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REVIEW

Metabolic changes associated with tumor metastasis, part 1: tumor pH, glycolysis and the pentose phosphate pathway

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Abstract Metabolic adaptations are intimately associated with changes in cell behavior. Cancers are characterized by a high metabolic plasticity resulting from mutations and the selection of metabolic phenotypes conferring growth and invasive advantages. While metabolic plasticity allows cancer cells to cope with various microenvironmental situations that can be encountered in a primary tumor, there is increasing evidence that metabolism is also a major driver of cancer metastasis. Rather than a general switch promoting metastasis as a whole, a succession of metabolic adaptations is more likely needed to promote different steps of the metastatic process. This review addresses the contribution of pH, glycolysis and the pentose phosphate pathway, and a companion paper summarizes current knowledge regarding the contribution of mitochondria, lipids and amino acid metabolism. Extracellular acidification, intracellular alkalinization, the glycolytic enzyme phosphoglucose isomerase acting as an autocrine cytokine, lactate and the pentose phosphate pathway are emerging as important factors controlling cancer metastasis.

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Abbreviations

6PGD	6-Phosphogluconate dehydrogenase
AE2	Anion exchanger 2
AMF	Autocrine motility factor = PGI
AP-1	Activator protein-1
CA	Carbonic anhydrase
CTC	Circulating tumor cell
ECM	Extracellular matrix
EMT	Epithelial-to-mesenchymal transition
ETC	Electron transport chain
HGF	Hepatocyte growth factor
HIF-1	Hypoxia-inducible factor-1
Hyal-2	Hyaluronidase 2
IL	Interleukin
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinase
MCT	Monocarboxylate transporter
MIBG	Metaiodobenzylguanidine
MMP	Matrix metalloproteinase
MT1-MMP	Membrane-type 1 matrix metalloproteinase
NF-κB	Nuclear factor-κB
NHE	Sodium-proton exchanger
PGI	Phosphoglucose isomerase = AMF
PHD2	Prolyl-hydroxylase 2
pHe	Extracellular pH
pHi	Intracellular pH
PKM	Pyruvate kinase M
PPP	Pentose phosphate pathway
ROS	Reactive oxygen species
TKT	Transketolase

TKTL1	Transketolase-like 1
uPA	Urokinase
VEGF	Vascular endothelial growth factor

Introduction

It is currently estimated that about one out of three people will develop cancer in his/her lifetime in Western countries and at least one out of five patients will die of the disease. Metastasis represents the ultimate step of tumor progression and accounts for ~90 % of cancer-associated deaths [1].

Cancer refers to a group of heterogeneous diseases that originate from different tissues and affect different cellular subtypes. Influenced by interactions with the host, patient lifestyle and therapy, cancers can evolve in different ways. In 2000, Hanahan and Weinberg [2] proposed a general framework detailing common hallmarks of cancer that served as a cornerstone to facilitate the biological understanding of the pathology. It was updated in 2011 [3]. Cancer hallmarks comprise aberrant metabolic activities and the ability to metastasize at distant sites from a primary tumor to generate secondary tumors. The relationship between cancer metabolism and metastasis is the focus of this review.

Deregulation of metabolic fluxes is one of the earliest distinctive features of cancers that was described almost one century ago when Otto Warburg [4] reported that some cancer cells convert glucose to lactate aerobically, whereas most normal cells in the body use glucose for oxidative metabolism. Nowadays, it is becoming increasingly clear that many hallmarks of cancer are under metabolic control and that the execution of the aggressive tumor agenda requires specific rewiring of metabolic fluxes. Metabolic characterization has a high potential to lead to new therapeutic applications against cancer [5, 6].

Metastasis (from Greek μετά [*beyond*] and στάσις [*standing still*]) is characterized by the acquisition of invasive and colony-formation capabilities by cancer cells. It is a critical hallmark of cancer as it defines the switch from benign tumor to malignant cancer. Metastasis is a rather inefficient process comprising several consecutive steps, with only a small proportion of cancer cells among those gaining access to the circulation being able to successfully generate a metastatic lesion. Metastatic progenitor cells possess distinctive characteristics corresponding to specific traits that are required for the formation of metastases in distant organs [1].

On their metastatic route, cancer cells have to cross a first barrier formed by the extracellular matrix and stromal cells, which necessitates the activation of proteases, such as matrix metalloproteinases (MMPs) [1]. Metastatic progenitor cells must also acquire migratory activities to get access to the blood or lymphatic circulation. Acquisition of these capabilities is often preceded by the epithelial-to-mesenchymal transition (EMT), during which cancer cells of epithelial origin activate transcription factors TWIST1 and SNAIL as main drivers of cell elongation [7]. During EMT, cancer cells adopt a fibroblastoid morphology. EMT is critical for metastasis formation: it provides mobility and increases resistance to apoptosis [8], and EMT pathways are tightly connected to those driving stemness/tumor initiation [9]. When getting access to the circulation (intravasation), cancer cells must successfully cope with reoxygenation-associated redox stress, shear stress, immune attacks and the absence of anchorage that would normally induce anoikis (from Greek ἄνοικος [*homelessness*]), a form of apoptotic cell death induced by the loss of prosurvival signals following cell detachment from the extracellular matrix (ECM) [10, 11]. Cancer cells that successfully reach the blood stream are termed 'circulating tumor cells' (CTCs). They represent a heterogeneous population of cells with various molecular markers and phenotypes [12–15]. Among CTCs, some can leave the circulation (extravasation) and restore their proliferative activity to establish a tumor at a secondary site. Cells that had initially undergone EMT must also be able to (partially) reverse EMT in a process called mesenchymal-to-epithelial transition [16]. Only a minority of CTCs successfully undergoes these changes, with about 0.01 % of them being able to successfully form metastases [17]. Cancer metastasis is thus a complex process, as further detailed in references [1, 18, 19].

