased production of lactate and to its accumulation in the extracellular space. Far from being just a waste product, lactate has established roles in tumor progression and maintenance. First, lactate can act as a carbon source, as lactate secreted in the tumor microenvironment can be used by cancer cells to fuel oxidative phosphorylation (Reverse Warburg effect)[40]. Second, lactate accumulation in the microenvironment affects the immune response. Lactic acidosis triggers an inflammatory response that recruits immune cells to the tumor site; among them, macrophages secrete cytokines and growth factors that drive tumor cell growth, invasion and metastasis, whereas the function of tumor-infiltrating lymphocytes is impaired by acidosis, thus disabling immunosurveillance[42,63]. Third, lactate promotes tumor angiogenesis. Indeed, lactate acts as signaling molecule in endothelial cells: it activates the VEGF/VEGFR2 signaling pathway[64], and its influx through monocarboxylate transporter MCT1 supports NF-kappaB/IL-8 pathway to induce endothelial cell migration and tube formation[65]. In the same line, it has been recently proposed that extracellular gradients of metabolites within the tumor might act as tumor morphogens, which induce tumor-associated macrophages (TAMs) to differentiate into distinct subpopulations and ultimately shape the tumor architecture built by cancer, stromal, and immune cells[66]. TAMs integrate lactate and oxygen levels and accordingly activate endothelial cells, promoting angiogenesis to restore blood perfusion of ischemic tumor areas[66]. Additionally, it has been discovered that cancer cells influence the nutrient microenvironment by reprograming the metabolism of stromal cells. For instance, PDAC cells can reprogram stromal cells to provide them with alternative carbon sources[67]. Indeed, PDAC cells induce autophagy in stroma-associated pancreatic stellate cells, with the consequent release of non-essential amino acids (NEAA), which can in turn fuel the growth of PDAC by fueling the TCA cycle and NEAA and lipid biosynthesis[67]. Hence, by exploiting the contribution of the stroma, PDAC reduce their dependence on glucose and serum-derived nutrients, which are limited in the pancreatic tumor microenvironment[68,69]. Likewise, both ovarian and breast cancer cells can induce cancer-associated adipocytes to release fatty acids in order to sustain rapid tumor growth[70,71]. Indeed, upregulation of lipoprotein lipase (LPL)[72], very low density lipoprotein receptor (VLDLR)[73] and fatty acid

binding proteins (FABPs)[74–76] grants breast cancer cells the ability to uptake dietary lipids or exogenous fatty acids released by cancer-associated adipocytes. The increased fatty acid uptake, in turn, fuels fatty acid oxidation (FAO), allowing breast cancer cells to survive under conditions of nutrient deprivation[71]. In the same line, glucose utilization is decreased as a source of acetyl-CoA, while uptake of exogenous fatty acids and FAO is increased, under conditions of chronic acidosis[77], a hallmark of most human tumors. These collective findings show that the link between metabolism and the microenvironment is bidirectional.
In conclusion, both the cell origin and the microenvironment are important determinants of cancer cell metabolism that need to be considered when developing metabolism-based anti-cancer drugs.

Matrix detachment defines metabolism

Cancer cells require a high adaptation potential during progression towards metastasis formation, resulting in shifts between different cellular phenotypes[62,78]. During the growth of the primary tumor the tumor environment is changing. Cancer cells adapt to these changes and some of them will acquire the ability to survive and grow without matrix attachment[79]. These phenotypes support cancer cells dissemination to distant organs. Once cancer cells have reached a distant organ they can undergo a dormancy period, but eventually they will overcome this quiescent state and colonize the organ, eventually leading to metastasis formation[62]. Thus, during metastasis formation cancer cells modify their phenotype shifting from a proliferative to a colonizing or a dormant state. Indeed, it becomes evident that metabolic alterations are not only a mere consequence of the proliferative state of the cells, but they are enabling cancer progression[5,8].

