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The metabolism of cancer cells during metastasis

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Abstract

Metastasis formation is the major cause of death in most patients with cancer. Despite extensive research, targeting metastatic seeding and colonization is still an unresolved challenge. Only recently, attention has been drawn to the fact that metastasizing cancer cells selectively and dynamically adapt their metabolism at every step during the metastatic cascade. Moreover, many metastases display different metabolic traits compared with the tumours from which they originate, enabling survival and growth in the new environment. Consequently, the stage-dependent metabolic traits may provide therapeutic windows for preventing or reducing metastasis, and targeting the new metabolic traits arising in established metastases may allow their eradication.

The metastatic establishment of cancers at distant organs is largely uncurable and primarily contributes to the deaths of cancer patients. Nonetheless, meta stasis formation itself is a rare event in tumours because cancer cells need to overcome multiple environmental hurdles before they can successfully manifest themselves in other organs[1,2]. As a first step, cancer cells need to become motile, invasive and intravasate the tumour vasculature to enter the bloodstream, either directly or via the lymphatic system. Although numerous cancer cells can find their way into the circulation, the majority will succumb during their journey, with only few able to extravasate, expand and successfully colonize other organs[3]. Clinical observations and multiple experimental studies have led to several propositions that can explain the acquired metastatic traits of cancer cells (as described and summarized elsewhere[2–7]). Regardless, whether a sequential acquisition of random mutations in tumours may provide and select for metastatic traits in rare tumour clones[8] or the metastatic predisposition of a tumour is already imprinted in the majority of cancer cells[4–7], cancer cells need to be able to continuously adapt to changing environments during metastasis.

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Thus, only very few metastatic cancer cells with the appropriate adaptations will colonize at distant sites[2,9]. This knowledge supports the proposition that cancers do not randomly metastasize but, rather, dependent on the tumour type, seed to specific organs. Around 1900, James Ewing argued that tumour cells seed in a circulation-dependent manner[10], while Stephen Paget proposed the 'seed and soil' hypothesis, stating that metastasizing cancer cells 'seed' only in certain especially hospitable tissues, akin to seeding in 'fertile soil'[11]. Since then, subsequent studies have revealed several classes of metastasis genes whose altered activities are needed at individual steps along the metastatic process[9]. The expression of gene clusters associated with metastasis initiation and progression has been shown to confer a selective advantage to cancer cells both at primary and secondary sites as well as in the circulation, whereas protein products of metastatic virulence genes only promote survival and proliferation during colonization at the metastatic site, suggesting also a specific role in their mediation of organ-specific metastases[12–14]. Organotropism is facilitated by multiple factors including tumour-intrinsic factors, organ-specific niches and the interaction between tumour cells and the host microenvironment[15,16]. Furthermore, through the secretion of specific factors and extracellular vesicles, tumours are able to establish a pre-metastatic niche in distant organs[17,18]. These factors, for example, can activate distal lymphangiogenesis, recruit bone marrow-derived cells and promote extracellular matrix remodelling by activating stromal fibroblasts, which creates a permissive microenvironment for cancer cell colonization[19–21].

Metastatic virulence genes

Genes or factors that confer proliferation and/or survival advantages to metastasizing cancer cells at the secondary site without affecting primary tumours.

Only recently, attention has been drawn to the fact that metastatic cells also need to have or gain certain metabolic traits to be able to survive and grow in the new soil[22–24], which may substantially vary in its nutrient and oxygen availability from the primary site. Such metabolic rewiring can be controlled transcriptionally, for example through epigenetic alterations, but also post-translationally or through metabolite availability to enzymes[25]. In this Review, we discuss growing evidence for the dynamic metabolic landscape metastasizing cells display to survive and propagate during the different steps of the metastatic cascade.

Metabolic adjustments during metastasis

Accumulating evidence supports the presence of dynamic changes in the metabolism of metastasizing cells, contributing to their ability to successfully transition through the changing microenvironments of the metastatic cascade. Here, we link the metabolic changes in cancer cells that traverse the metastatic cascade to the two intertwined concepts of metabolic plasticity and metabolic flexibility (FIG. 1). Similar to and extending beyond the previously described divergence in metabolism[24,26], metabolite plasticity describes metastasizing cells that can use one metabolite to fuel the various metabolic requirements of different steps of the metastatic cascade. In contrast, metabolite flexibility builds on the well-described nutrient flexibility of cells[27,28] and refers to metastasizing cancer cells that

can use different metabolites to meet the same metabolic requirement imposed by a specific step of the metastatic cascade.

Metabolic plasticity

A metabolite can be used for multiple purposes.

Metabolic flexibility

Several metabolites can be used for the same purpose.

Divergence in metabolism

Divergent properties appear in distinct molecular subsets of cancer and contribute to metabolic heterogeneity.

Metabolite plasticity

It is conceivable that many nutrients can contribute to metabolite plasticity during metastasis formation. Yet the majority of metabolic research has focused on the early metastatic steps of tumour migration and invasion whereas only few nutrients have been analysed at several metastatic steps. Here, we discuss the implication of four such nutrients, namely lactate, pyruvate, glutamine and fatty acids, in regulating tumour invasion, survival in the circulation and colonization at a secondary site (FIGS 2,3; TABLE 1).

Pyruvate and lactate metabolism

Pyruvate is produced from glucose and other nutrients that fuel the glycolytic pathway, and can give rise to lactate in a one-step reaction catalysed by lactate dehydrogenase (LDH). In addition, pyruvate and, consequently, lactate can be produced from glutamine and other amino acids that fuel the tricarboxylic acid (TCA) cycle via malic enzyme (ME) or pyruvate carboxy kinase (PCK), which funnel TCA cycle-derived carbon towards the lower part of glycolysis. Pyruvate (and lactate) can be reversibly converted to alanine, resulting either in the production of alanine from pyruvate or vice versa.

Additionally, pyruvate and, consequently, lactate can be oxidized in the mitochondria to acetyl-CoA by the enzyme pyruvate dehydrogenase (PDH) or converted by the enzyme pyruvate carboxylase (PC) to oxaloacetate; the latter replenishes the TCA cycle (anaplerosis). Yet both lactate and pyruvate are also nutrients that can be taken up by cells directly from the environment.

Anaplerosis

Refilling of the tricarboxylic acid (TCA) cycle with carbon.

Pyruvate and lactate metabolism in invading and motile cancer cells.—One of the first metastatic attributes of cancer cells is their switch from a proliferative to a migrating phenotype. Multiple studies have provided evidence that metabolic changes in cancer cells modulate activity of signalling pathways and global gene expression programmes driving migration and invasion (for example, an epithelial to mesenchymal transition (EMT))[23]. These metabolic adaptations are not just consequential bystander effects because metabolites such as pyruvate and lactate can directly promote the invasion and migration ability of cancer cells (FIG. 2a). In breast cancer cells, the anaplerotic entry of pyruvate via PC into the TCA cycle promoted an invasive phenotype by increasing their motility, although the underlying molecular mechanism remains elusive[29,30]. Pyruvate oxidation requires PDH activity. The resulting acetyl-CoA production and further accumulation of acetyl-CoA, through phosphorylation-induced inhibition of its consuming enzyme acetyl-CoA carboxylase 1 (ACC1)[31], led to the acetylation of the transcription factor Smad2, which is a known inducer of mesenchymal gene expression patterns[31]. Gene expression analysis of 20 different human cancers has shown that poor survival was associated with inhibition of at least one mitochondrial pathway. Oxidative phosphorylation was the most affected pathway in patients with a low versus high survival rate, and most frequently coincided with downregulated gene expression of the genes encoding the subunits of Complex I and IV of the respiratory chain[32]. Functionally, expression of genes contributing to oxidative phosphorylation showed a negative correlation with EMT[32]. Accordingly, metastasis-derived cell lines in vitro and metastases analysed ex vivo from a lung cancer mouse model had reduced mitochondrial functionality compared with non-metastatic primary tumours[33]. Although these changes in mitochondrial metabolism will have multiple effects, they may indicate a shift towards glycolytic energy production that requires lactate synthesis. Indeed, inhibition of lactate dehydrogenase A (LDHA) expression impaired invasion and migration in in vitro assays in renal cell carcinoma (RCC), pancreatic cancer and prostate cancer[34–36] and decreased metastasis in an orthotopic renal xenograft model[36]. Moreover, downregulation of microRNA30a-5p suppressed LDHA expression and thereby inhibited gallbladder cancer progression as well as metastasis formation in breast cancer mouse models[37,38], whereas knockdown of the oncogene c-MYC decreased LDHA expression in in vitro models of pancreatic cancer[35]. LDHA has also been found to be phosphorylated by HER2 and SRC[39]. Inhibition of this phosphorylation resulted in decreased invasiveness of head and neck as well as breast cancer cell lines in vitro and metastatic potential in breast cancer xenograft mouse models[39]. Interestingly, LDHA silencing could be rescued by lactate and the antioxidant *N*-acetylcysteine in in vitro assays, suggesting a redox metabolism-dependent mechanism[39].

The transporters monocarboxylate transporter 1 (MCT1) and MCT4 preferentially exchange lactate between the extracellular and intracellular space[40]. Both proteins are independent prognostic markers for progression-free survival in clear cell RCC[41], whereas high levels of MCT1 expression have been associated with a lower survival rate in patients with bladder cancer[42]. MCT1 expression also increased during glucose starvation in cervical cancer cell lines[43] and during starvation-induced autophagy in hepatocellular carcinoma cell lines[44], which are conditions that may occur in poorly perfused and hypoxic regions of the tumour microenvironment. Mechanistically, increased levels of MCT1 in cervical cancer cell

lines can lead to the formation of a heterocomplex with CD147 (REF.[43]), a protein that stimulates secretion of matrix metalloproteinases (MMPs) and cytokines in many cancers[45]. This interaction was shown in oxidative phosphorylation-dependent tumour cells where it increased migratory capacities in vitro[43]. Notably, MCT1, independent of its lactate transport function, has been shown to activate NF-κB, which is an upstream regulator of a pro-invasive EMT in cervix squamous carcinoma cell lines and an experimental mammary carcinoma mouse model[46], and promoted migration and invasion in osteosarcoma cell lines[47]. Interestingly, pH changes, which may result from lactate production, also activated NF-κB in a breast cancer cell line[48]. In addition, lactate has been shown to promote breast cancer progression by supporting chemoattraction, which stimulated cancer cell migration in a breast cancer cell line[49]. Accordingly, intraperitoneal administration of lactate to mice increased lung metastasis formation resulting from intravenously injected breast cancer cells[49]. Recently, lactate has also been identified as a precursor of histone lactylation[50]. It will be very interesting to reveal whether this post-translational histone modification provides another regulatory mechanism in tumour invasion.

Taken together, the studies described above support the notion that lactate and pyruvate metabolism can directly promote cancer invasion by inducing various signalling pathways and molecules that drive and facilitate tumour cell migration and invasion.