Knowledge concerning the metabolic aspects of tumor transformation and progression is increasing, which is notably reflected by a number of drugs targeting tumor metabolism that recently entered into clinical trials [6]. Still, little is known about the specific metabolic changes associated with the metastatic process. In the absence of validated markers, it is indeed particularly challenging to isolate and analyze rare metastatic progenitor cells and, in our opinion, illusory to try to define a metabolic phenotype typical of metastasis as a whole. In fact, a succession of metabolic adaptations is more likely needed to promote each step of the metastatic process (Fig. 1), with the possibility that some changes at an early step may not be required or could even be detrimental for later steps. Thus, an adapted analytical approach is needed to dissect the metastatic process from mechanistic and metabolic standpoints. Here, we detail the current understanding of the metabolic changes occurring during metastatic progression, with a special focus on tumor pH, glycolysis and the pentose phosphate pathway. A companion paper reviews the contributions of mitochondria, lipid and amino acid metabolism to cancer metastasis.

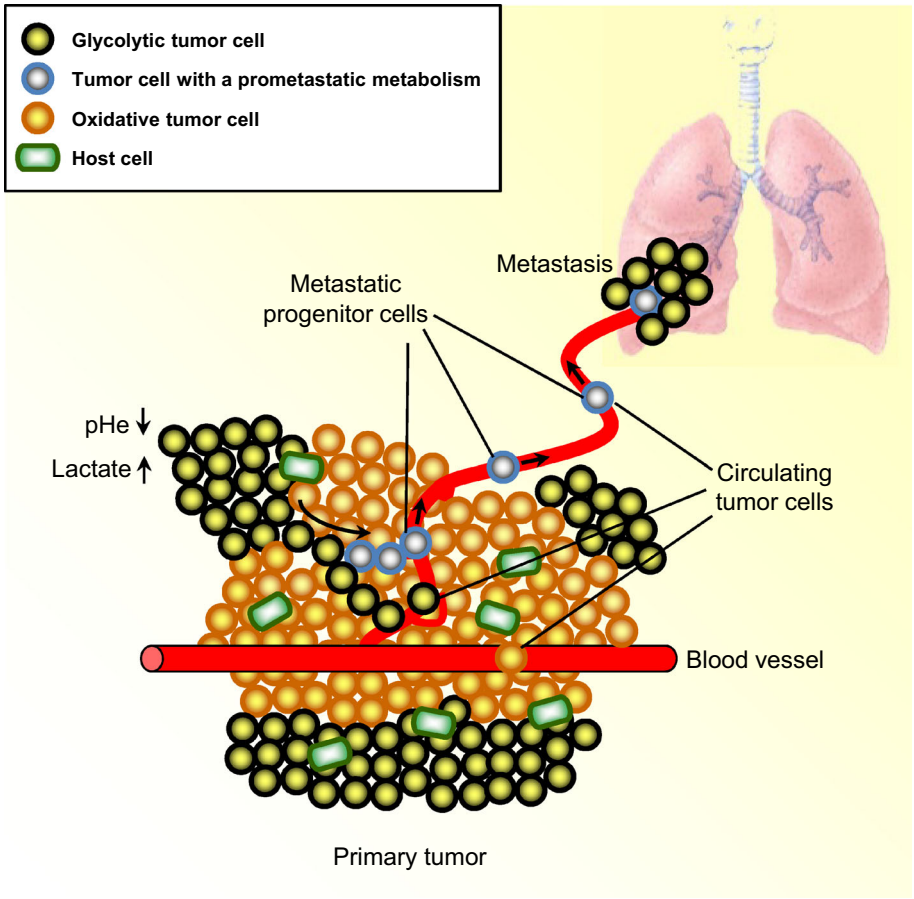

Fig. 1 Model depicting potential metabolic changes associated to cancer metastasis. Tumors are composed of malignant and host cells that are highly heterogeneous metabolically. According to the model, metastatic progenitor cells could evolve from glycolytic tumor cells in the glycolytic compartment of a primary tumor where a low extracellular pH (pHe), lactate and other microenvironmental parameters trigger tumor cell migration and invasion. On their metastatic route, tumor cells would acquire different metabolic features comprising, e.g., increased pentose phosphate pathway activity and an enhanced production of mitochondrial reactive oxygen species further promoting migration and invasion, facilitating intravasation, survival in the blood stream and extravasation, and conferring stem cell capabilities to metastatic progenitor cells. At the secondary site in a distant organ such as the lungs, metastatic progenitor cells would revert to a more glycolytic phenotype associated with cell proliferation (the Warburg effect) and metastasis formation. Of note, according to the model, metastatic progenitor cells would constitute a distinct population/populations of cells among all circulating tumor cells