The metabolism of colonizing cells

Our current understanding of the metabolic difference between proliferating and colonizing (cancer) cells is largely based on *in vitro* experiments in 2D versus 3D cultures[80]. It has been shown that matrix detachment enforced by 3D cultures leads to a metabolic defect in non-transformed cells. In particular a decrease of glucose uptake has been observed. This results in a reduction of pentose phosphate pathway (PPP) flux, with a consequent deficiency in the antioxidant response, and decreased activity of pyruvate dehydrogenase (PDH), with a consequent impairment in glucose oxidation and mitochondrial respiration[81,82]. Moreover, the decreased antioxidant response has been shown to result in ROS accumulation, which could in turn inhibit fatty acid oxidation, further adding to the energy defect[83]. In contrast, one of the peculiarity of cancer cells is their ability to rewire metabolic fluxes in order to cope with extracellular matrix detachment (Figure 2a)[8,53]. Indeed, depending on the cellular context, different oncogenic signaling pathways seem crucial for the metabolic remodeling required to avoid the metabolic stress caused by loss of extracellular matrix attachment[81–85]. In particular, it has been shown that oncogenic signals such as PI3K/AKT and MEK/ERK are able to rescue glucose uptake rates, scavenge ROS and thus reactivate fatty acid oxidation (Figure 2a)[81,83]. Furthermore, metabolic flux modeling revealed that ERK signaling could sustain PDH flux (Figure 2a)[81]. The application of metabolic flux modeling further led to the identification of an unexpected metabolic response that allows cancer cells to counteract mitochondrial ROS when facing detachment from the extracellular matrix. Indeed, whereas matrix-attached cancer cells rely on reductive carboxylation of glutamine for lipid production, matrix-detached cancer cells depend on reductive glutamine metabolism for shuttling cytosolically produced NADPH into the mitochondria, where it can be used for ROS scavenging (Figure 2b)[86]. Accordingly, *in vivo* evidences

suggest that increased antioxidant metabolism supports metastasis formation in melanoma mouse models[87]. Moreover, it has been recently discovered by Elia et al. that colonizing breast cancer cells upregulate proline catabolism via the enzyme proline dehydrogenase (PRODH) to meet the increased energy demands imposed by this cellular phenotype (Figure 2b)[88]. Interestingly, in breast cancer patients PRODH expression was increased in metastases compared to primary breast cancer tissue, and inversely correlated with MYC expression[88], which is a known negative regulator of PRODH[89]. Accordingly, MYC expression was found to be lower in micrometastasis than in macrometastasis using single-cell-based gene expression analysis[90]. This evidence suggests a possible prevalent role of proline catabolism in sustaining the earlier steps of metastasis formation. Thus, it becomes evident that the phenotypic state of cancer cells can induce a rewiring of metabolic fluxes that creates new and potentially unique metabolic vulnerabilities. The mechanistic understanding of phenotype-driven metabolic changes has the potential to reveal a whole new area of therapeutic targets. For instance, recent *in vivo* data suggest the inhibition of proline catabolism as promising and safe drug target for impairing metastasis formation[88]. Moreover, studying metabolic changes induced by the cellular phenotype is not only important for the discovery of new metabolic drug targets, but can be exploited to rationally combine existing therapies. In particular, it has been found using flux modeling that NSCLC with loss of liver kinase B1 (LKB1) tumor suppressor exhibit reduced metabolic flexibility during matrix detachment[84]. As a consequence cancer cells deficient for LKB1 were more sensitive to combination treatments with glutaminase inhibitors and phenformin[84].
The above discussed findings suggest that metabolic vulnerabilities arising due to a change in the cancer cell phenotype can open a window of opportunity for novel, metabolism based treatments against metastasis formation.

The metabolism of quiescent cells

In tumors, the proliferation of the cancer cells is counteracted by different mechanisms, such as apoptosis, impaired vascularization, immunosurveillance and oxidative stress[91]. Thus, cancer cells can show a (macroscopic) stop in proliferation and enter a (pseudo) dormant state that can even last for years[91]. As proliferating and dormant cancer cells likely rely on different metabolic programs, dormant cancer cells often evade therapeutic approaches that were developed against proliferating cancer cells. Thus, elucidating the metabolic differences between proliferating and dormant cancer cells might reveal new weaknesses of tumor cells during the different stages of cancer progression and help improving the current therapeutic strategies. Yet, even in non-transformed cells very little is known about the metabolic requirements of a proliferating versus quiescent state. For instance, lymphocytes and fibroblasts are normally in a quiescent state until they are activated and can start proliferating[92,93]. Evidently, this phenotypic switch requires sustaining a diversity of cellular processes, including metabolic adaptations. Interestingly, a metabolic rewiring involving increased glucose uptake and glycolytic flux is crucial to sustain the switch from inactive quiescent to active proliferating T lymphocytes[94,95]. Fibroblasts on the other hand rely on similar high glycolytic rates in proliferation and quiescence, yet they exploit glycolysis to fuel different metabolic needs. In proliferating cells glucose is used to mainly produce biomass, whereas in quiescent fibroblast it is used to support NADPH production, renew their protein and lipid pool and secrete specific extracellular matrix proteins[96]. Moreover, it has been found that proliferation and quiescence of non-transformed mammary epithelial cells are supported by a different glutamine metabolism. Highly proliferating cells suppress glutamate dehydrogenase (GLUD) activity and rely on transaminases to convert glutamate, whereas quiescent cells show an inverse dependency on these