Pyruvate and lactate metabolism in circulating tumour cells.—Cancer cells need to increase their antioxidant defence in the circulation[51–53] to avoid matrix detachment-induced cell death (anoikis, reviewed elsewhere[54,55]). Lactate and pyruvate metabolism may potentially contribute to resistance of matrix-detached cells against reactive oxygen species (ROS) (FIG. 2b). Studies in vitro and in patients revealed that pyruvate concentrations correlate with matrix detachment and invasiveness. As such, pyruvate levels were increased intracellularly in matrix-detached 293T cells[56], whereas highly invasive ovarian cancer cells exhibited an increased pyruvate uptake in matrix-detached conditions compared with less invasive ovarian cancer cells[57]. In patients, levels of pyruvate were elevated in the plasma of individuals with metastatic carcinoma of different origin compared with healthy individuals[58]. Congruently, pyruvate concentrations in serum of patients with aggressive metastatic breast cancer were higher compared with those of patients with early-stage breast cancer[59]. Interestingly, a study in non-malignant and malignant cells indicates that pyruvate itself can act as an antioxidant via a non-enzymatic reaction with hydrogen peroxide[60]. Moreover, it has been demonstrated that the serum lactate concentration was higher in patients with metastatic compared with non-metastatic colorectal cancer[61]. Thus, these observations suggest that pyruvate and lactate are available to circulating tumour cells and could support their successful transition through the blood circulation. Corroborating the idea that pyruvate and lactate metabolism can protect against ROS, the disruption of intracellular conversion of pyruvate via PC to oxaloacetate in cultured breast cancer cells resulted in a decreased ratio of $NADPH/NADP^+$ and glutathione (GSH)/GSSG, indicating reduced ROS scavenging capacity and, consequently, causing elevated oxidative stress[62]. Striking evidence has been provided for an important role of lactate metabolism in circulating tumour cells. In particular, MCT1-mediated uptake of lactate facilitated

melanoma metastasis in patient-derived xenografts by promoting pentose phosphate pathway flux and a consecutive ROS defence[63].

Matrix-detached cells can induce hypoxia via cell clustering to reduce ROS[56]. Interestingly, intracellular pyruvate or lactate accumulation can induce a hypoxic response by stabilizing hypoxia inducible factor 1α (HIF1α)[64–66]. In the case of lactate, this can also occur via *N*-Myc Downstream-Regulated Gene 3 Protein (NDRG3) stabilization[67]. Thus, it is tempting to speculate that increased lactate and pyruvate concentrations in the blood circulation may support circulating tumour cells and cell clusters by facilitating a hypoxic response.

In conclusion, there is supporting evidence that lactate and pyruvate uptake can help circulating tumour cells to survive by enhancing their resistance against oxidative stress.

Pyruvate and lactate metabolism during metastatic colonization.—Once cancer cells reach a distant organ, they need to adapt to the new environment and also create a permissive niche in order to proliferate (FIG. 3; TABLE 1). Colonizing cancer cells are likely exposed to different types and/or levels of nutrients at the secondary site compared with the primary site. For example, pyruvate is enriched in lung interstitial fluid compared with plasma[68], with the lung being an organ of frequent metastasis across several cancer types. Thus, it is conceivable that cancer cells utilize pyruvate when undergoing metastatic colonization in the lung. Indeed, there is evidence from mouse models that breast cancer cells colonizing in the lung require extracellular pyruvate, but not lactate, to create a permissive niche through extracellular matrix remodelling[69]. In particular, increased levels of pyruvate facilitated transamination between glutamate and pyruvate (catalysed by the mitochondrial enzyme alanine aminotransferase 2 (ALT2; also known as GPT2)), leading to the generation of α-ketoglutarate and alanine[69]. In turn, α-ketoglutarate boosted activity of the enzyme collagen prolyl-4-hydroxylase (P4HA)[69], which independent studies have reported to be essential for collagen deposition and remodelling[70]. Yet, the role of pyruvate in the lung environment may go beyond collagen hydroxylation because expression of PC was required for breast cancer-derived lung metastases but not for extrapulmonary metastases in an experimental mouse model[71]. Moreover, pyruvate availability has been shown to increase activity of the serine biosynthesis pathway, which potentiated mTORC1 signalling in lung metastases but not primary breast tumours[72].

Besides the above described findings, the number of studies that particularly investigate the impact of pyruvate and lactate metabolism in metastatic colonization in an in vivo setting is rather limited. However, supporting evidence for the importance of these two nutrients for metastatic colonization has been provided in vitro via colony formation assays, which may to some extent mirror a similar metabolic programme to that of in vivo metastatic colonization[73]. For example, pyruvate supplementation can stimulate in vitro colony formation in breast cancer cells by fuelling mitochondrial respiration[74] and MCT1 inhibition with AR-C155858 had a cytostatic effect on colony growth in different cancer cell lines[75]. Similarly, blocking lactate and pyruvate entry into the mitochondria through inhibition of the mitochondrial pyruvate carrier (MPC) with 7ACC2 elicited cytotoxic effects on colony growth in different cell lines[75]. Whereas there is evidence that pyruvate and lactate fuel in vivo tumour proliferation[68,76], the full extent to which lactate and

pyruvate are important nutrients for cancer cells to seed and colonize in the metastatic niche is largely unknown.

Glutamine metabolism

Glutamine is the most abundant free amino acid in the plasma. Many cancer cells take up glutamine, which contributes to non-essential amino acid as well as nucleotide synthesis through carbon or nitrogen metabolism. Moreover, glutamine can be converted in the mitochondria to replenish the TCA cycle (anaplerosis) or can be fully oxidized to produce ATP (glutaminolysis).

Glutaminolysis

Full oxidation of glutamine.

Glutamine metabolism in invading and motile cancer cells.—Glutamine metabolism has been extensively studied in proliferating cancer cells[77]. Emerging evidence suggest that glutamine metabolism is also important for invasion (FIG. 2a; TABLE 1). Invasive, but not non-invasive, ovarian cancer cells displayed a dependency on glutamine availability in vitro[78], and metastatic melanoma cells exhibited elevated glutamine oxidation[79]. Moreover, mRNA expression levels of glutaminase 1 (*GLS1*), which catabolizes glutamine to glutamate, correlated with lymph node metastasis of colorectal cancer, and GLS1 expression was required for hypoxia-mediated cancer cell migration in vitro[80]. Moreover, SOX12 overexpression compared with control was found to promote colorectal cancer metastasis via GLS1 in experimental mouse models[81]. The isoenzyme GLS2 repressed hepatocellular carcinoma metastasis based on protein interactions with the small GTPase RAC1 (REF.[82]) and with Dicer[83]. The latter interaction inhibited the pro-invasive regulator and EMT transcription factor Snail[83]. Specifically, GLS2 binds to small GTPase RAC1 and inhibits its interaction with the RAC1 activators guanine-nucleotide exchange factors, which in turn inhibits RAC1 to suppress cancer metastasis[82]. Moreover, GLS2 also interacts with Dicer and stabilizes Dicer protein to facilitate miR-34a maturation, and subsequently represses Snail expression[83]. Both interactions are independent of the catalytic function of GLS2.

Glutamine-derived glutamate can have different fates, including secretion via the cystine antiporter xCT (also known as SLC7A11), expression of which has been identified as a predictive marker of recurrence, tumour invasion, lymph node metastasis and venous invasion in patients with colorectal cancer[84]. Mechanistically, secretion of glutamate via xCT can lead to paracrine activation of the metabotropic glutamate receptor GRM3, which in turn upregulates Rab27-dependent recycling of the transmembrane membrane type 1 (MT1)-MMP to promote the invasive behaviour of breast cancer cells[85]. Accordingly, xCT inhibition with sulfasalazine impaired lung metastasis through a ROS-dependent p38 MAPK activation in breast cancer and oesophageal squamous cell carcinoma mouse models[86,87]. In the latter scenario, disruption of xCT enhanced homotypic cell–cell adhesion and attenuated cell–extracellular matrix adhesion[87].

Thus, cancer cells can rely on glutamine and cystine metabolism to alter cell adhesion and activate invasive cues.

Glutamine metabolism in circulating cancer cells.—So far, evidence of glutamine metabolism in supporting circulating tumour cells is sparse (FIG. 2b,c). Reduced glutamine concentrations and markedly elevated glutamate concentrations have been identified in the plasma of patients with oesophageal squamous cell carcinoma presenting lymph node metastasis compared with those who did not present with metastasis[88]. In line with this, circulating tumour cells with tumour-initiating capacity ($CD44^{+/high}$ versus $CD44^{-/low}$) from patients with gastric cancer have increased expression of xCT[89]. Thus, it is conceivable that increased xCT expression may explain the above discussed elevated levels of glutamate in the plasma of patients with metastatic oesophageal squamous cell carcinoma because xCT overexpression may favour the secretion of glutamate. Yet, the intracellular metabolism of glutamate seems also to be linked to metastatic cancers. As such, glutamate dehydrogenase (GDH), which converts glutamate to α-ketoglutarate, has been identified as a prognostic marker of colorectal cancer metastasis[90]. Indeed, GDH knockdown or inhibition with R162 attenuated anoikis resistance and decreased tumour metastasis through CamKK2–AMPK signalling in an LKB1-deficient lung cancer mouse model[91]. Thus, different fates of glutamine may affect circulating tumour cells. For a broader understanding and interpretation of the above described observations, further investigations are required.

Glutamine metabolism during metastatic colonization.—Recent studies have described changes in glutamine metabolism of colonizing cancer cells (FIG. 3; TABLE 1). In this respect, inhibition of the glutamine transporter ASCT2 (also known as SLC1A5) with short hairpin RNA knockdown was shown to impair primary prostate cancer growth and lung, but not liver, metastasis in a mouse model[92]. Accordingly, treatment of the VM-M3 mouse model of systemic metastatic cancer with the glutamine analogue 6-diazo-5-oxo-l-norleucine (DON) reduced metastasis to the liver, lung and kidney[93]. Moreover, anti-xCT vaccination inhibited arising and established lung metastasis nodules in breast cancer mouse models[94]. On the molecular level, xCT activity regulated cancer stem cell self-renewal and the intracellular redox balance in breast cancer cells[94]. These data show that glutamine and cystine metabolism can support cancer cells to settle in distant organs.

Fatty acid metabolism

Fatty acids are an important fuel in anabolic and catabolic processes. Fatty acids can be synthesized de novo or taken up from the extracellular space. Newly synthesized fatty acids are often further desaturated to monounsaturated fatty acids, whereas the generation of most polyunsaturated fatty acids requires the uptake of essential fatty acids such as linoleic acid. Fatty acids are thereby important building blocks of lipids in cell membranes, and their desaturation status and double-bond position can define physical, chemical and biological properties such as membrane fluidity and peroxidation sensitivity[95,96]. Additionally, fatty acids can serve as important signalling molecules[25,97]. Moreover, many fatty acids can be oxidized in the mitochondria to acetyl-CoA, whereas specific long-chain and branched-chain fatty acids are oxidized in the peroxisomes.

Peroxidation

A chemical reaction between mainly unsaturated fatty acids and the reactive forms of oxygen.

Fatty acid metabolism in invading and motile cancer cells.—Multiple studies have linked obesity to cancer progression, metastasis formation and mortality in several cancer types, including prostate cancer, melanoma and breast cancer[98–101]. Although several mechanisms can contribute to this correlation, lipids have been functionally implicated in several steps of the metastatic cascade (FIG. 2a; TABLE 1). Indeed, a metabolomics analysis of meta static versus non-metastatic oral squamous carcinoma cell lines showed differences in lipid metabolism[102]. In various cancers, increased fatty acid uptake, lipid accumulation and/or overexpression of genes encoding fatty acid transporters or other fatty acid metabolism genes induced invasive and migratory traits of cancer cells compared with control conditions, elevated the seeding capacity of tumour cells in distant organs in several mouse models[103–106] and was associated with metastatic progression as well as poor prognosis in patients with various cancers[105].

