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Review

Plasticity of cancer invasion and energy metabolism

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Energy deprivation is a frequent adverse event in tumors that is caused by mutations, malperfusion, hypoxia, and nutrition deficit. The resulting bioenergetic stress leads to signaling and metabolic adaptation responses in tumor cells, secures survival, and adjusts migration activity. The kinetic responses of cancer cells to energy deficit were recently identified, including a switch of invasive cancer cells to energy-conservative amoeboid migration and an enhanced capability for distant metastasis. We review the energy programs employed by different cancer invasion modes including collective, mesenchymal, and amoeboid migration, as well as their interconversion in response to energy deprivation, and we discuss the consequences for metastatic escape. Understanding the energy requirements of amoeboid and other dissemination strategies offers rationales for improving therapeutic targeting of metastatic cancer progression.

Highlights

Mechanochemical strategies deployed by cancer cells to invade tissues depend upon different amounts of energy production and consumption.

Whereas collective and mesenchymal migration is bioenergetically demanding, amoeboid migration is energetically favorable.

Under conditions of energy deprivation, invading cancer cells adapt both their cytoskeletal activity and metabolism to save energy and secure migration.

Recently identified transitions in response to oxygen and energy deprivation include collective-to-amoeboid and mesenchymal-to-amoeboid transitions.

Understanding the crosstalk between bioenergetic and cell migration pathways will aid the identification of intervention points to interfere with tumor cell dissemination and metastasis.

Cancer invasion and nutrient deprivation

Cancer cell invasion and metastasis result from cytoskeletal activation in tumor cells that initiates migration and detaches cells from the primary tumor followed by dissemination through interstitial tissue and across vessel walls [1,2]. As a consequence of migration, tumor cells can spread via blood and lymphatic vessels to other organs and initiate metastatic regrowth. To migrate through tissues, tumor cells deploy migration strategies used by other cells during physiological processes such as morphogenesis, wound healing, and inflammation. Three distinct but interconvertible migration programs have been identified which differ in cell–cell and cell–matrix adhesion, cytoskeletal organization, and mechanochemical tissue interactions. **Collective movement** (see Glossary) depends on cell–cell adhesion of variable stability and represents an important migration mode in embryonic morphogenesis, vascular spouting, wound healing, and cancer cell metastasis [3–5]. **Mesenchymal migration** of individual cells involves prominent cell–matrix adhesions, actomyosin contractility, and proteolytic remodeling of the tissue, as detected in fibroblasts and tumor cells that have undergone the epithelial-to-mesenchymal transition [6]. **Amoeboid migration** is mediated by cortical actomyosin contractility, weak cell–matrix adhesion, and low pericellular proteolysis. This type of movement is mediated by changes in cell shape as detected in leukocytes, lymphoma cells, and more rarely in solid tumors [7].

During cancer progression, cancer cells must adapt their energy production and consumption to local conditions of the primary or metastatic tumor microenvironments. Rapidly growing tumors display high energy demands and consumption of energy carriers ATP and GTP but simultaneously suffer from significant local perfusion deficits [8]. The resulting metabolic challenges include hypoxia and nutrient depletion, as well as an accumulation of cell-derived toxic metabolites [8,9]. Energy deprivation induces a cascade of adaptation responses in tumor cells to reduce energy consumption and make use of alternative nutrient sources and metabolic pathways so as to avoid cell death [10–12]. In addition, energy deprivation activates programs that induce migration and enable cell escape from perturbed tissue [13,14]. However, whether and by

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which mechanisms energy deprivation causes either arrest, activation, or switching of invasion programs has been unclear. Recent progress in applying live-cell microscopy in 3D tissue culture and tumor models *in vivo* has revealed the astounding adaptability of tumor cell migration programs in response to hypoxia and/or energy deprivation. As an outcome, an integrated program consisting of metabolic adaptation, growth control, and plasticity of tumor cell migration towards an energy-conserving amoeboid escape mode has been identified [15,16].

We summarize here the bioenergetic pathways engaged in cancer cell invasion, the commonalities and differences in energy metabolism in collective, mesenchymal, and amoeboid modes, and their interconversion in response to energy deprivation. We discuss the adaptation of energy metabolism in response to oxygen and nutrient deficiency and the resulting adaptation of migration strategies. Lastly, we highlight conversion to amoeboid dissemination as an integrated program that secures both cancer cell dissemination through 3D tissue and survival. Understanding the shared programs of energy metabolism and invasion mechanisms offers new perspectives for therapeutic intervention to combat metabolic resilience and metastatic escape.

Cell migration modes and metabolism

In moving cells, energy demands are tightly linked to cytoskeletal activity. ATP consumption results from **actin filament** formation, as well as cyclic phosphorylation and dephosphorylation of regulatory and adaptor proteins. These energy-consuming processes are necessary to build actin networks, regulate actin filament dynamics, and contract actin filaments by myosin motor activity [17]. Consequently, together with enzymatic remodeling of extracellular matrix (ECM) structures, all actin-dependent steps of cell migration, including cell–cell and cell–matrix adhesion, change of cell shape, cell contraction, and force generation, consume significant amounts of ATP and GTP [17]. To effectively deliver energy at a subcellular scale to sites where the cytoskeleton is actively being rearranged, mitochondria and **glycolytic** enzymes interact with the actin cytoskeleton to ensure energy production near the site of consumption [18–22].