pathways[97]. This dependency profile was also found in highly proliferating cancer cells[97]. Thus, one can speculate that inhibition of GLUD could be effective not only in non-transformed quiescent cells, but also against dormant cancer cells. In addition, it has been found that the presence or absence of certain metabolic enzymes enforces a non-proliferative and thus potentially quiescent/dormant state in (cancer) cells. For instance, integration of metabolomics with metabolic flux modeling data linked the proliferation inhibition caused by overexpression of the pyruvate kinase isoform M1 to reduced *de novo* nucleotide production, in non-transformed proliferating fibroblasts[16]. Moreover, it has been suggested that loss of enzyme activity in succinate dehydrogenase (SDH) (which can be found in tumors, but also neurodegenerative diseases) leads to proliferation inhibition unless it is combined with an additional loss of complex I of the respiratory chain[98]. Accordingly, it has been discovered that sole loss of SDH activity, and combined loss of SDH and complex I activity resulted in very different and distinct changes in mitochondrial metabolism[98]. Thus, despite our limited knowledge on the metabolism of dormant cancer cells, therapeutic interventions that target these cells might be an interesting way to prevent cancer relapse and metastasis formation.
Overall, the understanding of the metabolic vulnerabilities imposed by the cellular phenotype has an exciting potential for the development of new therapeutic and preventative strategies.

Metabolite concentrations define the epigenetic state

The interplay between metabolism and its regulators is bidirectional: whereas metabolism is regulated by various stimuli such as the microenvironment, metabolism can in turn also regulate itself (recently reviewed by Macpherson & Anastasiou[99] (allosteric regulation) and by Lorendeau at al.[8] (regulation via post-translational modifications)), cellular signaling (recently reviewed by Lorendeau at al.[8]), and the cellular epigenetic state.
The best described epigenetic marks are DNA as well as histone methylation and histone acetylation[100,101]. In humans, DNA methylation of cytosine residues in CpG islands in promoter regions is commonly associated with transcriptional inhibition. Histones can be modified by mono-, di- and trimethylation on lysine or arginine residues. As a consequence, activation or repression of gene expression can occur, depending on which residue is modified and on the number of methyl groups[102]. Cancers frequently display global DNA hypomethylation compared with their healthy tissue counterparts, resulting in genomic instability[103]. In addition, this global hypomethylation is often combined with local hypermethylation of genomic regions in which tumor suppressor genes reside[104–107]. Moreover, cancer cells exhibit increased variability in DNA methylation compared with their corresponding normal tissues[108,109]. Nearly half of the known histone methyltransferases (HMTs) have been associated with cancer[110–112]. SAM is the primary methyl group donor for both DNA and histone methylation[101]. Cellular SAM concentrations are a function of the orchestrated interplay between serine, glycine and one-carbon metabolism with the folate cycle and the methionine cycle (Figure 3a)[103,113]. Interestingly, changes in dietary folate intake as well as methionine restriction have been shown to modulate gene expression by acting both on DNA and histone methylation[114–116]. Moreover, variation in the enzymes of the serine, glycine and one-carbon metabolism have been linked to several tumors types in which altered DNA methylation is an acknowledged player[103,117–120]. Indeed, enzyme kinetics have revealed that the K_m values of many HMTs lies in the range of intracellular SAM levels[114], suggesting that fluctuations in SAM levels might modulate their activity (Figure 3a). Furthermore, integrative modeling of DNA methylation, obtained by computational and statistical analysis of multi-platform data from human tumors, recently

highlighted the role of the metabolic genes involved in the methionine cycle in inter-tumor variability of DNA methylation[121].