To explore a potential mechanistic link between fatty acid availability and metastasis formation, commonalities in gene expression across fatty acid-rich and cancer-associated environments have been analysed. Thereby, CD36, a transmembrane protein that facilitates the import of fatty acids into the cell, was found to be induced in ovarian cancer cells when co-cultured with adipocytes[107], in cervical cancer of mice in response to a fat-enriched compared with control diet[108] and in vitro in oral squamous carcinoma cells exposed to palmitate (compared with non-palmitate) supplementation in the culture media[103]. Congruently, the breast-associated adipocyte secretome enabled in vitro fatty acid uptake and invasiveness in breast cancer cells via induction of CD36 expression[109]. Moreover, CD36 was highly expressed in several metastasis-initiating compared with non-metastatic cancer cells[103]. Highlighting the relevance of these observations, elevated CD36 expression levels predicted poor prognosis in patients with clear cell RCC[110] as well as glioblastoma[111], and accelerated gastric cancer metastasis in experimental mouse models[112,113]. Conversely, CD36 inhibition (with Nobiletin, sulfo-*N*-succinimidyl oleate or CD36–short hairpin RNA-mediated knockdown) impaired angiogenesis as well as migration and invasion of breast cancer cell lines[114,115]. Silencing of CD36 in preclinical mouse models of prostate cancer also reduced fatty acid uptake, as well as the abundance of oncogenic signalling lipids, and slowed cancer progression compared with control conditions[116]. Different mechanisms have been identified as potentially responsible for the ability of CD36 to facilitate cancer cell invasion and motility. CD36-associated fatty acid uptake also promoted an EMT in hepatocellular carcinoma cell lines[106], whereas the pro-metastatic effect of CD36 in cervical cancer was synergistic with a TGFβ-induced EMT in cell lines[117].

Fatty acid binding proteins (FABPs) can likewise facilitate fatty acid uptake by cells. In this respect, expression of FABP4 (also known as A-FABP) was associated with metastatic potential, cancer progression and mortality of patients with ovarian cancer, and

expression of FABP5 (also known as E-FABP) was accompanied by these parameters in patients with clear cell RCC and colorectal cancer[118–120] (FIG. 2b). FABP5 induced a pro-metastatic EMT phenotype in hepatocellular carcinoma cell lines with FABP5 overexpression whereas knockdown cell lines showed the opposite phenotype[121]. Moreover, FABP5 silencing impaired MMP expression in cervical cancer cells in vitro and in vivo[122], and decreased invasion and migration of gastric cancer cell lines in vitro[123]. Additionally, FABP5 expression was required in conjunction with fatty acid synthase (FASN) and monoacylglycerol lipase (MAGL) to support prostate cancer progression in mouse models[124]. Similarly, overexpression of the liver-specific FABP1 (also known as L-FABP) promoted angiogenic properties and migration in hepatocellular carcinoma cell lines and increased the number of liver metastases[125]. Further, FABP1 is highly expressed in 44% of patients with melanoma, enabling melanoma cells to increase their invasive potential through uptake of adipocyte-derived lipids[126].

Cancer cells can also synthesize fatty acids de novo from various nutrients. In this respect, there is some evidence that de novo fatty acid synthesis contributes to the invasion and migration capacity of cancer cells. Inhibition of FASN with the compound orlistate impaired melanoma-induced metastases and angiogenesis in a mouse model[127], and silencing of FASN attenuated CD44 expression-induced signalling and metastasis formation in colorectal cancer mouse models[128]. Mechanistically, FASN-mediated de novo lipogenesis regulated expression of CD44 at a post-transcriptional level, possibly by altering its palmitoylation[128]. In an independent study, the proliferation, adhesion and migration of colorectal cancer cells in vitro was promoted by FASN-driven sphingolipid metabolism modulating focal adhesion signalling[129].

Fatty acids, regardless of whether they are taken up or synthetized de novo, can be further processed in the cell. This includes monodesaturation via the isoenzymes stearoyl-CoA desaturase 1 (SCD1) and SCD5, both producing the same fatty acids, for example palmitoleate, or via the enzyme fatty acid desaturase 2 (FADS2). Although FADS2 is mainly known for its function in polyunsaturation, it was recently discovered as an alternative metabolic route of monodesaturation in cancers leading to the production of the fatty acid sapienate[130,131]. SCD1 and SCD5 have been already studied in the context of cancer cell invasion and migration. High levels of SCD1 expression were prognostic in patients with colorectal cancer and supported cancer cell migration in vitro through monounsaturated fatty acid production compared with SCD1-silenced cancer cells[132,133]. In line with this, blocking fatty acid desaturation with the SCD inhibitor CAY10566 and the FADS2 inhibitor SC-26196 impaired NF-κB signalling, resulting in decreased stemness properties of ovarian cancer cells compared with vehicle-treated controls[134]. By contrast, blocking SCD1 with A939572 compared with vehicle treatment triggered endoplasmatic reticulum stress in several melanoma cells and ovarian cancer cells, which then led to enhanced invasion and metastatic dissemination to the lung of mice[135]. Thus, SCD1 inhibition has led to controversial results, either reducing or increasing metastasis formation in melanoma (TABLE 1), which may be dependent on the presence of an endoplasmatic reticulum stress response and the Melanocyte Inducing Transcription Factor (MITF) status. Expression of SCD5 was suggested to reduce melanoma-derived metastasis by impairing the secretion of

the extracellular matrix-modifying protein SPARC[136]. To date, it remains to be determined to what extent FADS2-produced sapienate plays a role in metastasis formation.

Beyond desaturation, other fatty acid modifications can occur that foster cancer progression. For example, there is evidence that inhibition of cholesterol esterification through targeting cholesterol acyltransferase (ACAT) suppressed the development and growth of metastatic lesions in prostate cancer[137] as well as pancreatic cancer[138] mouse models and reduced in vitro cell migration in Lewis lung carcinoma cells[139]. In prostate cancer cell lines, inhibition of cholesterol esterification blocked secretion of Wnt3a through reduction of mono unsaturated fatty acids, which limited Wnt3a acylation and, consequently, cancer cell migration[137], whereas in pancreatic cancer cell lines it induced endoplasmatic reticulum stress through cholesterol accumulation[138].

Fatty acids can be oxidized to generate acetyl-CoA. Major fatty acid oxidizing organelles are the mitochondria into which many long-chain fatty acids, such as palmitate, are transported depending on carnitine palmitoyltransferase 1 (CPT1). Elevated fatty acid oxidation has been observed in breast and ovarian cancer cells in co-culture with adipocytes compared with sole monocultures[140,141], whereas CPT1A-silenced breast cancer cells lacked the ability to effectively drive tumour-associated lymphangiogenesis because of decreased VEGF-C and VEGF-D expression[142]. Further, a splice variant of *CPT1A* supported cancer cell survival and invasiveness by promoting histone deacetylase (HDAC) activity in the nucleus through protein–protein interaction with HDAC1 (REF.[143]). In addition, fatty acid oxidation can generate mitochondrial ROS, which is known to facilitate an EMT in different cancer cell lines[144]. Finally, the breakdown of odd-chain fatty acids, cholesterol and certain amino acids can lead to the production of methyl malonic acid (MMA). This metabolite was recently found to be elevated in the serum of old compared with young human individuals and to drive cancer progression in breast cancer mouse models due to the induction of a Sox4-mediated EMT[145].

In summary, fatty acid metabolism can increase tumour cell migration and invasion by altering cellular signalling cues and regulation of epigenetic modifiers.

Fatty acid metabolism in circulating cancer cells.—Higher lipid serum concentrations have been detected in colorectal and breast cancer patients with distant metastases compared with patients without metastases[146,147]. There was, however, no correlation between lipid serum levels and metastases in patients with oral squamous cell carcinoma[148]. Thus, it appears that, at least in certain tumour types, metastasizing cancer cells can be exposed to higher lipid levels in the blood circulation (FIG. 2b,c). Given that exogenous lipids could be metabolized by circulating cancer cells, this could suggest that their metabolism can potentially support survival in the circulation. Congruently, blocking fatty acid oxidation by CPT1A silencing led to ROS accumulation in matrix-detached colorectal cancer cell lines[149]. The accumulation of ROS has been shown to be detrimental for matrix-detached cells in vitro[150] and circulating tumour cells in vivo[51,52,151]. In line, fatty acid oxidation-generated acetyl-CoA supported calcium/calmodulin-dependent kinase II (CaMKII) activity in prostate cancer cells that resulted in reduced anoikis and cell migration[152]. Thereby, the presumed mechanism may depend on acetyl-CoA-derived

CoA, generated locally by a yet to be identified acetyl-CoA hydrolysing reaction, and consecutive binding to the calmodulin (CaM)-binding domain of CaMKII to promote CaM binding and activation of CaMKII at basal calcium concentrations[153]. Moreover, a recent study demonstrated in mice bearing patient-derived melanomas that cancer cells metastasizing through the lymphatic system conveyed reduced levels of ROS-induced ferroptosis compared with cells metastasizing through the blood vascular system[154]. This was based on increased availability of oleic acid-containing vesicles in the lymph compared with blood that allowed circulating cancer cells to take up oleate, resulting in less desaturated cell membranes and reduced sensitivity to lipid peroxidation[154]. Interestingly, cancer cells that disseminated through the lymph and then entered the blood circulation were protected from ferroptosis compared with cancer cells directly disseminating into the blood circulation[154]. Thus, fatty acids may be available to and protective of certain circulating cancer cells, depending on the metastatic route.

Fatty acid metabolism during metastatic colonization.—Little is known about the availability of fatty acids in the different sites of metastasis relative to the primary tumour tissues but there is ample evidence that fatty acid uptake and metabolism can boost the nesting of metastasizing cancer cells in multiple organs (FIG. 3; TABLE 1). Whereas targeting CD36 had only minor to no effects on primary tumour growth, it dramatically impaired metastasis formation in the lungs and lymph nodes (and likely other organs) of mice bearing oral carcinomas[103] (FIG. 3a,b). In line with this observation, long non-coding RNA LNMICC promoted lymph node metastasis through FABP5-mediated fatty acid metabolism in cervical cancer mouse models[155], whereas FABP4 inhibition increased α-ketoglutarate concentrations and, consequently, increased DNA demethylation through regulation of ten–eleven translocase (TET) enzymes in vitro and reduced omental colonization in a mouse model of ovarian cancer[156] (FIG. 3c).

In addition to fatty acid uptake, de novo fatty acid synthesis has also been implicated in the ability of cancer cells to colonize a distant organ. Accordingly, FASN overexpression resulted in increased peritoneal metastasis of ovarian cancers in mice, and promoted cellular colony formation and metastatic ability in vitro[157] (FIG. 3c). CD147 knockout in hepatocellular carcinoma cells decreased fatty acid synthesis by impairing the Akt/mTOR signalling pathway and upregulated peroxisome proliferator-activated receptor-α (PPARα), resulting in increased proliferation and metastasis formation compared with control in cell lines and a mouse model[158]. Similarly, hyperactivation of sterol regulatory element-binding protein (SREBP), a downstream target of mTOR, by *Pten* and *Pml* double-null compared with *Pten*-null genetic modification of mouse prostates was capable of promoting prostate cancer metastasis by upregulating de novo fatty acid synthesis[98] (FIG. 3b). In addition, the fatty acid monodesaturating enzyme SCD1 was induced in melanoma cells when they were co-cultured with lung fibroblasts, and genetically silencing SCD1 in cancer cells impaired metastasis formation and prolonged their survival of mice injected with melanoma cells[159]. Yet it has also been shown that an increased ratio of monounsaturated to saturated fatty acids can result in mitochondrial dysfunction and, consequently, reduced breast cancer metastasis to the lung[160] (FIG. 3a). In line with this and as discussed above, elevation of saturated fatty

acids, for example through SCD1 inhibition, increased melanoma-derived lung metastasis through inducing invasion in a mouse model[135].