Intracellular and extracellular pH

A common feature of the tumor microenvironment is extracellular acidosis, and a low extracellular pH (low pHe) promotes tumor growth and cancer progression (Fig. 2) [20–22]. Extracellular acidosis results from exacerbated metabolic activities of cancer cells (associated or not with an elevation of the glycolytic flux [23]), a high activity of proton transporters and carbonic anhydrases, and a poor extracellular proton clearance rate [6, 24, 25]. The origins of the low pHe are still ill-defined as it involves the metabolic contributions of different cancer cell and host cell populations in tumors. Glycolytic tumor cells convert glucose to lactate at a high rate, and lactate is exported together with a proton by monocarboxylate transporters (MCTs), among which MCT4 is often the main contributor [26–28]. Glycolytic tumor cells comprise hypoxic cells and proliferating cells that adopt a glycolytic metabolism either as an adaptation to hypoxia or as preferred metabolic platform allowing to rapidly alternate between energy production and biosynthetic phases associated with the cell cycle [6]. Transcription factors hypoxia-inducible factor-1 (HIF-1) and c-Myc cooperate to upregulate the expression of glucose transporters, glycolytic enzymes, lactate and proton transporters, and carbonic anhydrases [6, 25]. Oxidative cancer cells produce CO_2 that can contributes to tumor acidification, and additional sources of acidity comprise the metabolism of amino acids (especially glutamine) [29] and ATP hydrolysis [30].

While driver mutations causing cancers have been associated with the number of divisions of stem cells in healthy tissues and to a lower extent with environmental