Methylation is a reversible and dynamic epigenetic mark[122], which is regulated not only by methylating, but also by demethylating enzymes. Demethylation of histones and DNA is mainly catalyzed by Jumonji-C (JmjC) domain-containing histone demethylases (JHDMs) and Ten-eleven translocation (TET) enzymes, respectively (Figure 3b). Both are Fe^{II}-, O_2-, and α-ketoglutarate (αKG)-dependent dioxygenases, which share a common enzymatic mechanism of action, exploiting molecular oxygen and αKG as substrates (Figure 3b). Oxidative decarboxylation of αKG to CO_2 and succinate leads to the formation of a highly reactive oxyferryl species (Fe^{IV}=O) that subsequently hydroxylates the methyl group on lysine or cytosine. Hydroxymethyl-lysine is unstable and spontaneously reverts to lysine releasing formaldehyde, whereas 5-hydroximethyl-cytosine (5hmC) undergoes additional rounds of TET-mediated oxidation, whose products are reverted to cytosine via base excision repair[101]. As mentioned above, αKG is a substrate for JMDH and TET enzymes, and its intracellular concentration can modulate DNA and histone methylation by directly influencing the activity of these enzymes. For instance, αKG-mediated histone and DNA demethylation has recently been shown to be important for the maintenance of mouse embryonic stem cell pluripotency[123,124]. Whereas αKG promotes the activity of JMDH and TET enzymes, the resulting product of the reaction, namely succinate, inhibits their activity via mass action kinetics. Thus, rather than αKG concentrations on their own, the ratio between intracellular αKG and succinate can regulate the epigenetic state of cells[125]. Moreover, other metabolites, such as fumarate and 2-hydroxyglutarate (2-HG), which share structural similarities with succinate and αKG respectively, are competitive inhibitors of DNA and histone demethylation[126–128]. Most dramatic variations of these metabolites, resulting in DNA and histone hypermethylation, are observed in cancers with mutations in TCA cycle enzymes: mutations in SDH leading to the loss of enzyme expression, and consequently to succinate accumulation, are frequently found in paraganglioma, pheochromocytoma, gastrointestinal stromal tumor (GIST), and renal carcinoma[129,130]. Similarly, loss of fumarate hydratase (FH) expression, with consequent fumarate accumulation, is found in the familial cancer syndrome HLRCC (hereditary leiomyomatosis and renal cell cancer), as well as in paraganglioma and pheochromocytoma[131]. Gain-of-function mutations of isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2), which lead to the production of 2-HG from αKG, are found in glioma, chondrosarcoma, cholangiocarcinoma, and acute myeloid leukemia (AML)[132–135].

A second class of histone demethylases, the lysine demethylase 1 (LSD1) family of amine oxidases, catalyzes the removal of methyl groups from histone H3 lysine residues via an FAD-dependent mechanism, which results in formaldehyde production[136]. It has been postulated that these histone demethylases use their covalently bound THF molecule for protection against the destructive effects of formaldehyde, by formation of 5,10-methylene-THF[137]. In line with this hypothesis, dietary folate deficiency is associated with increased levels of methylated lysine 4 on histone H3[137].

Concentrations of TCA cycle metabolites are not the only modulators of DNA and histone methylation. As mentioned above also molecular oxygen (O_2) is a substrate for the reaction catalyzed by JMDH and TET enzymes. Interestingly, in many tumor areas pathological O_2 limitation (hypoxia) is observed, raising the question whether hypoxia influences the epigenetic state. Recently it was found that hypoxia induces DNA hypermethylation in cancer cells by diminishing the activity of TET enzymes (independently of their expression, of αKG concentrations, and of HIF1α stabilization) suggesting O_2 tension as a novel modulator of the epigenetic state in tumors (Figure 3b)[138]. Moreover, it was recently shown that in stem cells also vitamin C and proline availability in the nutrient microenvironment can modulate DNA methylation[139].

The effect of vitamin C is well explained via its need as co-factor to the TET enzyme-catalyzed reaction. Yet, the mechanism by which proline availability influences DNA methylation, and to which extent this mode of regulation occurs beyond stem cells, remains to be explored. Thus, DNA and histone methylation and consequently gene expression are highly regulated by metabolism.

Another widely described epigenetic mark is histone acetylation, which is the reversible transfer of acetyl groups from AcCoA to the lysine residues on histone tails (Figure 3c). Acetylation neutralizes the positive charge of lysine on histone tails, thus loosening the interaction with negatively charged DNA, and promotes the formation of docking sites for transcription factors and transcriptional regulators[101,140]. AcCoA is the sole donor of acetyl groups used by histone acetyltransferases (HATs) for acetylation in eukaryotic cells[140,141], and histone acetylation is sensitive to alterations in AcCoA levels depending on the nutritional and signaling status. In yeast, fluctuations of AcCoA in response to glucose availability influence histone acetylation, in turn modulating the expression of a wide set of genes involved in cell growth and cell cycle progression[142,143]. Further highlighting the interplay between the genetic landscape, cellular metabolism and the epigenome, it has been found that driver mutations in human tumors can directly affect AcCoA homeostasis. Indeed, both MYC and AKT, two oncogenes that extensively contribute to metabolic rewiring[13,144], promote AcCoA production through ATP-citrate lyase (ACLY)[145,146]. In particular, high AKT activity enables cancer cells to maintain histone acetylation even in glucose-depleted conditions[147], thus preventing histone acetylation from fluctuating with nutrient availability and maintaining pro-proliferative gene expression programs in a harsh microenvironment[140]. Recently, ^{13}C tracing combined with acetyl-proteomics identified lipid-derived AcCoA as a major source of carbon for histone acetylation[148], strengthening the established interconnection between this epigenetic mark and fatty acid homeostasis[35,149]. Furthermore, quantitative analysis of acetate utilization fluxes via stable isotope tracing revealed that, in hypoxic tumor areas, acetate is recycled via AcCoA synthetase 2 (ACSS2) in the nucleus, where it maintains histone acetylation by replenishing the nuclear AcCoA pool. The importance of this regulation is highlighted by the observation that deletion of ACSS2 reduces tumor burden in mouse models[54]. Yet, AcCoA is not only a substrate for histone acetylation in the nucleus, but also for fatty acid synthesis in the cytosol. Interestingly, despite the assumption that most metabolites can freely equilibrate between the cytosol and nucleus, AcCoA exchange appears to be fairly limited, which could result in different AcCoA concentrations in the nucleus and the cytosol[150]. Such compartment specific AcCoA concentrations could allow the decoupling of histone acetylation from the biosynthetic needs of cells[151–153].