Little is known about the role of fatty acid oxidation in metastatic colonization. In a mouse model of ovarian cancer, salt-inducible kinase 2 (SIK2)-phosphorylated ACC and SIK2 overexpression compared with control promoted fatty acid oxidation required for effective metastasis formation[141] (FIG. 3c). Moreover, inducible silencing of YAP in melanoma cells in mice showed that cancer cells were critically dependent on YAP-induced fatty acid oxidation to seed in the lymph node, but not in the lung[161] (FIG. 3b).

In summary, fatty acid uptake, synthesis and modification fosters the colonization of the metastatic niche through multiple mechanisms.

Additional metabolic rewiring

The studies described above infer several metabolic liabilities that can be potentially targeted while cancer cells transition through the metastatic cascade (TABLE 1). Notably, there are additional metabolic vulnerabilities that have been evaluated only for a limited number of metastatic steps, and thus were not possible to include as examples for metabolite plasticity. This includes, but is not limited to, acetate, serine, alanine, proline and asparagine metabolism, which are highlighted below (FIGS 2,3; TABLE 1). We do not address the metabolic rewiring of glucose metabolism during metastasis formation, which is covered in other recent reviews[22,162,163].

Acetate metabolism.—Acetate is a nutrient that can be converted to acetyl-CoA or produced from acetyl-CoA via different enzymes. Acetyl-CoA concentrations can be increased through downregulation of the enzyme acyl-CoA thioesterase 12 (ACOT12), which converts acetyl-CoA to acetate. Consequently, ACOT12 downregulation by genetic silencing and subsequent acetyl-CoA accumulation in hepatocellular carcinoma cell lines facilitated histone acetylation-induced activation of Twist2 (REF.[164]), a known inducer of EMT. Phenotypically, elevated acetyl-CoA concentrations led to increased invasive properties of hepatocellular carcinoma cells[164], and similar observations have been made in breast cancer cells[31,164] (FIG. 2a). By contrast, inhibition of the cytosolic enzyme acetyl-CoA synthase 2 (ACSS2; which converts acetate to acetyl-CoA), resulted in increased invasiveness and migration of hepatocellular carcinoma cells due to a HIF2α-dependent induction of an EMT[165] (FIG. 2a). Acetate is available to tumours via the circulation and, potentially, through production by other organs such as the liver[166,167]. As such, through conversion to acetyl-CoA and contribution to the TCA cycle, acetate can serve as a bioenergetic fuel for breast cancer, non-small cell lung cancer, clear cell RCC, melanoma and endometrial cancer-derived brain metastases[168] (FIG. 3d). Thus, further studies are needed to evaluate the role of acetate metabolism and acetyl-CoA concentrations in metastasis formation.

Serine, alanine, proline and asparagine metabolism.—Serine, alanine, proline and asparagine are non-essential amino acids that can be produced by cells but are also available to differing extents in body fluids. Serine is produced from the glycolytic intermediate 3-phosphoglycerate, alanine is made from pyruvate, and proline and asparagine

are often generated from glutamine. Serine, alanine and proline, but not asparagine, can be catabolized by human cells, resulting in pyruvate production (serine, alanine) and TCA and/or urea cycle fuelling and/or glutamate/glutamine production (proline).

It has been reported that the expression levels of all serine biosynthesis enzymes were elevated in MDA-MB-231 breast cancer cells with enhanced bone metastatic abilities compared with the parental cell lines[169]. Expression levels of the first enzyme of the serine biosynthesis pathway, namely phosphoglycerate dehydrogenase (PHGDH), has been associated with lymph node metastasis in patients with non-small cell lung cancer or pancreatic cancer[170,171] and high expression levels of PHGDH have been linked to shorter overall survival of patients with breast cancer-derived liver metastasis[172]. Serine conversion to glycine (indicated by high levels of serine hydroxymethyltransferase 2 (SHMT2) expression) has been prognostic in patients with breast cancer-derived lung metastasis[172]. In mice, PHGDH activity facilitated breast cancer-derived lung metastasis formation through maintaining mitochondrial redox homeostasis[173] (FIG. 3a). Moreover, PHGDH inhibition by genetic silencing or PH-755 treatment in mice impaired breast cancer-derived brain metastases due to low availability of serine in the brain[174] (FIG. 3d) and it decreased mTORC1 signalling in lung meta stases but not in primary breast cancers[72] (FIG. 3a). These observations indicate a role of serine metabolism in metastasis formation.

The biosynthesis and catabolism of alanine depends on the reversible reaction catalysed by ALT1 (cytosolic) or ALT2 (mitochondrial), which convert alanine and α-ketoglutarate to pyruvate and glutamate or vice versa. In a mouse model of primary breast cancer, inhibition of alanine catabolism by silencing *ALT2* impaired primary tumour growth, in particular, leading to elevated α-ketoglutarate concentrations, resulting in HIF1α degradation and subsequent reduced sonic hedgehog signalling[175]. Conversely, *ALT2* silencing compared with control led to inhibition of alanine synthesis from pyruvate in breast cancer-derived lung metastasis, thereby reducing the metastatic burden in an experimental mouse model, based on decreased α-ketoglutarate concentrations impairing P4HA activity and, consequently, collagen hydroxylation[69] (FIG. 3a). Interestingly, these data suggest that primary breast cancers rely on alanine catabolism, whereas breast cancer-derived lung metastases rely on alanine biosynthesis.

Asparagine and glutamine metabolism are intertwined because limited glutamine availability makes asparagine an essential amino acid[176]. Interestingly, reducing the bioavailability of asparagine to cancer cells through knockdown of asparagine synthetase (ASNS), treatment with L-asparaginase or dietary asparagine restriction reduced lung metastasis without affecting the growth of the primary breast tumour in mice[177]. Mechanistically, ASNS silencing impaired the number of circulating tumour cells as well as invasiveness by preventing the induction of mesenchymal gene expression patterns[177] (FIG. 2a).

Proline is synthesized and catabolized by different enzymes coupled to different cofactors, namely proline dehydrogenase (PRODH) and pyrroline-5-carboxylate reductase 1 (PYCR1)[178]. PYCR1 is highly expressed in patients with invasive ductal breast carcinoma[179] and its levels have been predictive for lymph node metastasis in patients with non-small cell lung cancer[180]. Moreover, PYCR1 silencing reduced invasiveness in

neuroblastoma cell lines with overexpression of MZF1-AS1 in vitro[181], whereas proline starvation impaired clonicity in different cancer cell lines[182]. Accordingly, the proline cycle consisting of catabolism via PRODH and synthesis via PYCR1 is required for breast cancer-derived lung metastasis in mice[73] (FIG. 3a).

Taken together, nutrients such as lactate, pyruvate, glutamine and lipids (and likely others) appear to be crucial metabolites in many steps of the metastatic cascade — that is, their use is plastic. Although the evidence presented here supports the concept of metabolite plasticity, several questions arise. For example, it remains elusive as to how the cancer cell origin impacts the different aspects of metabolite plasticity-driven metastasis formation. The largest body of evidence for metabolite plasticity is based on a wide range of different in vitro and in vivo models across multiple cancer types. Thus, additional studies are required that follow metabolite plasticity within the same cancer model to link changes in the metabolization of a particular nutrient to phenotypic switches of the cancer cells. Moreover, it will be important to understand how concentration changes of one and the same metabolite can arise across different organelles, tissues, tumours and patients and how these contribute to the ability of cancer cells to leverage these metabolites during metastasis formation. Interestingly, some of the targets discussed above also affect primary tumours, whereas others only affect metastasis formation (BOX 1; TABLE 1). The latter supports the proposition that the metastatic phenotype and/or the site of metastasis defines the metabolic vulnerabilities of cancer cells.

Metabolite (in)flexibility

Multiple studies demonstrate that cancer cells rely on different nutrients to fuel the same metabolic requirements, a phenomenon termed metabolite flexibility, which might allow them to overcome the hurdle of a specific step in the metastatic cascade. Here, we will discuss metabolite flexibility in light of circulating and colonizing tumour cells. We decided to cover these two specific steps in the metastatic cascade because there is evidence that circulating cancer cells require certain metabolic products (that is, NADPH, glutathione) to control ROS levels, whereas cancer cells that seed and colonize the metastatic niche have an increased need for the metabolic product ATP compared with proliferating cancer cells in the primary tumour[23,24,183,184]. ATP and NADPH can be produced from various nutrients; however, ATP and NADPH in metastasizing cancer cells seem to be produced from only a limited range of nutrients (nutrient inflexibility).

Nutrient inflexibility

A dependence on one nutrient despite the fact that multiple nutrients can lead to the production of a certain metabolite.

ROS defense in circulating tumour cells

Antioxidant metabolism and resistance to ROS is essential for the survival of cancer cells in the circulation[23,24]. Cancer cells can rely on different nutrients to avoid ROS-induced cell deaths such as ferroptosis (FIG. 4). As described above, pyruvate can act as an extracellular antioxidant[60] whereas lactate-driven pentose phosphate pathway activity[63] and

fatty acid oxidation[185] generate NADPH. NADPH is required for the recovery of the ROS scavenger GSH in melanoma and colorectal cancer, and potentially other cancers. As mentioned above, oleic acid uptake reduced the susceptibility of lymph-circulating melanoma cells to ferroptosis in blood circulation through the reduction of peroxidation-sensitive double bounds in lipids[154]. In addition, glutamine reductive carboxylation and glucose fermentation induced through cell detachment protected cells from anoikis by NADPH regeneration in the mitochondria and reduced mitochondrial ROS production in various cancer cell lines[150,186]. Moreover, glucose-fuelled folate metabolism reduced oxidative stress in circulating melanoma cells based on low-dose methotrexate treatment, ALDH1L2 knockdown or MTHFD1 knockdown inhibiting distant melanoma metastasis but not primary tumours in mouse[51]. These findings highlight that numerous nutrients, including pyruvate, lactate, fatty acids, glutamine and glucose, can contribute to ROS resistance in circulating tumour cells.

Reductive carboxylation

A metabolic pathway in which α-ketoglutarate is converted to citrate through a reaction with carbon dioxide.

Glucose fermentation

A biological process in which glucose is converted to lactate.