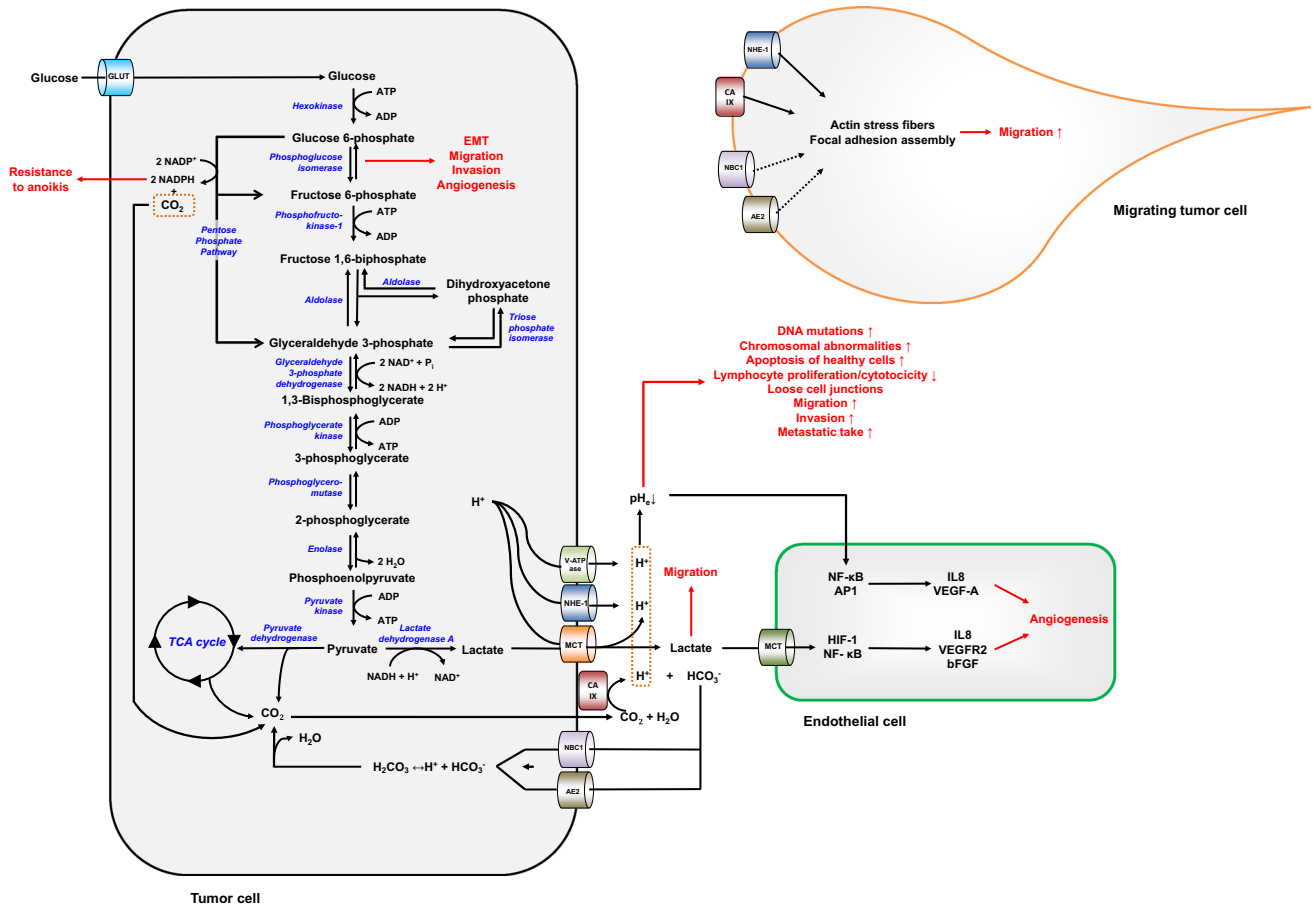

Fig. 2 Simplified scheme highlighting the contribution of tumor pH, glycolysis and the pentose phosphate pathway to tumor metastasis. Enzymes are represented in *italicized blue* font and their substrates in *bold black*. Tumor cells avidly take up glucose, which is progressively broken down during glycolysis to form pyruvate, a metabolite that fuels the tricarboxylic acid (TCA) cycle. Pyruvate can also be converted to lactate during lactate fermentation, and lactate is released from the cell along with protons, inducing intracellular alkalinization and extracellular acidification. This process and other ion exchangers involved in cellular pH regulation promote tumor cell migration and metastasis (shown in the migrating tumor cell on *top right*). Lactate and a low extracellular pH (pHe) can also promote tumor angiogenesis by activating several signaling pathways represented in the endothelial cell shown on *bottom right*. The glycolytic enzyme phosphoglucose isomerase acts as an autocrine signaling factor that triggers the epithelial-to-mesenchymal transition (EMT), migration, invasion and angiogenesis. The pentose phosphate pathway promotes tumor cell survival upon detachment. Other abbreviations: *AE2* anion exchanger 2, *AP1* activator protein 1, *bFGF* basic fibroblast growth factor, *CA IX* carbonic anhydrase IX, *GLUT* glucose transporter, *IL8* interleukin 8, *MCT* monocarboxylate transporter, *NBC1* sodium bicarbonate cotransporter 1, *NF-κB* nuclear factor-κB, *NHE-1* sodium-proton exchanger-1, *pHi* intracellular pH, *VEGF-A* vascular endothelial growth factor-A

factors and inherited predispositions [31], the low pHe of established tumors facilitates the acquisition of passenger DNA mutations and chromosomal abnormalities during tumor progression [32–34]. A low pHe further triggers the apoptosis of healthy cells at the periphery of the tumor and selects for acid- and/or apoptosis-resistant cancer cell clones [35–37]. It decreases lymphocyte activity and proliferation [38, 39], limits patient response to therapy [40] and promotes tumor angiogenesis, tumor cell migration, invasion and lung colonization following intravenous injection in animal models (i.e., metastatic take, also known as 'experimental metastasis') [25]. This review will discuss the aspects that are directly related to the metastatic process.

Tumor cell detachment from neighboring cells is a prerequisite for migration. A low pHe has been shown to decrease the abundance of adherent junctions between hepatoma cells by promoting c-Src-induced β-catenin phosphorylation and the subsequent disruption of β–catenin/E-cadherin interaction [41].

Once cells have detached, cell migration necessitates cycles of cellular elongation and contraction with the formation of a lamellipodium at the migrating edge of the cell during the elongation phase. During these cycles, pH regulators sodium-proton exchanger 1 (NHE1, a member of the SLC9 family of sodium-proton antiporters expressed at the plasma membrane of most cancer cells [42]), carbonic anhydrase IX (CA IX, the major membrane-bound CA

isoform catalyzing the extracellular conversion of CO_2 and water to bicarbonate and proton [43]), sodium bicarbonate transporter 1 and anion exchanger 2 (AE2, a pH-regulated chloride-bicarbonate antiporter [44]), which all act as extracellular acidifiers, are preferentially localized at the lamellipodium [45–47]. There, NHE1 was shown to assist the formation of actin stress fibers and focal adhesion clusters, at least in part through alkalinizing intracellular pH (pHi) [46, 48]. This process could also theoretically be controlled by other pH regulators expressed in lamellipodia. At the outer side of the membrane, extracellular acidification controls cell adhesion to the ECM by modulating the interaction of integrin $\alpha_2\beta_1$ with collagen. In a melanoma cell line, Stock et al. [49] identified an optimal pHe range promoting cell migration, with a too alkaline pHe ($\sim$7.5) resulting in cell adhesion that was too weak to promote migration and a too acidic pHe ($\sim$6.6) resulting in cell adhesion that was too strong. Extreme pHe values thus impaired migration. In prostate cancer cells, lowering pHe below physiological values stimulated the production of reactive oxygen species (ROS) and triggered ROS-mediated cell migration, which could involve decreased E-cadherin expression and modulation of the expression of integrins [50]. Conversely, targeting NHE1 [45, 46, 49, 51, 52], CA IX [47] and AE2 [45] with pharmacological inhibitors has been well documented to repress tumor cell migration. The promigratory activities of these proteins depend on their ability to regulate pH, but not only. At least in the case of NHE1 and CA IX, interactions with membrane proteins and the cytoskeleton have been described to control cell migration [48, 53].