Similar to histone methylation, histone acetylation is highly dynamic with an average acetyl-lysine half-life of 2-3 min[154,155]. Sirtuins catalyze the deacetylation of histones in a NAD^+ dependent reaction yielding O-acetyl-ADP-ribose, the deacetylated substrate, and nicotinamide as products (Figure 3d). In physiological conditions, circadian oscillations in NAD^+ levels correlate with fluctuations in SIRT1 histone deacetylase activity[101]. Although the role of sirtuins in histone deacetylation during tumorigenesis is complex and far from being fully understood[156], nicotinamide phosphoribosyltransferase (NAMPT) inhibitors, which reduce NAD^+ salvage pathways, are tested against cancers and partially act via altered sirtuin activity[157,158] and, potentially, a consequent histone deacetylation.

Thus, understanding the interaction between metabolism and the epigenetic state of the cell is a first step towards the coordinated manipulation of cellular programs that are driven by gene expression patterns.

Conclusions

The aberrant rewiring of cellular metabolism is a hallmark of cancer. Thus, targeting the metabolic network has become an attractive area for the development of new therapeutic strategies. However, due to the complexity of the disease, our mechanistic understanding of cancer metabolism is still fragmented, thus limiting the discovery of novel and effective metabolism-based therapies.

Further studies are needed in order to better define the influence of the microenvironment on cancer cellular metabolism. Recreating nutritional microenvironments *in vitro* that are more resembling the *in vivo* situation might be, depending on the context, a crucial factor to identify more physiological mechanisms. Moreover, increasing the complexity of *in vitro* systems by integrating the interactions between cancer cells and other cell types present in the *in vivo* microenvironment might drive our functional understanding a step forward.

Cancer is a dynamic disease resulting in temporal and spatial changes in the cellular phenotype. Thus, understanding the metabolic requirements of cancer cells associated to different cellular phenotypes has the potential to reveal novel therapeutic strategies, especially against cancer progression towards metastasis and dormancy-acquired drug resistance. On this matter, the development of reliable models recapitulating the *in vivo* situations and further technical advances to perform metabolomics and flux analysis in small *in vivo* cell population, are crucial in order to study and characterize the different metabolic vulnerabilities arising during the metastatic process.

Changes in specific metabolite concentrations are the basis of metabolic regulation, which influences (in addition to cell signaling) also the epigenetic state of cancer cells, generating gene expression patterns that sustain cancer progression. However, accounting for just the cell average metabolite concentrations is a simplification of a more complex and fine-tuned regulation: Nutrient sensing, metabolite concentrations and subcellular localization can be determinants of the epigenetic state, conferring selective advantages to cancer cells. Thus, further advances in techniques that allow investigating metabolism on the sub-cellular (i.e. the compartment level) are needed.

Tackling these challenges through the application of integrative experimental and computational approaches will be important for the design of new therapeutic strategies targeting the metabolic vulnerabilities of cancer cells during the different stages of cancer development and progression.