ATP production in seeding tumour cells

Energy generation has emerged as an important metabolic output for cancer cells establishing tumours in distant organs[23]. The reason for increased energy requirement of cancer cells colonizing in distant organs is unknown; however, it is conceivable that this could relate to protein as well as extracellular matrix production and trafficking, which are both highly energy-demanding processes[187] and required to form a permissive metastatic niche. Cancer cells can rely on different nutrients to increase their ATP availability during metastatic colonization (FIG. 4). Fatty acids have been identified as important nutrients in this respect. In breast cancer, metastatic triple-negative breast cancer cells compared with benign cell lines required fatty acid-driven energy metabolism for the formation of distant metastasis based on the inhibition of fatty acid oxidation with etomoxir or the perturbance of fatty acid metabolism through inhibition of CUB-domain containing protein 1 (CDCP1) activity with an engineered blocking fragment in experimental mouse models[188,189]. The rewiring of energy metabolism yielded more ATP that was needed to activate Src through autophosphorylation[188]. Moreover, both proline catabolism and glucose metabolism-derived ATP contributed to energy production during metastatic colonization of breast cancer cells in the lung of mice[68,190]. Interestingly, breast cancer-derived lung metastases used glucose to fuel glycolysis and mitochondrial metabolism to account for their energy needs[190], whereas breast cancer-derived liver metastases only relied on glycolytic energy production[191]. In mouse models of colon cancer, colon cancer-derived liver metastases scavenged ATP from the extracellular space[192]. In particular, colon cancer cells released creatine kinase brain-

type into the liver extracellular space, which converted creatine into phosphocreatine in an ATP-driven reaction[192,193]. Phosphocreatine was then taken up by the colonizing colon cancer cells and used for intracellular phosphorylation of ADP to ATP. In summary, there is evidence that cancer cells, in principle, can rely on several metabolites (for example, fatty acids, proline, glucose) to accommodate their energy needs when they colonize distant organs.

The fact that cancer cells rely on multiple metabolites and nutrients to produce NADPH, GSH and ATP is not surprising, especially as these metabolic products are substrates and products of multiple reactions across the entire metabolic network. It is, however, surprising that targeting only one of the nutrients producing these molecules is sufficient to exhibit therapeutic efficacy in mouse models; for example, leading to reduction of the number of circulating tumour cells or reduced metastasis formation (FIG. 4). Thus, metastasizing cancer cells entail a certain metabolic rigidity, that is, nutrient inflexibility that seems to depend on the organ of metastasis, cell state, cell of origin, microenvironment or (epi) genetic landscape, which opens a therapeutic window to target these metabolic vulnerabilities. Accordingly, proline catabolism inhibition through blocking PRODH or inhibition of lactate uptake through MCT1 did not impair primary tumour growth, whereas it had dramatic effects on metastasis formation due to the different cell state and/or microenvironment during metastasis[63,73].

Consequently, interesting questions arise such as which patients would benefit most from such treatments; and whether an anti-metabolic therapy would be more effective and eventually less toxic than a standard chemopreventative strategy in counteracting the metastatic seeding, or would also be considered for patients who already have developed metastatic disease. If organ-specific metastases may indeed demonstrate specific metabolic vulnerabilities that are targetable, anti-metabolic therapy of patients with established metastases might become an achievable goal. Considering the possibility that some of the vulnerabilities of metastasizing cancer cells, such as increased energy needs during seeding or sensitivity of circulating tumour cells to oxidative stress, may be lost in established metastases further argues for distinct therapeutic windows in which metabolic targeting therapies would be most effective. Clinical trials in the neoadjuvant setting may thus be a promising strategy to evaluate metabolic therapeutics given that tumour spread has become an accepted clinical trial end point[194].

Metabolic evolution of metastases

Once cancer cells have successfully metastasized to a distant organ, the secondary tumour shows a similar behaviour (that is, phenotype) to the primary tumour — both grow, proliferate and can reseed[2,9]. One could then argue that primary tumours and established metastases are metabolically similar, or at least depend on the same metabolic pathways or enzymes. Some examples discussed above are in line with this proposition because, in some cases, disrupting metabolic activity leads to effects in cancer cells regardless of whether they grow as metastases or primary tumours (TABLE 1). However, there is an increasing body of evidence that suggests a metabolic evolution from primary tumours to metastases in different organs. Accordingly, some metabolic vulnerabilities discussed above only target

metastases (sometimes only in specific organs) but not primary tumours (TABLE 1). Here, we summarize the current evidence suggesting that primary cancers and their metastatic progenies differ in their metabolic profiles.

Over the last decade, expression analysis of a limited number of metabolic genes has revealed distinct expression profiles between primary and secondary lesions. For example, a study on patients with pancreatic adenocarcinoma and corresponding metastatic lesions demonstrated common but also highly distinct metabolic gene expression profiles between primary tumours and metastases[195]. A recent single-cell RNA-sequencing study of metastatic patient-derived xenograft tumours confirmed a clear intra-tumour and inter-tumour heterogeneity in metabolic expression profiles in distinct cancer cell populations in primary breast tumours, and lung and lymph node micrometastases[196]. Additionally, single-cell transcriptional profiling of tumour tissue samples from six patients with triple-negative breast cancer showed subclonal heterogeneity of malignant cells shared by various tumours with multiple signatures of treatment resistance and metastasis as characterized by elevation of glycosphingolipid metabolism and associated innate immunity pathways[197]. Moreover, lung micrometastases from breast cancer patient-derived xenograft mouse models displayed a distinct metabolic gene expression signature of mitochondrial oxidative metabolism compared with the corresponding primary breast cancers[196]. These results support the notion that metastases can be metabolically different compared with their corresponding primary tumours. Notably, whereas the evidence presented here and below is mainly based on transcriptional changes, post-translational modifications and metabolite concentration-driven metabolic rewiring in primary tumours versus metastasis is likely to occur, yet is less demonstrated due to technological limitations.

Another layer of complexity is observed by inter-metastatic metabolic heterogeneity in relation to their metastatic site. Data from Flura-seq (fluorouracil-labelled RNA sequencing), a technique that determines which genes are active in small clusters of cells in a tissue, showed that lung micrometastases had differential transcriptional activity compared with brain micrometastases and primary mammary tumours in mice[198]. This may suggest that metabolic requirements exist in the lung microenvironment that are distinct from brain or breast tissue. Accordingly, mitochondrial electron transport chain genes were higher expressed in lung metastases compared with both brain metastases and orthotopic mammary tumours[198]. In addition, a proteomics study on patient-derived breast cancer cells observed a unique metabolic protein profile of brain metastasis compared with bone metastasis in mice, which suggests either a selection of predisposed cells or bioenergetic adaptation of the tumour cells to the brain environment[199]. In line with this, melanoma-derived brain metastases displayed an enrichment for oxidative phosphorylation-associated gene expression patterns in comparison with patient-matched extracranial metastases[200].

Although these observations reiterate the existence of differential metabolic traits between primary tumours and metastases, and across metastases, the need for these metabolic alterations are less understood. At least two fundamental principles could lay the foundation for these alterations (FIG. 5). Either the (epi)genetically or metabolically heterogeneous tumour cell pool in a primary tumour provides a selection for a distinct cancer cell subpopulation that is optimally suited to flourish in a specific organ environment, or several

cancer cell subpopulations may be able to adapt to a certain organ environment. It is conceivable that both selective and adaptive processes occur, which may again depend on the tumour origin and metastatic site.

Regardless of whether selection or adaptation is the determinant factor for successful growth of secondary tumours, emerging evidence shows that the (metabolic) environment matters. An interesting experiment compared the influence of in vitro and in vivo environments. Thereby, brain and lung metastases as well as mammary tumours from mice were directly analysed after harvesting the tissue[198]. In parallel, an aliquot of these tissue samples was dissociated into single cells, cultured for 1–2 weeks and subjected to RNA sequencing. Strikingly, several thousand genes were distinctly expressed across the different tissues whereas the same cells showed differential expression of only a few hundred genes when cultured in vitro[198]. Moreover, in vitro culture of lung cancer cells versus in vivo growth of the same tumour cells resulted in a different metabolic phenotype[201]. Moreover, microenvironmental differences in per-fusion also correlated with intra-tumour metabolic differences in patients with lung cancer[202]. Such experiments demonstrate the environmental dependency of metabolism.

In addition, there is also circumstantial evidence that the nutrient availability in the environment is important. For example, breast cancer-derived brain metastases, similar to glioblastoma, can use acetate for propagation[168,203] (FIG. 3d). This observation was based on ^{13}C tracer infusions in humans and mice, which assess in vivo nutrient contribution to metabolism[204,205]. Moreover, there is evidence that secreted factors from primary breast tumours increased the glucose availability in the metastatic niche, which elevated the effectiveness of metastatic seeding in the lungs of mice[206] (FIG. 3a).

Thus, nutrient availability in the metastatic niche is important and can be altered to increase the permissiveness of the niche. Data from ^{13}C-glucose infusions in mice harbouring metastatic primary breast cancer demonstrated an increase in PC-dependent anaplerosis in lung metastases compared with primary tumours, which was recapitulated in vitro by adding pyruvate to the media[68]. Additionally, the dependence of breast cancer-derived brain metastases on PHGDH activity can be explained through the very low serine availability in the brain environment[174] (FIG. 3d). Finally, there is also some evidence that the microenvironment may prepare cancer cells en route metabolically for another environment because melanoma cells that traversed through the oleate-enriched environment of the lymph system before entering the blood circulation were better prepared to avoid cell death, and consequently more successful in seeding in distant organs of mice[154] (FIG. 2b).

The studies described above support the notion that primary tumours and their metastases differ in their metabolic attributes because metastases adapt or have growth advantages for specific environments (FIG. 5). Although limited evidence exists, metabolic priming of the metastatic niche[206] and by a certain environment[154] may also exist. Whereas the here presented evidence suggests a role of the nutrient environment, additional factors of the microenvironment such as the presence of stromal cells may also contribute to environment-dependent metabolic requiring. Thus, it is tempting to speculate that metastases of differing

origin growing in the same organ or taking the same route of metastasis may metabolically be more similar to each other than metastases of the same origin growing in different organs.

It is important to note that the environment is certainly not the only determinant of metabolic rewiring and that other factors such as the cancer cell origin, that is the cell lineage from which the cancer cell arises, may override the influence of nutrient availability (as suggested for PCG1α in breast versus prostate cancer[23]). Moreover, some metastatic niches may be more permissive for cancer cells with different genetically embedded metabolic programmes to successfully proliferate. Finally, some metabolic traits such as the reliance on CD36 activity in metastasis-initiating cells seem to be essential across multiple cancers and metastatic sites[103], making them very attractive targets for cancer therapy.

Further studies that dissect the metabolic dependencies of metastases will be instrumental in determining and mechanistically understanding when the influence of the microenvironment outweighs the cellular origin, cell state (that is, phenotype) and (epi)genetic landscape in imposing metabolic constrains on metastasis growing in different organs.

Conclusion

Emerging evidence depicts specific metabolic vulnerabilities in cancer cells during their metastatic journey that could be exploited to potentially halt metastatic growth and even prevent successful seeding. These vulnerabilities are not just manifested intrinsically in a tumour type-specific manner but, rather, dynamically dependent on the stage of and location during their metastatic journey. Importantly, the success of metastatic manifestation may be influenced by the nutrient composition of the respective organ to which metastatic tumour cells need to adapt. These observations fit well with the ‘seed and soil’ hypothesis in which tumour cells require an appropriate soil with the necessary metabolites and nutrients in order to expand successfully. Nonetheless, the complexity of metabolic traits and their necessities in maintenance and propagation, not only of tumour cells but of every normal cell, makes therapeutic targeting of metabolic pathways challenging and requires a more detailed understanding of the effects of metabolite inhibition in the context of the heterogeneous cell types in metastases and the organ in which they seed.