During cell invasion, a low pHe stimulates the secretion and/or activation of several hydrolases that degrade ECM components. Among them, cathepsins B, D and L are cysteine proteases characterized by an optimal activity at low pH and a broad spectrum of substrates. They promote tumor cell invasion. Cathepsins B and L degrade several types of collagen, laminin and fibronectin in a process facilitated by a low pHe [54–56]. When pHe decreases, cathepsins are increasingly expressed at the plasma membrane of tumor cells and/or secreted as zymogens or active proteinases [57–60]. A low pHe indeed promotes the redistribution of lysosomes towards the cell periphery where they can release active cathepsin B [58, 61–63]. The molecular events orchestrating this response have not been fully identified to date. They implicate the activity of NHE1 in invadopodia, extracellular and intracellular acidification, and activation of RhoA GTPase that controls microtubule assembly [59, 62–64]. Procathepsins can also be released, and their autoactivation can theoretically occur in the tumor interstitial fluid at low pH ($\sim$4.5 to $\sim$6.5) [65–67]. In addition to a direct effect on the ECM, cathepsin B can cleave pro-urokinase (pro-uPA) into uPA, which then converts plasminogen to plasmin, a serine protease that degrades the ECM and promotes tumor cell invasion [57]. Besides cathepsins, matrix metalloproteinases MMP2 and MMP9 are proteinases secreted by tumor cells as zymogens that, upon activation, degrade collagen and promote tumor cell invasion. A low pHe increases their expression [68–70] and activity [60, 71], and can promote their release [60, 71, 72]. With respect to MMP9, a low pHe was found to activate voltage-dependent calcium channels that increased intracellular levels of free calcium, which was associated to activation of phospholipase D, mitogen-activated protein kinase (MAPK) kinase 1/2, p38 MAPK, and transcription factor nuclear factor-κB (NF-κB) directly controlling MMP9 transcription [69]. Activation of acidic sphingomyelinase was further involved [70]. Other MMPs are anchored in the plasma membrane, including membrane-type 1 MMP (MT1-MMP), which is under the control of NHE-1 [73, 74]. Via a still unknown mechanism, NHE-1 upregulates MT1-MMP expression and promotes its localization at the plasma membrane where MT1-MMP stimulates tumor cell invasion. Not only proteinases but also glycosidases participate in invasion. A close association between hyaluronan receptor CD44, NHE-1 and hyaluronidase 2 (Hyal-2) allows cooperative activity for ECM degradation [59]. In the complex, binding of hyaluronan to CD44 recruits Rho kinase to the plasma membrane, where it phosphorylates/activates NHE-1. NHE-1 contributes to extracellular acidification, which stimulates Hyal-2-induced hyaluronan catabolism and cathepsin B-dependent ECM degradation, both of which contribute to the invasive phenotype. Furthermore, the secretion of heparanase, an enzyme degrading proteoglycans of the ECM with an acidic pH optimum, was reported to correlate with invasion and lung colonization after injection of mouse melanoma cells in the tail vein of mice [75, 76]. Although a low pHe stimulates tumor cell invasion through multiple mechanisms, some studies failed to show activation of the invasive process after acidic priming, suggesting that these mechanisms may not necessarily be common to all cancer cell types [77]. Still, pharmacological inhibition of proton transporter NHE and extracellular CAs impaired tumor cell invasion in several studies [51, 59, 62, 73, 74, 78].

After tumor cell detachment, migration and invasion, metastatic take is also influenced by pH. Acidic priming of tumor cells was repeatedly reported to increase their capacity to colonize the lungs after injection into the tail vein of mice [50, 60, 72, 79]. While in the study of Schlappack et al. [79] the metastatic potential was further increased after a recovery period of 24–48 h at pH 7.4, the effect of acidic priming was only transient in the work of Rofstad et al. [60]. This observation suggests that different mechanisms with distinct kinetics could be involved,

depending on the cell line and/or cancer type considered. It also raises the question of whether low pHe exposure allows stable phenotype selection. In this context, there is evidence that growth at low pHe increases melanoma cell migration and invasion even after recovery at physiological pH for several passages [80].

Experimental metastasis investigates the ability of tumor cells to survive in the blood stream, to extravasate and to colonize distant organs. To understand whether interstitial acidosis is sufficient to promote the metastatic process as a whole, Kalliomaki et al. [81] used a model of spontaneous metastasis in mice in which acidosis was induced by administration of glucose and metaiodobenzylguanidine (MIBG, an inhibitor of complex I of the mitochondrial electron transport chain [ETC]). The treatment decreased pHe in primary tumors, but did not increase their metastatic potential, which can be related to the fact that full ETC inhibition can repress the metastatic process [82, 83] (see also companion paper).

Abnormal neovessels produced during tumor angiogenesis facilitate the metastatic process. The production and release of vascular endothelial growth factor (VEGF)-A and interleukin (IL)-8, two potent inducers of angiogenesis, have been found to be stimulated by a low pHe in various cancer cell lines following activation of the transcription factors NF-κB and activator protein-1 (AP-1) (Fig. 2) [60, 84–89]. Similar to activation of NF-κB by a low pHe [69], activation of AP-1 was found to involve the upstream activation of Ras and of the MAPK pathway, supporting enhanced *VEGF-A* transcription via AP-1 [89]. When binding to its receptors, VEGF-A promotes vascular permeability [90, 91] and activation of MMP1 [92] and uPA in migrating vascular endothelial cells [93]. IL-8 further stimulates the expression of MMP2 in melanoma cells [94] and was found to enhance the formation of liver metastasis in an orthotopic model of pancreatic cancer [84].

Pharmacological inhibition of CA IX was found to repress breast cancer metastasis formation in experimental and spontaneous metastatic models in mice [95, 96]. However, because CA IX inhibition also reduced tumor growth in spontaneous metastasis experiments [96], decreased metastasis detection could potentially reflect primary tumor growth impairment. Other approaches aim to buffer pH with alkaline compounds such as sodium bicarbonate, imidazole-based molecules or lysine. With respect to cancer metastasis, systemic pH buffering was shown to inhibit metastatic take in the lungs and spontaneous metastatic dissemination in several different mouse models of cancer [97–99]. Buffering tumors with alkaline compounds can indeed impair cathepsin B activity and the ability of tumor cells to extravasate [97]. Some cancer cells were also shown to be dependent on extracellular acidification for efficient proteolytic activities and lung colonization, whereas others were not [100]. The latter were insensitive to pH buffering treatments, thus implying that extracellular acidification is not an obligatory requirement for a tumor to successfully metastasize.

Glycolytic enzyme phosphoglucose isomerase

As stated above, established tumors contain tumor cells with different metabolic phenotypes, among which glycolytic tumor cells are characterized by elevated glucose uptake and lactate production resulting from the upregulation of most glycolytic enzymes as well as glucose and lactate transporters. In addition to catalyzing the reversible isomerization of glucose-6-phosphate to fructose-6-phosphate, glycolytic enzyme phosphoglucose isomerase (PGI) can act as a prometastatic signaling agent. PGI is also known as 'autocrine motility factor' (AMF), an autocrine cytokine that promotes tumor cell migration, invasion, experimental metastasis and, overall, metastasis as a whole (Fig. 2) [101–109].