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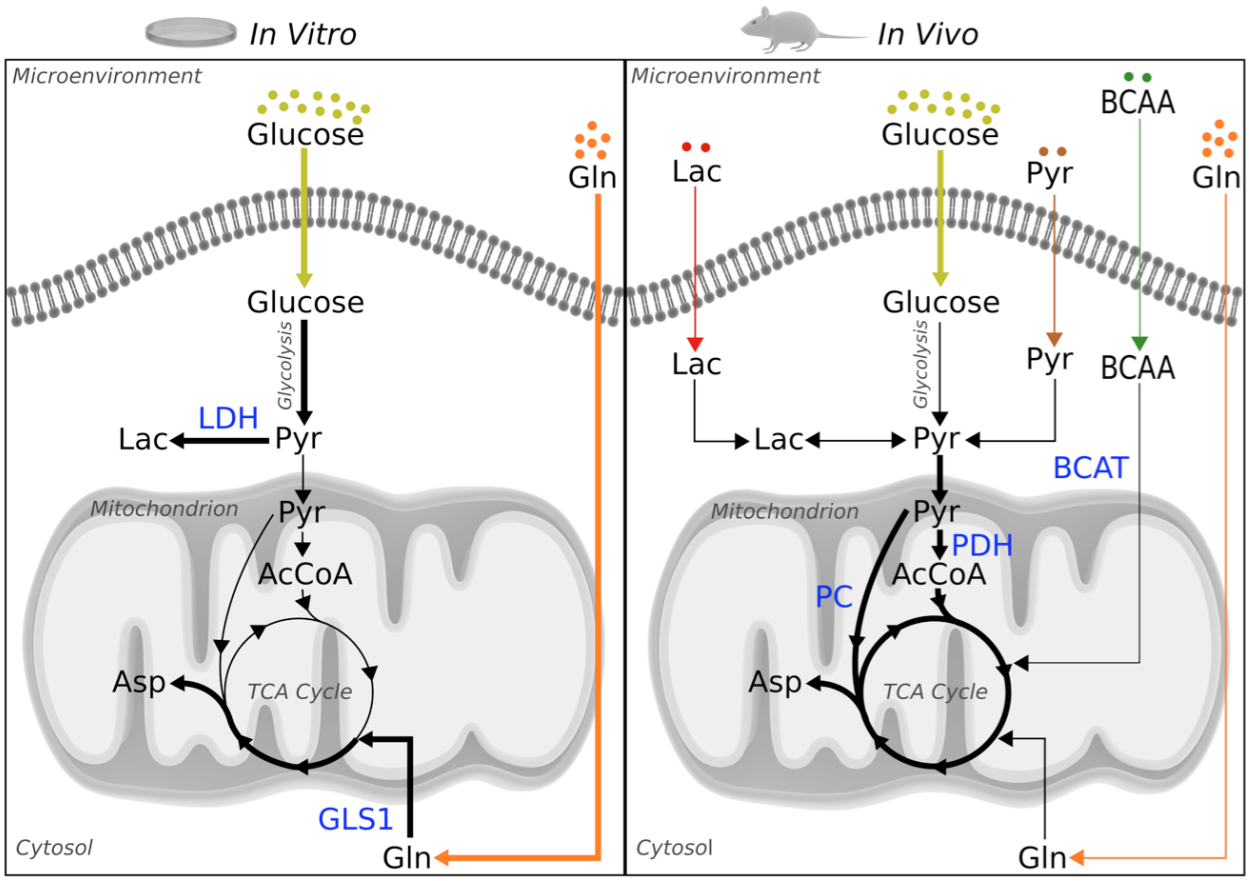

Figure 1. Metabolism is defined by the microenvironment.

The *in vitro* and *in vivo* microenvironment enforces a different nutrient usage in cancer cells. The depicted *in vivo* changes have been observed when cancer cells (originating from breast or lung cancers) proliferate in the lung microenvironment. *Arrows thickness* depicts flux magnitude. *Black arrows* represent metabolic pathways. *Colored arrows* represent the contribution of different extracellular nutrients to intracellular metabolism. Metabolic enzymes connected to the observed flux changes are depicted in *blue*. Only a selection of metabolic reaction within central metabolism is depicted.

Abbreviations: AcCoA, acetyl-CoA; Asp, aspartate; BCAA, branched-chain amino acids; BCAT, branched-chain amino acid transaminase 1; Gln, glutamine; GLS1, glutaminase1; Lac, lactate; LDH, lactate dehydrogenase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; Pyr, pyruvate; TCA cycle, tricarboxylic acid cycle.