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Competing interests

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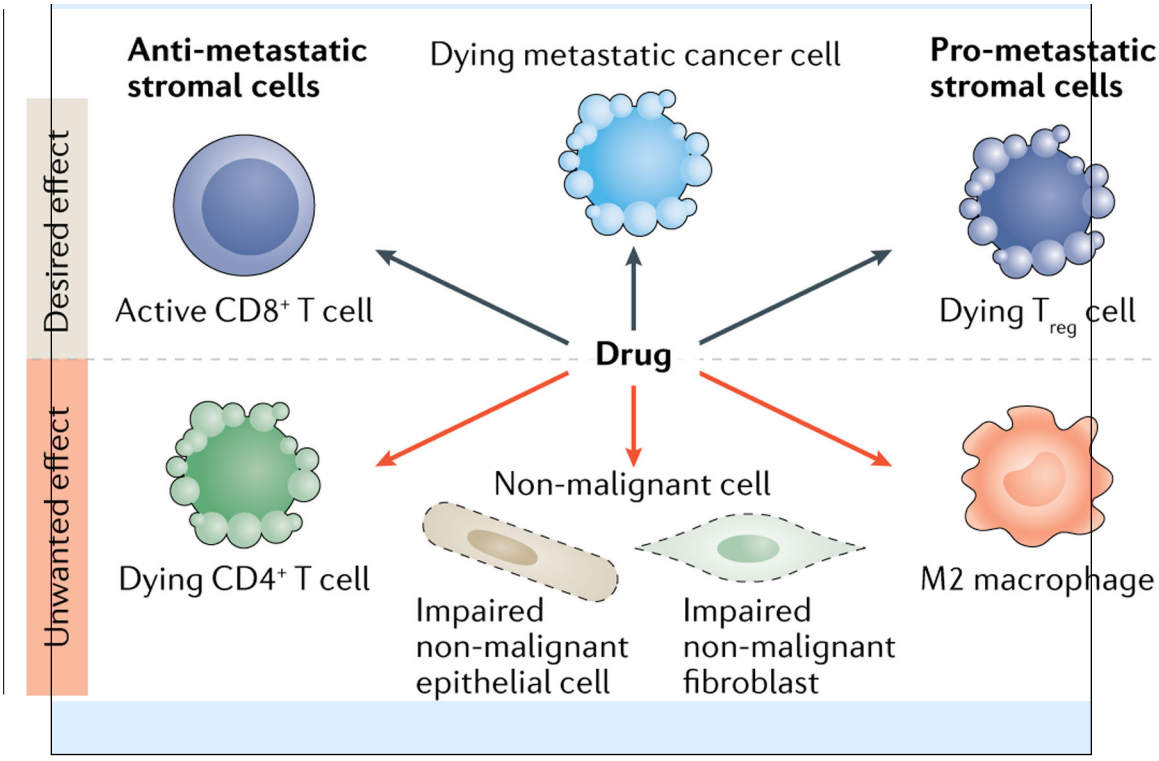
Box 1 |

Targeting metabolism in the context of the tumour microenvironment

It is important to note that targeting metabolism in cancer in general, or metastasis formation in particular, can lead to (un)wanted systemic effects of a drug on stromal cells within the tumour microenvironment. Notwithstanding, targeting similar metabolic traits in the tumour microenvironment (for example, the tumour vasculature, immunosuppressive cells) and cancer cells can also lead to improved beneficial effects (see the figure). For example, many enzymes implicated in fatty acid uptake are active in stromal cells[207]. Immunosuppressive regulatory CD4 T cells (T_{reg} cells) have been shown to take up fatty acids[208]. Accordingly, genetic ablation of the fatty acid transporter cD36 in T_{reg} cells suppressed tumour growth and enhanced antitumour activity in tumour-infiltrating lymphocytes without disrupting immune homeostasis[209]. Moreover, tumour-associated compared with non-tumour-associated macrophages showed upregulation of cD36 expression[210] and lipid accumulation in natural killer cells limited their antitumour response[211,212]. Thus, targeting lipid uptake through cD36 inhibition may have positive effects on stromal cells during cancer therapy. In contrast, lipid uptake activity through fatty acid binding protein 4 (FaBP4) and FaBP5 was required for the long-term survival of memory $cD8^+$ T cells[213]. It is not known how FaBP4 and FaBP5 inhibition impacted memory $cD8^+$ T cells in a tumour context, but double knockouts were less effective at protecting mice from cutaneous viral infection[213], which may suggest undesired side effects in cancer treatment. This example highlights the challenge but also the potential benefit of targeting metabolic traits in metastasizing cancer cells in the context of the tumour microenvironment. Yet our current knowledge regarding stromal cells is often limited to observations in primary tumours or even non-cancerous situations, making it difficult to predict the overall effects in the metastatic setting. In addition, it remains elusive to which extent immune cell populations differ between metastatic sites. For example, distinct immune profiles have been documented between brain and extracranial metastases[200], whereas lung metastases shared a common immune profile regardless of tumour origin[214]. Therefore, further studies are urgently needed that consider the effect of metabolism targeting drugs on metastasizing cancer and stromal cells.



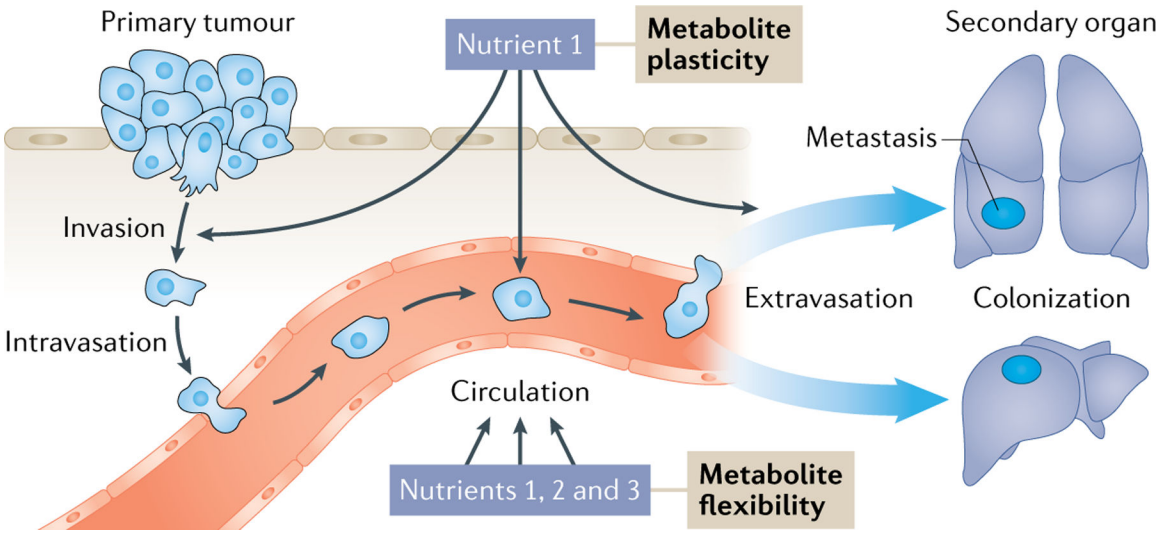


Fig. 1 |. Metabolite plasticity and flexibility in metastasizing cancer cells.

Metastasizing cells undergo dynamic metabolic changes to adjust to the differing microenvironments while travelling to distant organs. Thereby, metastasizing cells can exhibit metabolic plasticity in which they use one metabolite to fuel the various metabolic requirements of the different steps in the metastatic cascade. Alternatively, they can display nutrient flexibility by using multiple metabolites to meet the same metabolic requirement imposed by a specific step of the metastatic cascade. In cancer cells, both phenomena, metabolic plasticity and metabolic flexibility, contribute to metastasis formation and may be targeted for therapy.

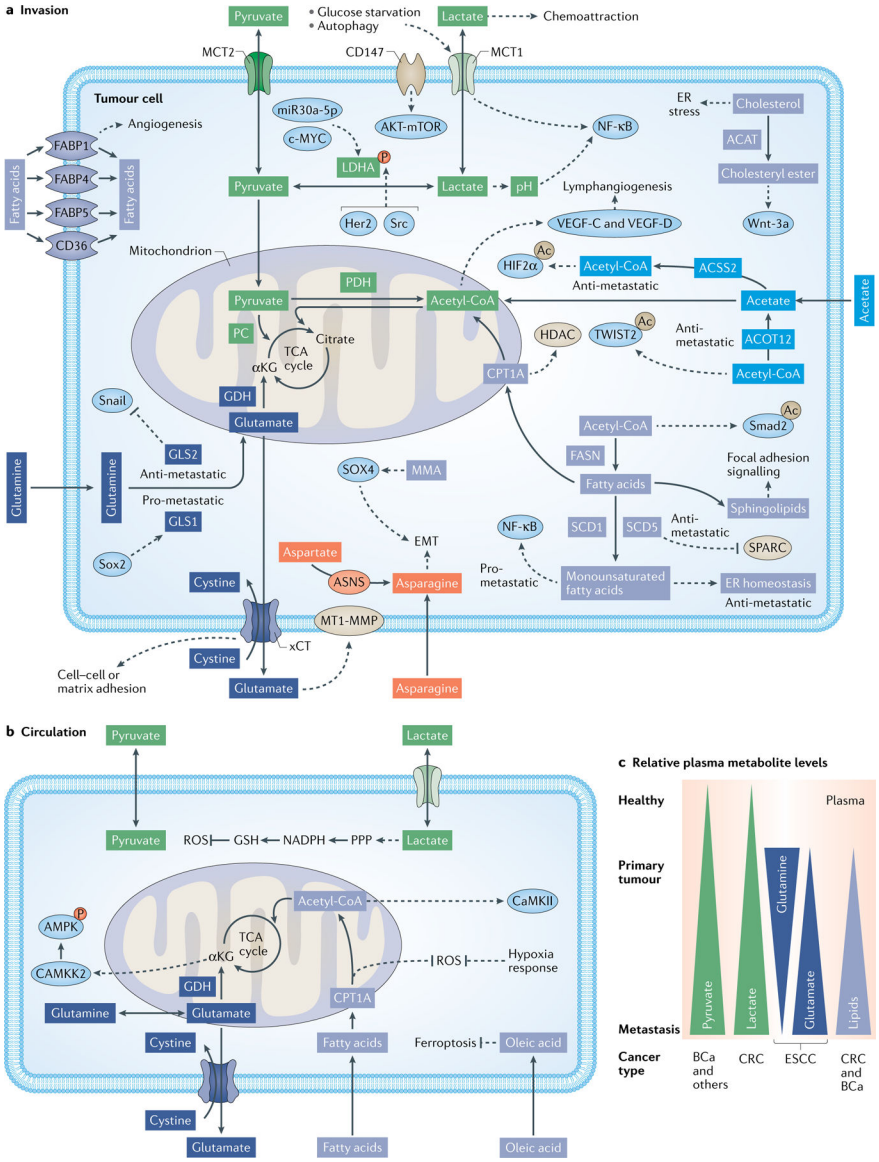


Fig. 2 |. The metabolism of invading and circulating (detached) cancer cells.