PGI lacks a signal sequence for secretion and is therefore exported from cells through non-classical pathways activated according to the PGI expression level [107, 110, 111]. *PGI* transcription is indirectly induced by hypoxia-inducible factor-1 (HIF-1) through the VEGF pathway [108], and HIF-1 is itself a main inducer of the glycolytic switch in cancer. Once exported, PGI binds to membrane receptor gp78 to activate intracellular effectors [102], resulting in (1) relocalization of RhoA and Rac1 small GTPases, two master regulators of actin dynamics, to filopodia and lamellipodia [104, 112]; (2) increased expression of integrins $\alpha_2\beta_3$ and $\alpha_5\beta_1$ that translocate to the cell surface in order to regulate cell adhesion and to stimulate MMP2 activity [103, 104]; and (3) stimulation of EMT through NF-κB activation, upregulation of SNAIL, ZEB1 and ZEB2 transcription factors and downregulation of mir-200, leading to the loss of E-cadherin [106, 113, 114]. PGI/AMF can also bind to endothelial cells expressing gp78 to promote angiogenesis and vascular permeability, thereby facilitating tumor cell intravasation [115–117]. In patients, high PGI/AMF and gp78 expression in primary tumors and elevated serum levels of PGI were reported to positively correlate with metastasis in colorectal carcinoma, esophageal squamous cell carcinoma and lung adenocarcinoma [118–121].

Besides PGI, no other glycolytic enzyme has been directly involved in the promotion of tumor metastasis. Nevertheless, several glycolytic enzymes have been found in invadopodia [122], and glycolysis has been proposed to be a main source of ATP for tumor cell survival upon detachment and during migration [123–125].

Glycolytic end-product lactate

Glycolytic cancer cells secrete large amounts of lactate that notably serves as a counter-ion for proton export via MCTs (Fig. 2) [126, 127]. Once exported, lactic acid readily dissociates in lactate and protons. High glycolytic and glutaminolytic activities in combination with poor clearance thus result in lactate accumulation in the tumor interstitium [128], with lactate concentrations ranging from 1 to 40 μmol/g in human tumors (median value = 8 μmol/g) [129]. More than a mere metabolic waste, lactate can serve as an energetic fuel for oxidative tumor cells [28] and a signaling molecule that promotes angiogenesis (see below), chronic inflammation [130, 131], inhibits the immune system [132–135] and contributes to tumor resistance to radiotherapy [136]. In patients, high lactate levels have been positively correlated with metastasis in head and neck cancer [137, 138], rectal adenocarcinoma [139], and cervix carcinoma [140, 141].

Some studies have proposed a direct role of lactate in the metastatic process. Through a yet unknown molecular mechanism, lactate can indeed stimulate the production of hyaluronan, a high molecular weight glycosaminoglycan polymer of the ECM, and the expression of its receptor CD44 in tumor-associated fibroblasts, creating a favorable environment for tumor cell motility [142]. Lactate can also act directly on tumor cells. Addition of lactate to head and neck carcinoma cells in culture dose-dependently stimulated cell migration [135]. In glioma, Baumann et al. [143] reported that lactate promotes cell motility and EMT. This response to lactate was attributed to a LDHA-dependent induction of transforming growth factor-β2 that upregulated the expression of β_1-integrin, a subunit of ECM protein receptors controlling cell adhesion, and of MMP2. Bonuccelli et al. [144] further reported that 10 mM of lactate increased the in vitro migration of MDA-MB-231 human breast carcinoma cells, and daily intraperitoneal injection of lactate to mice promoted the metastatic take of these cells in the lungs of the animals. The same group showed that 10 mM of lactate further enhanced the clonogenicity (i.e., the ability of isolated tumor cells to generate a clonal population of daughter cells on soft agar) of MCF7 human breast carcinoma and induced a genetic signature associated with stemness [145].

Lactate also stimulates angiogenesis and could therefore indirectly contribute to the metastatic process (Fig. 2). Lactate can indeed be taken up by oxidative tumor cells and vascular endothelial cells via MCT1, after which it is oxidized to pyruvate by lactate dehydrogenase 1 (LDH1/LDHB). Pyruvate consequently accumulates in these cells where it competes with α-ketoglutarate to inhibit prolyl-hydroxylase 2 (PHD2), resulting in HIF-1 and NF-κB activation, increased *VEGF-A* transcription in oxidative tumor cells and increased transcription of VEGF receptor-2, basic fibroblast growth factor and IL-8 in vascular endothelial cells [146–152]. Interestingly, HIF-1 and NF-κB are inducers not only of angiogenesis but also of tumor metastasis [153, 154], suggesting that lactate could promote tumor metastasis by supporting PHD2 inhibition. This possibility still requires further investigation.