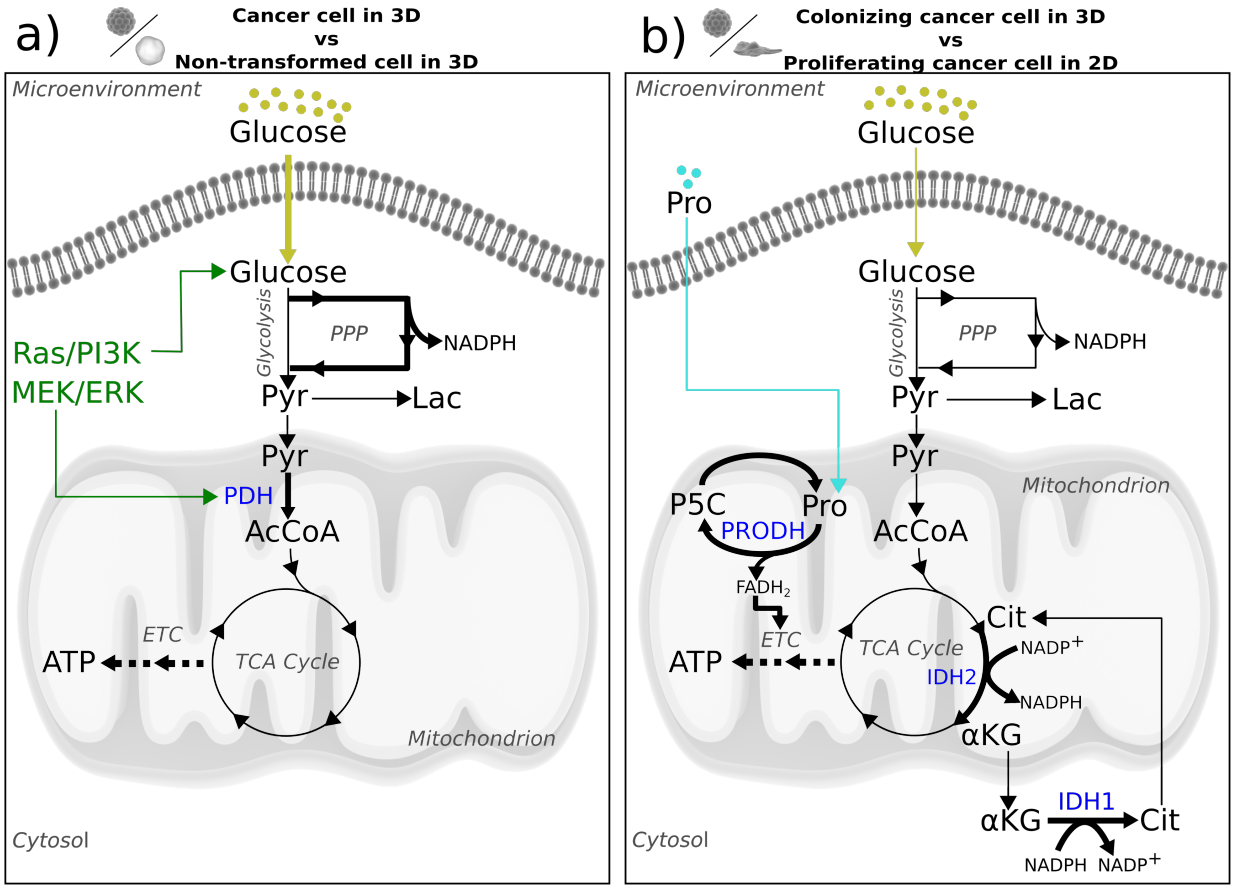

Figure 2. Metabolism is defined by the cellular phenotype.

a) Metabolic differences observed in colonizing cancer cells compared to colony forming non-transformed cells grown in 3D culture are depicted.

b) Metabolic differences observed in colonizing (3D) cancer cells compared to proliferating (2D) cancer cells are depicted. *Black arrows* represent metabolic pathways of central carbon metabolism. *Arrows thickness* depicts flux magnitude. *Colored arrows* represent the contribution of different extracellular nutrients to intracellular metabolism. Metabolic enzymes connected to the observed flux changes are depicted in *blue*. Oncogenic signaling pathways are depicted in *green*. Only a selection of metabolic reaction within central metabolism is depicted.

Abbreviations: αKG, α-ketoglutaric acid; AcCoA, acetyl-CoA; Asp, aspartate; ATP, adenosine triphosphate; BCAA, branched-chain amino acids; BCAT, branched-chain amino acid transaminase 1; Cit, citrate; ETC, electron transport chain; Gln, glutamine; GLS1, glutaminase1; Lac, lactate; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, reduced $NADP^+$; P5C, pyrroline 5 carboxylic acid; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PPP, pentose phosphate pathway; Pro, proline; PRODH, proline dehydrogenase; Pyr, pyruvate; TCA cycle, tricarboxylic acid cycle.