Multiple nutrients and metabolites facilitate the invasiveness and migratory abilities of cancer cells (part **a**) and their survival in the circulation (part **b**). Differential availability of nutrients and metabolites in the circulation of healthy individuals, patients with non-metastatic cancer (primary tumour) or patients with metastatic cancer have been reported. In the circulation, increased pyruvate levels have been observed in patients with non-metastatic versus metastatic breast cancer (BCa) and for patients with multiple types of cancer compared with healthy individuals. Increased lactate levels have been observed in patients with metastatic colorectal cancer (CRC). Low glutamine and high glutamate levels have been observed in patients with oesophageal squamous cell carcinoma (ESCC). Enhanced lipid levels have been observed in patients with colorectal and breast cancer, but not patients with oral cancer (part **c**). Fatty acid metabolism is depicted in light indigo. Lactate and pyruvate metabolism are depicted in green. Glutamine metabolism is depicted in dark

indigo. Acetate metabolism is depicted in indigo. Asparagine metabolism is depicted in orange. Proteins whose function is regulated by metabolism pathways are shown in light blue. Enzymes depicted in box shape and membrane transporters not coloured in beige have been discussed as targets in the main text. Dashed lines indicate regulatory events. Multiple metabolic reactions are summarized for clarity reasons. Ac, acetylation; ACAT, acetyl-coenzyme A acetyltransferases; ACOT12, acyl-CoA thioesterase 12; ACSS2, acyl-coenzyme A synthetase short-chain family member 2; AMPK, adenosine monophosphate-activated protein kinase; αKG, α-ketoglutarate; ASNS, asparagine synthetase (glutamine-hydrolysing); CAMKK2, calcium/calmodulin-dependent protein kinase kinase 2; c-MYC, MYC proto-oncogene; CoA, coenzyme A; CPT1A, carnitine palmitoyltransferase 1; CTSB, cathepsin B; EMT, epithelial to mesenchymal transition; ER, endoplasmic reticulum; FABP1, fatty acid-binding protein 1; FASN, fatty acid synthase; GDH, glutamate dehydrogenase; GLS1, glutaminase 1; GSH, glutathione; HDAC, histone deacetylases; Her2, Erb-B2 receptor tyrosine kinase 2; HIF2α, hypoxia-inducible factor 2α; LDHA, lactate dehydrogenase A; MCT1, monocarboxylate transporter 1; miR-21, microRNA 21; MMA, methylmalonic acid; MT1-MMP, membrane type 1 matrix metalloproteinase; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κb, nuclear factor κ-light-chain-enhancer of activated B cells; P, phosphorylation; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase 1; Smad2, mothers against decapentaplegic homologue 2; Sox2, SRY (sex determining region Y)-box 2; SPARC, secreted protein acidic and cysteine rich; TCA, tricarboxylic acid; TWIST2, twist family BHLH transcription factor 2; VEGF, vascular endothelial growth factor; Wnt-3a, wingless-type MMTV integration site family, member 3A; xCT, solute carrier family 7 member 11.

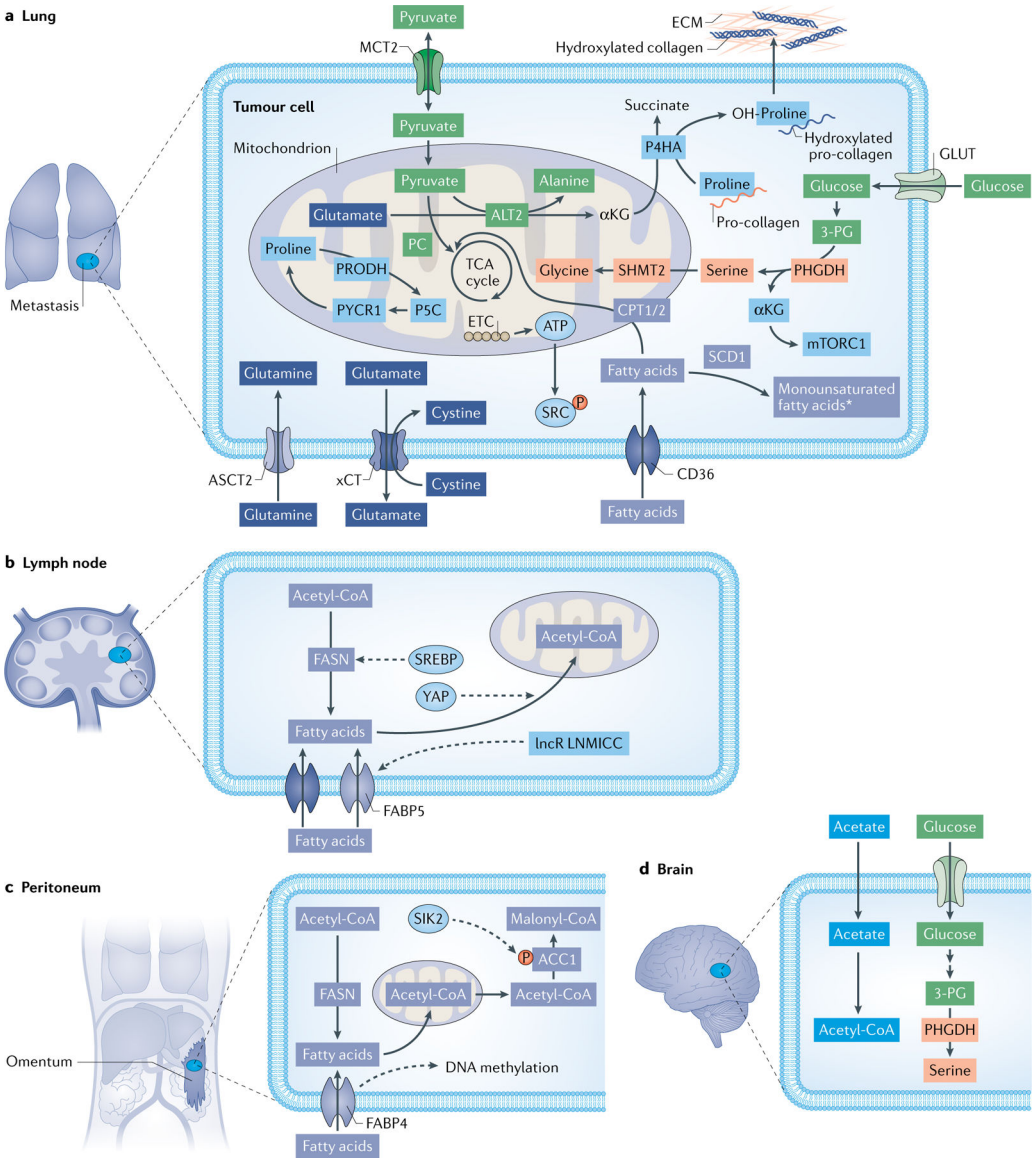


Fig. 3 |. The metabolism of cancer cells colonizing in distant organs.

Different nutrients and metabolic pathways are supporting the nesting of metastasizing cancer cells in the lung (part **a**), lymph node (part **b**), peritoneum (part **c**) and brain (part **d**). In the lung, breast cancer cells take up extracellular pyruvate via monocarboxylate transporter 2 (MCT2) to generate α-ketoglutarate (αKG) from glutamate to activate the enzyme collagen prolyl-4-hydroxylase (P4HA), which is essential for extracellular matrix deposition and remodelling (part **a**). Uptake of glutamine via ASCT2 is important for the growth of prostate cancer cells and their metastatic seeding to the lung (part **a**). Elevated fatty acid metabolism is associated with a higher propensity of several cancer cell types to metastasize. Inhibition of CD36 and fatty acid binding proteins (FABPs), which both import fatty acids into the cell, as well as inhibition of fatty acid synthase (FASN) to block de novo fatty acid synthesis has been shown to restrain lung metastases (part **a**). In lymph nodes, melanoma and breast cancer cells undergo a metabolic shift towards

fatty acid oxidation (FAO). Transcriptional co-activator yes-associated protein (YAP) is selectively activated in lymph node metastatic tumours, leading to the upregulation of genes in the FAO signalling pathway (part **b**). In the peritoneum, visceral adipocytes promote ovarian cancer progression and metastases in the peritoneal cavity by providing fatty acids and increasing CD36 expression in cancer cells (part **c**). Salt inducible kinase 2 (SIK2)-mediated ACC and SIK2 phosphorylation and overexpression promote fatty acid oxidation required for effective metastasis formation (part **c**). In the brain, acetate was discovered as an alternative energy source to glucose for human and mouse brain glioblastoma and lung and breast cancer-derived brain metastases (part **d**). As the brain exhibits low serine levels, inhibiting serine production by genetic phosphoglycerate dehydrogenase (PHGDH) silencing or PH-755 treatment in mice impairs breast cancer-derived brain metastases. *The metabolism of monounsaturated fatty acids can have a pro-metastatic or anti-metastatic function (part **a**). Fatty acid metabolism is depicted in light indigo. Lactate and pyruvate metabolism are depicted in green. Glutamine metabolism is depicted in dark indigo. Acetate metabolism is depicted in indigo. Serine metabolism is depicted in light orange. Proteins whose function is regulated by metabolism pathways are shown in light blue. Enzymes depicted in box shape and membrane transporters have been discussed as targets in the main text. Dashed lines indicate regulatory events. Multiple metabolic reactions are summarized for clarity reasons. ACC1, acetyl-CoA carboxylase 1; ALT2, alanine aminotransferase 2; CoA, coenzyme A; CPT1/2, carnitine palmitoyltransferase 1/2; ECM, extracellular matrix; ETC, electron transport chain; lncR LNMICC, link RNA LNMICC; P5C, 1-pyrroline-5-carboxylic acid; P, phosphorylation; PC, pyruvate carboxylase; PRODH, proline dehydrogenase; PYCR1, pyrroline-5-carboxylate reductase 1, mitochondrial; SCD1, stearoyl-CoA desaturase 1; SHMT2, serine hydroxymethyltransferase 2; TCA, tricarboxylic acid; xCT, solute carrier family 7 member 11.