Lactate signaling involves lactate exchange through the plasma membrane, a process facilitated by the lactate-proton co-transporters of the MCT family. MCTs recently emerged as new therapeutic targets in cancer [25, 155]. In the context of tumor metastasis, silencing and pharmacological inhibition of MCT1 or MCT4 have been shown to inhibit the migration and invasion of various cancer cell lines in several independent studies [156–159]. The anti-metastatic potential of MCT inhibition is further supported by clinical studies having found higher levels of the MCT1 protein in metastatic lesions *versus* primary tumors of non-small cell lung carcinoma patients [160] and a positive correlation between MCT4 expression in primary colorectal cancers and the number of distant metastases in patients [161]. Still, to date, a formal demonstration that MCT inhibition represses the metastatic process is lacking. Such investigation should further dissect the respective contributions of lactate and protons. A differential response to lactate and acidity would not be trivial. For example, human mammary epithelial cells exposed to lactic acid or to HCl (both at pH 6.7) showed a similar genetic signature, which was different from that obtained after exposure to sodium lactate (pH 7.4) [162]. For the future development of MCT inhibitors, direct inhibition of lactate flux versus indirect effects should also be investigated. MCT1 and MCT4 indeed interact with CD147, a chaperone protein that is known to induce the secretion and activation of MMPs [163, 164]. Overall, whether lactate and proton influx, efflux or both promote metastasis, depending on the cell type, the tumor region and the cell compartment, remains an open question.

Pentose phosphate pathway

Glycolysis is connected to other metabolic pathways, notably the pentose phosphate pathway (PPP) (Fig. 2). As a major contributor to anabolism, the PPP has been well described to support tumor cell proliferation [165]. Interestingly, a further enhancement of the PPP has been reported in metastatic breast cancer cells with high tropism for the brain compared to the general population of CTCs [166], and in metastatic lesions compared to primary tumors in renal cell carcinoma [167]. Although correlative,

these observations indicate that the PPP could facilitate tumor metastasis.

The PPP consumes glucose-6-phosphate that fuels the sequential oxidative and non-oxidative arms of the pathway. The oxidative PPP produces ribulose-5-phosphate and, as side products, 1 CO_2, 3 protons and 2 NADPH per molecule of glucose-6-phosphate consumed. CO_2 and protons contribute to tumor acidification [168–170], and NADPH is a necessary cofactor for glutathione peroxidase and fatty acid synthesis. Ribulose-5-phosphate is either used for nucleic acid synthesis or fuels the non-oxidative arm of the PPP. The associated production of glyceraldehyde-3-phosphate and fructose-6-phosphate generates a metabolic shunt bypassing the PGI-catalyzed step of glycolysis (Fig. 2). Accordingly, overexpression of the PPP enzyme transketolase-like 1 (TKTL1) was shown to increase pyruvate and lactate production, resulting in HIF-1α stabilization [171].

Evading anoikis is a prerequisite for tumor cells to metastasize, a capability that can be acquired by several mechanisms including a switch in the pattern of integrin expression, EMT, prosurvival signaling and metabolic adaptations [11]. Because it is a main provider of NADPH for glutathione reduction, increasing the metabolic flux of the PPP is one of the strategies that tumor cells can select to counter ROS-induced anoikis upon cell detachment [124, 172]. Several studies have observed a requirement for PPP to afford tumor cell growth without anchorage [171, 173, 174]. Mechanistically, coupling between glycolysis and the PPP is controlled by the glycolytic enzyme pyruvate kinase M (PKM), catalyzing the conversion of phosphoenolpyruvate and ADP to pyruvate and ATP. Compared to differentiated cells that primarily express PKM1, tumor cells often express the embryonic PKM2 isoform resulting from alternative splicing: *PKM* gene transcription is induced by HIF-1 and alternative splicing to the PKM2 isoform by c-Myc that induces the expression of specific ribonucleoproteins [175–178]. Unlike PKM1, PKM2 can form active tetramers or inactive dimers [178]. Oscillation between the two states is regulated allosterically by metabolites such as fructose-1,6-bisphosphate (that promotes active tetramer formation) and alanine (that promotes inactive dimer formation) and, independently, by oxidation [179]. When ROS levels increase (for example following cell detachment), Cys358 of PKM2 is oxidized, impairing tetramer formation and redirecting metabolic flux from glycolysis to the PPP where NADPH is produced. Elevated NADPH production promotes glutathione recycling, reduction of Cys358 and tetramer assembly to restore a high glycolytic flux and a lower PPP activity. PKM2 thereby acts as a redox sensor that controls the metabolic fate of glucose. Similarly, it has been shown in yeast that the activity of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase is reversibly inhibited by oxidation of Cys152, which controls the coupling between glycolytic and PPP fluxes [180]. Another regulator of the PPP is the tumor suppressor protein p53, which binds to and prevents the dimerization/activation of glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the oxidative arm of the PPP [181]. Together, these mechanisms control antioxidant defenses and promote cell survival in stress situations associated with increased ROS production, such as tumor cell detachment. Other important sources of NADPH are the malic enzyme reaction [182] and folate metabolism [183].

Although the contribution of the PPP to resistance to anoikis is well recognized, whether and how it influences tumor cell migration and invasion has not received much attention to date. Experimental evidence shows that silencing 6-phosphogluconate dehydrogenase (6PGD), the third enzyme of the oxidative arm of the PPP, reduces the in vitro migration of lung carcinoma cells upon hepatocyte growth factor (HGF) stimulation [184]. It does so by decreasing the tyrosine phosphorylation/activation of HGF receptor c-MET. Still, the molecular pathway linking 6PGD expression and/or PPP activity to c-MET phosphorylation has not been identified.