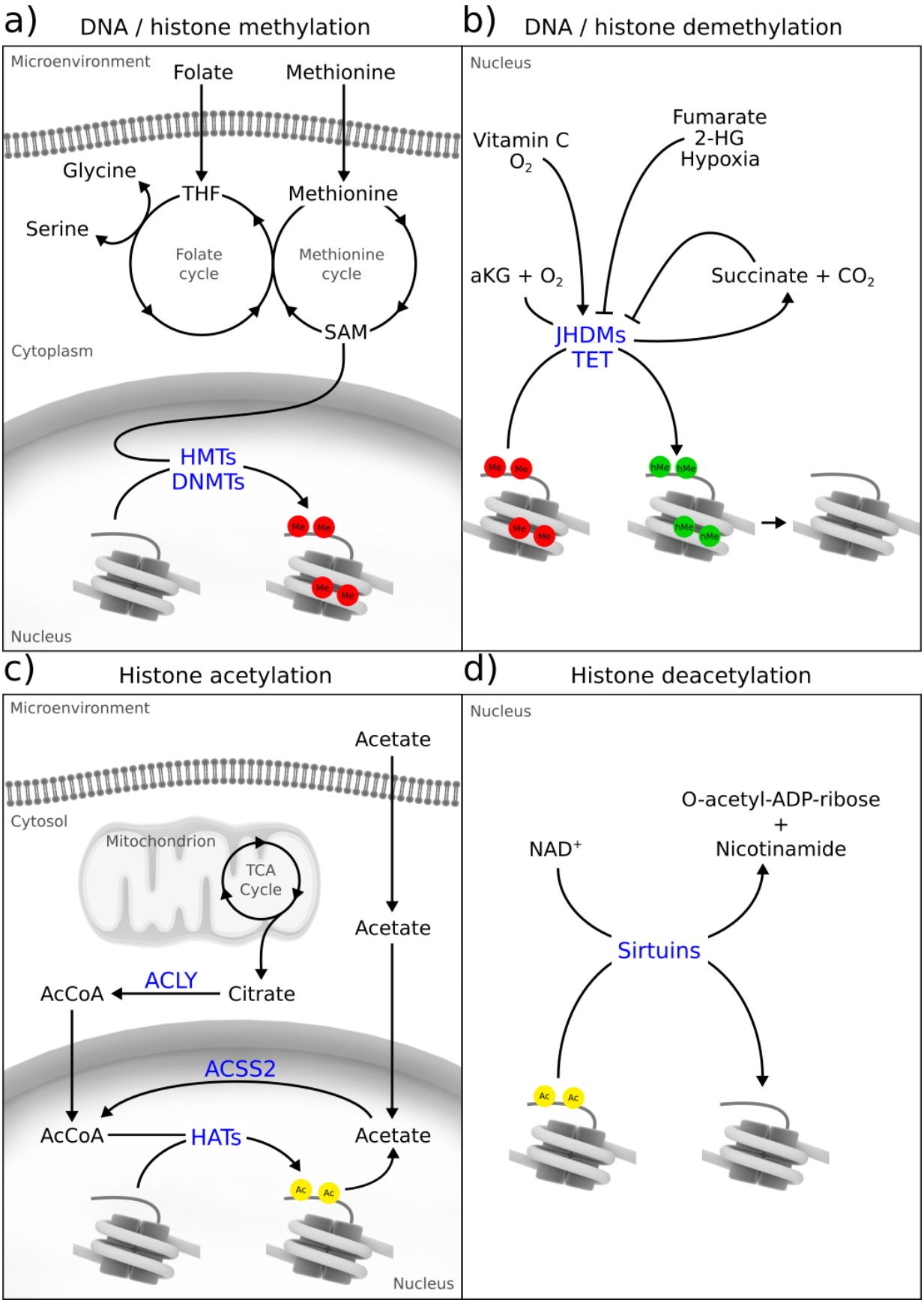
a)
DNA / histone methylation
Microenvironment
Folate
Methionine
Glycine
THF
Methionine
Serine
Folate cycle
Methionine cycle
SAM
Cytoplasm
HMTs
DNMTs
Me
Nucleus
b)
DNA / histone demethylation
Nucleus
Vitamin C
O_2
Fumarate
2-HG
Hypoxia
aKG + O_2
Succinate + CO_2
JHDMs
TET
Me
hMe
c)
Histone acetylation
Microenvironment
Acetate
Cytosol
Mitochondrion
TCA Cycle
Acetate
ACLY
AcCoA
Citrate
ACSS2
AcCoA
HATs
Acetate
Ac
Nucleus
d)
Histone deacetylation
Nucleus
O-acetyl-ADP-ribose
+
Nicotinamide
NAD^+
Sirtuins
Ac

Figure 3. Metabolism defines the epigenetic state.
a-b) Metabolic regulation of DNA/histone methylation and demethylation. c-d) Metabolic regulation of histone acetylation and deacetylation. TET enzymes and DMNTs target DNA, while JHDMs, HMTs, and HATs target histones. Enzymes are depicted in *blue*. Methylation is depicted in *red*. Hydroxymethylation is depicted in *green*. Acetylation is depicted in *yellow*. Only a selection of metabolic reaction within central metabolism is depicted.
Abbreviations: 2-HG, 2-hydroxyglutaric acid; AcCoA, acetyl-CoA; ACLY, ATP-citrate lyase; ACSS2, AcCoA synthetase 2; DNMTs, DNA methyltransferase; HATs, histone acetyltransferases; HMTs, histone methyltransferases; JHDMs, Jumonji-C (JmjC) domain-containing histone demethylases; NAD^+, nicotinamide adenine dinucleotide; SAM, S-Adenosyl methionine; TCA cycle, tricarboxylic acid cycle; TET, ten-eleven translocation enzymes; THF, tetrahydrofolate.

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Financial interest
The authors declare to have no competing financial interest.