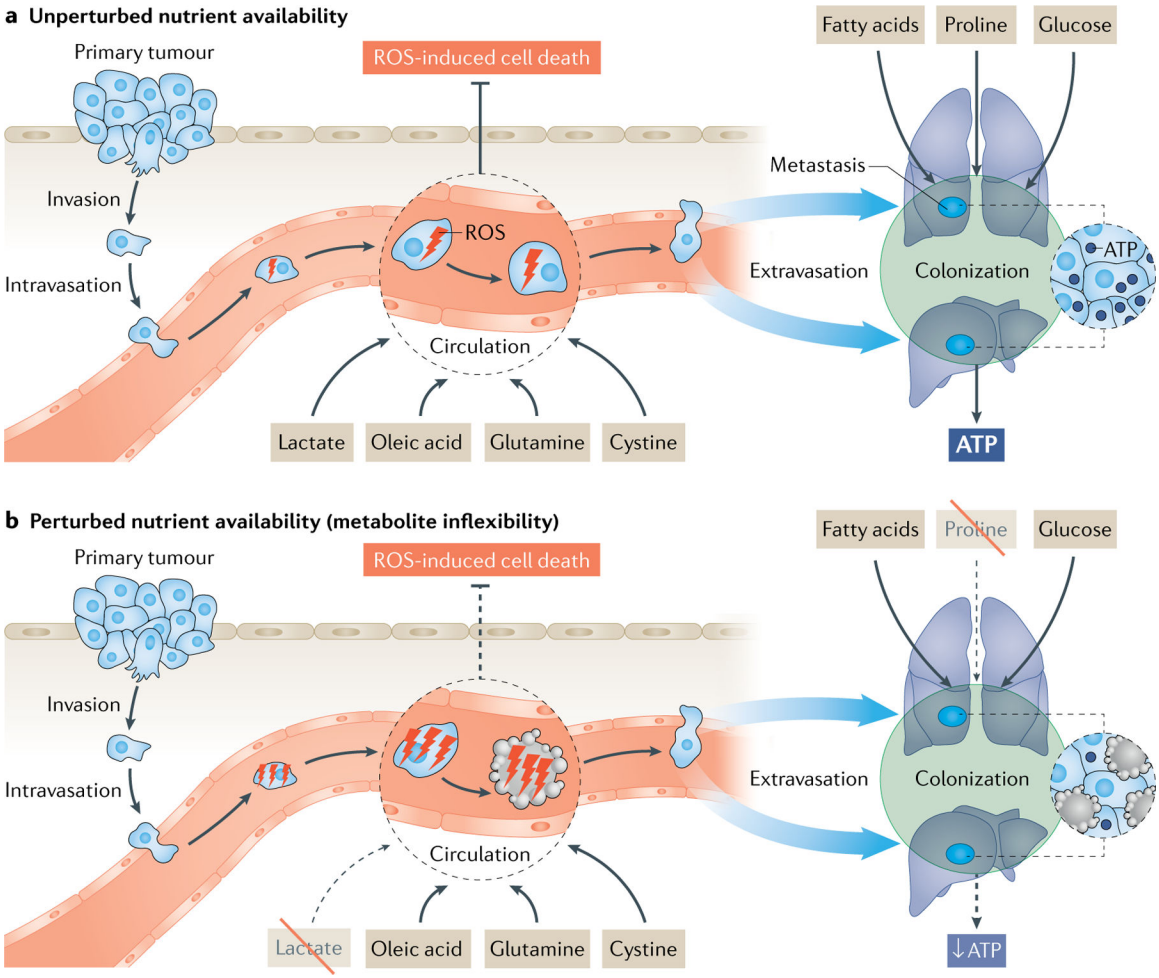


Fig. 4 |. Nutrient inflexibility during metastasis formation.

a | Cancer cells can use multiple nutrients to support the specific metabolic needs that arise when transitioning through the metastatic cascade. One example are circulating tumour cells that rely, for example, on lactate, glutamine, oleic acid and cystine to avoid reactive oxygen species (ROS)-induced cell death. Another example are colonizing cancer cells that rely, for example, on fatty acids, proline and glucose to generate sufficient ATP. **b** | Despite the fact that cancer cells can use multiple nutrients for this purpose, a therapeutically relevant nutrient inflexibility arises. For example, inhibition of lactate or proline uptake is sufficient to impair circulating and colonizing cancer cells, respectively by either inducing cell death or preventing cell division. Consequently, metastasis formation is reduced and may be prevented. Dashed lines in (**b**) indicate a decrease relative to (**a**).

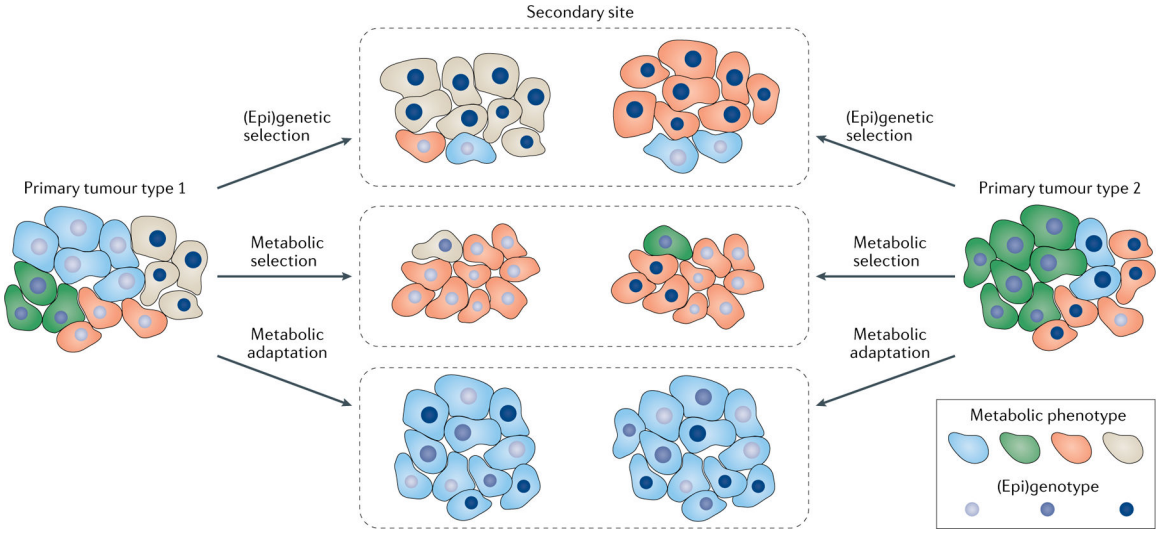


Fig. 5 |. Selection and adaptation processes contributing to the metabolic differences between primary tumours and metastases.

Cancer cells growing as a secondary tumour are challenged with a different environment. Either (epi) genetic or metabolic subpopulations of cancer cells from the primary tumour have a selective growth advantage in the new soil, or cancer cells may have the ability to adapt to the new soil. In the case of adaptation, the metabolic make-up of secondary tumours depends on the organ of metastasis rather than the cancer cell origin. In the case of (epi)genetic selection, a subpopulation of cancer cells selected by their (epi)genetic features grows in the secondary site, resulting in a different metabolism depending on the cancer origin. In the case of metabolic selection, a subpopulation of cancer cells selected by their metabolic features grows in the secondary site, resulting in the same metabolism regardless of the cancer origin. It is conceivable that likely a combination of these processes may occur in vivo.

Table 1 |

Metabolic targets for metastasis prevention and treatment discussed in this Review

Target	Cancer type	Investigated metastatic side	Model	Targeting strategy	Effect on primary tumour relative to control condition	Investigated therapeutic strategy	Refs
ACAT	Prostate, pancreas	Lung, liver, spleen, pancreas, lymph node	Human PC-3M, PC-3, MIA PaCa-2 cells	Avasimibe	Reduced invasion and proliferation	Metastasis prevention	137,138
ALT2	Breast	Lung	Murine 4T1 cells, human MCF7 cells	shRNA knockdown	Reduced tumour size	Metastasis prevention	69,175
ASCT2	Prostate	Liver (trend), lung (significant effect)	Human PC-3 cells	shRNA knockdown	Reduced tumour growth	Metastasis prevention	92
ASNS	Breast	Lung	Murine 4T1-T cells	shRNA knockdown, asparaginase, low-asparagine diet, combination of shRNA and drug or diet	No significant change in response to single treatments	Metastasis prevention	177
CD36	Head and neck, breast, skin	Lymph node, lung, liver, bone	Patient-derived SCC tumours, murine SCC Ln-7 cells, human MCF7 cells, human 501mel cells	shRNA knockdown, neutralizing antibodies FA6.152, JC63.1	No changes or slight reduction (one shRNA) of tumour growth	Metastasis prevention and treatment	103
CPT1A	Colon and/or rectum	Lung, liver	Human HCT15, HCT116	Etomoxir, shRNA knockdown	n.d.	Metastasis prevention	185
FABP4	Ovaries	Diaphragm, liver, spleen, lymph node, pelvic areas, omentum	Human HeyA8 MDR, Ovcar 5, OVCAR8 cells, murine ID8 cells	DOPC nanoliposomes containing siRNA injections, CRISPR knockout, BMS309403	No changes (BMS309403) or reduced tumour weight	Metastasis prevention and treatment	118,156
FABP5	Cervix, prostate	Lung, femur, lymph node	Human SiHa cells, PC-3	siRNA, shRNA knockdown	Reduced tumour size	Metastasis prevention	122,124
FASN	Ovarian, skin, colorectal	Peritoneum, lung, liver, lymph node	Human SKOV-3, KM-20, HT29 cells, murine B16-F10,	shRNA knockdown, orlistat	Reduced tumour growth	Metastasis prevention	127,128,157
GDH	Lung	Liver, lung	Human A549, H460	shRNA knockdown	n.d.	Metastasis prevention	91
GLS1	Colorectal	Lung, liver, kidney	Human HT29 cells, murine VM-M3 cells	shRNA knockdown, 6-diazo-5-oxo-L-norleucine (DON)	Decreased tumour volume and weight	Metastasis prevention	80,93
LDHA	Breast	Lung	Human MDA-MB-231 cells	shRNA knockdown	Reduced tumour growth	Metastasis prevention	37,39
MCT1	Skin	Lung	Patient-derived xenografts, murine YUMM1.7, YUMM3.3, YUMM5.2 cells	shRNA knockdown, AZD3965	No change (AZD3965) or slight (one shRNA) reduction of tumour size	Metastasis prevention	63
MCT2	Breast	Lung	Murine 4T1, EMT6.5 cells	CRISPR knockout, α-cyano-4-hydroxycinnamic acid	No (volume) to slight (weight) effect on tumour growth	Metastasis prevention	69
P4HA	Breast	Lung, lymph node	Human MDA-MB-231, MDA-MB-435 cells	shRNA knockdown, ethyl 3,4-dihydroxybenzoate	Reduced tumour volume	Metastasis prevention	70

Target	Cancer type	Investigated metastatic side	Model	Targeting strategy	Effect on primary tumour relative to control condition	Investigated therapeutic strategy	Refs
PC	Breast	Lung	Murine D2A1 cells	shRNA knockdown	No significant effect	Metastasis prevention	68
PHGDH	Breast, kidney	Lung, brain	Human MDA-MB-231, MDA-MB-231 BrM cells, patient-derived clear cell renal cell carcinoma cells	shRNA knockdown, PH-755	Similar to increased tumour mass	Metastasis prevention and treatment	173,174
PRODH	Breast	Lung	Murine 4T1, EMT6.5 cells	L-THFA	No significant effect	Metastasis prevention	73
SCD1	Skin	Lung	Murine B16-F10 cells, $MITF^{+}$ B16-F1	shRNA knockdown, CAY10566, A939572	n.d.	Metastasis prevention but also promotion	135,159
xCT	Breast, oesophagus, gastrointestinal tract	Lung	Murine 4T1, TUBO cells, human KYSE-150 cells	Anti-xCT vaccination, sulfasalazine	Reduced tumour size	Metastasis prevention and treatment	86,87,94

ACAT, cholesterol acyltransferase; ALT2, alanine aminotransferase 2; ASNS, asparagine synthetase; CPT1A, carnitine palmitoyltransferase 1A; FABP4, fatty acid binding protein 4; FASN, fatty acid synthase; GDH, glutamate dehydrogenase; GLS1, glutaminase 1; LDHA, lactate dehydrogenase A; MCT1, monocarboxylate transporter 1; n.d., not determined in the referenced metastasis study; P4HA, prolyl-4-hydroxylase; PC, pyruvate carboxylase; PHGDH, phosphoglycerate dehydrogenase; PRODH, proline dehydrogenase; SCC, squamous cell carcinoma; SCD1, stearoyl-CoA desaturase 1; shRNA, short hairpin RNA; siRNA, small interfering RNA; xCT, solute carrier family 7 member 11.