Evidence exists pointing at a control of tumor cell invasion and cancer metastasis by transketolase (TKT) and TKTL1 enzymes. TKT is the rate-limiting enzyme of the non-oxidative part of the PPP that catalyzes the reversible transfer of two carbon units between ketose- and aldose-phosphate [185]. On the other hand, TKTL1 has been proposed to have a different substrate affinity and a different catalytic activity: instead of transferring two carbon units, it would act similarly to bacterial phosphoketolase and produce ATP and either acetate or acetyl-CoA for lipid biosynthesis, thus providing a tumor growth advantage [186]. High TKTL1 expression has been reported in various human cancer types [171, 187–189] and was positively correlated with invasion in urothelial and colon carcinomas [187] and to metastasis in renal cell, ovarian and papillary thyroid carcinomas [190–192]. In colorectal carcinoma, TKTL1 levels correlated positively with lymph node positivity but negatively with distant metastasis [193]. However, data associating high TKTL1 expression to metastasis have to be interpreted with caution. The specificity of the anti-TKTL1 antibody used in correlative studies has indeed been called into question, and TKT has rather been proposed as the dominant enzyme expressed in malignant tumors [194, 195]. Thus, the relevance of the non-oxidative part of the PPP in tumor metastasis still remains to be demonstrated.

Concluding remarks

Nowadays, the clinical evolution of cancers to the metastatic stage is too often associated with patient death, reflecting the limits of the current therapeutic arsenal and, notably, the lack of specific pharmacological therapies targeting metastatic progenitor cells and metastatic lesions. Metabolic heterogeneity is a hallmark of cancer that impacts and can probably drive most phenotypic features of malignancy, including metastasis. Among these phenotypes, switching to a glycolytic metabolism is well known to promote hypoxic cell survival and offers the metabolic plasticity necessary for cells to rapidly switch from energy production metabolism to biosynthesis for cell proliferation [6]. But while the strongest evidence that glycolysis promotes tumor aggressiveness has been obtained in primary tumors, little is known regarding its contribution to the metastatic process.

Among glycolytic enzymes and metabolites, PGI/AMF and lactate single out as potential prometastatic agents that facilitate early phases of the process. Yet, clinical applications are still to be developed. Inhibiting PGI binding to gp78 could be of potential therapeutic interest for metastasis prevention, but, to our knowledge, is currently not under experimental evaluation. As an alternative, MCT inhibitors with clinically compatible pharmacological profiles have been identified [196–198], among which MCT1 inhibitor AZD3965 is currently entering a Phase I clinical trial for patients with prostate cancer, gastric cancer or diffuse large B cell lymphoma (ClinicalTrials.gov NCT01791595). The primary objective of this trial is to identify dose-limiting toxicities and its secondary objective is to objectivize anticancer effect(s) of the MCT1 inhibitor. Thus, prevention of metastasis, prolongation of metastasis-free survival and the assessment of metastasis regression are currently beyond the scope of clinical evaluation, which also reflects the fact that a strong, formal experimental demonstration that lactate and MCTs promote cancer metastasis is still lacking.

Glycolysis does not produce a high yield of protons *per se* but fuels proton-producing side pathways and reactions, including the PPP, ATP hydrolysis and oxidative metabolism [25, 199]. It also contributes to extracellular acidification with lactate serving as a counter-ion for proton export [24]. Although the invoked mechanisms can differ across studies, there is now good evidence that extracellular acidification and intracellular alkalinization can promote tumor cell detachment, migration, invasion, metastatic take, angiogenesis and, consequently, tumor metastasis. In theory, a low pHe, therefore, constitutes a therapeutic target *per se*, which is currently explored with pH buffering therapies, but resistance may occur by the selection of mechanisms that promote metastasis independently of a low pHe [100]. As an alternative, inhibition of the various transport systems facilitating lactate export could potentially repress metastatic dissemination, as probably best exemplified with CA IX inhibitors [96, 200]. Still, a thorough understanding of the specific *versus* redundant contributions of these proton transporters to tumor metastasis is needed in order to design inhibitors and identify most effective anti-metastatic strategies. Of note, while repressing tumor acidification is a potential therapeutic approach against cancer, exploiting tumor acidity to target drugs to tumors is also particularly appealing. In particular, pH-sensitive nanoparticles are currently being developed to selectively deliver anticancer drugs to tumors with enhanced efficacy and limited side effects [201, 202].

Despite some recent progress, our current understanding of the metabolic features associated to cancer metastasis is still fairly limited and many reported observations are purely correlative. Therefore, a main task to achieve in basic research is to discriminate metabolic changes driving tumor metastasis from those acquired as a secondary adaptation to phenotypic changes. Several metabolic intermediates (including lactate, succinate and fumarate [24, 203, 204]) have already been identified to act as signaling agents capable of activating prometastatic pathways, therefore directly enhancing tumor growth and metastasis. Their activities include modulation of enzymatic activities [205, 206] and binding to membrane receptors [207]. The list of metabolites endowed with signaling activity is most probably far to be exhaustive and could be extended by systematic characterization. Another area deserving attention relates to the nonmetabolic functions of metabolic enzymes, as illustrated here with PGI/AMF. Several enzymes could indeed promote tumor metastasis independently of their catalytic activity. For example, PKM2 has been found in cell nuclei where it can regulate the transcriptional activity of HIF-1 and promote EMT, glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase can activate the prosurvival Akt pathway, and aldolase can trigger EMT [208, 209]. Together, a better understanding of the nonmetabolic functions of metabolites and metabolic enzymes could allow the rationale design of new anti-metastatic approaches for therapy.

While increasing evidence indicates that a low pHe, glycolysis and the PPP can influence cancer metastasis, there is also good evidence that mitochondria, lipid and amino acid metabolism can facilitate the metastatic process. This is the topic of a companion paper. This argues for the existence of temporally well-defined metabolic adaptations along the metastatic route, the articulation of which is still largely beyond understanding.

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