Actin filament formation and contraction

Actin polymerization into filaments and their turnover are crucial for cell polarization and the formation of protrusions including lamellipodia, filopodia, focal adhesions, and stress fibers [23]. A large amount of ATP is consumed by the ATPase activity of actin in building actin filaments from monomers (Figure 1A). Actin filament formation and actin network dynamics are regulated by **actin-binding proteins (ABPs)** [24]. Depending on upstream regulation, including **Rho-family GTPases** and cooperating kinases and phosphatases, ATP-consuming actin dynamics extend three protrusion types at the leading edge with different morphologies, actin organization, and kinetics: **lamellipodia**, **filopodia**, and **blebs** [24] (Figure 1A). Actin **stress fibers** and actin filament networks become contracted by **non-muscle myosin II** [25] and by ATP-dependent myosin motor activity, and are regulated by ATP-dependent kinases that control Rho regulatory light-chain (RLC) activity (Figure 1A).

Lamellipodia and filopodia are actin polymerization-driven extensions of the plasma membrane that require respective branching and bundling of actin filaments and myosin contractility to engage with the extracellular substrate [24]. Alternatively, blebs initially form when the intracellular hydrostatic pressure increases and the plasma membrane locally detaches from the actin cortex [26]. Lamellipodia and filopodia depend upon actin turnover, whereas blebs form without actin polymerization [26]. Consequently, the energy demands incurred by each protrusion type may differ (Box 1) [24].

Cell–cell adhesion

Adherens junctions support cell–cell adhesion between collectively migrating tumor cells and mediate tumor cell interaction with stromal fibroblasts and/or macrophages during invasion

Glossary

Actin-binding proteins (ABPs): mediators of actin filament organization and dynamics, including elongation (by formins), branching of actin networks (by actin-related protein 2/3 complex, Arp2/3), network severing (cofilin), and disassembly into monomers (cofilin).
Actin filaments: flexible and thin microfilaments formed by the polymerization and depolymerization of actin monomers, and which determine cell adhesion, shape, stability, and movement.
Adherens junction: the initiator and stabilizer of **cell–**cell adhesion; junctions are composed of cadherin adhesion receptors, intracellular adaptors (e.g., β-catenin, α-catenin, p120-catenin), and actin filaments.
Amoeboid migration: a migration mode driven by rounded cell shape, blebbing, or pseudopodal protrusions, as well as by weak or absent cell–matrix adhesions.
AMP-activated protein kinase (AMPK): a central sensor of low intracellular ATP or high ADP and AMP levels which responds to energy deficiency by inhibiting ATP consumption and securing ATP production by favoring glycose uptake.
Autophagy: a catabolic process by which cellular components are engulfed in autophagosomes, degraded into sugars, fatty acids, and amino acids, and recycled to generate pyruvate and glucose to secure glycolysis and oxidative phosphorylation under conditions of energy deprivation.
Blebs: poorly adhesive roundish membrane protrusions that form through hydrostatic pressure, contain cortical actin, and frequently support amoeboid movement.
Collective movement: a motility mode of groups, sheets, or strands of cells that preserves cell–cell junctions and synchronizes their intracellular signaling and actin dynamics.
Epithelial–mesenchymal transition (EMT): a multistep activation and differentiation process by which epithelial cells achieve mesenchymal phenotypes, activate migration, and delay cell-cycle progression.
Fatty acid oxidation: the primary mitochondrial aerobic pathway of fatty acid catabolism to acetyl-CoA to produce proteins, carbohydrates, and lipids.

[3,27,28]. Cadherins transmit force to the actin and microtubule cytoskeleton through adaptor molecules and ABPs, and ATP is consumed by upstream regulators including Abelson tyrosine kinase (Abl), Src, RhoA, and Rac1 [29]. For example, Abl phosphorylates vinculin which then binds to actin, activates RhoA, and increases actomyosin contractility at cell–cell interactions [30]. Cadherin mechano-coupling activates metabolic signaling and energy production, including increased glucose uptake and ATP production [31].

Cell–ECM adhesion

Integrin activation, clustering, and mechano-coupling are mediated by adaptor proteins, including talin, vinculin, paxillin, filamin A, and α-actinin [32], under the control of ATP-consuming upstream kinases and phosphatases [33]. In concert, focal adhesion kinase (FAK), Src-family kinases, integrin-linked kinase (ILK), PAK Ser/Thr kinases, and tyrosine phosphatases SHP2 [34] and PTP-PEST [35] control integrin engagement, adhesion turnover, and migration [36]. Integrin interactions with actin filaments and mechano-coupling further depend on the localized activation of Rac and RhoA and engagement of myosin II in an ATP-dependent manner [33].

Nucleocytoskeleton connection

The nucleus is the largest and stiffest organelle, which becomes deformed, moved, and mechanically protected in migrating cells by perinuclear actomyosin networks in an ATP-dependent manner [37]. Rho GTPases Cdc42 and RhoA control the nucleo–actin connection by myosin II-mediated crosslinking and contraction (Figure 1D) [38–40]. Perinuclear actin couples to the nuclear envelope via the adaptors nesprin and SUN proteins under the control of Rac1 [41]. ATP is further necessary to assemble, deform, and disassemble the nuclear lamina consisting of a filamentous network of A/C- and B-type lamins under the control of kinases including protein kinase B (PKB/Akt) [42,43] and Src kinase [44]. Src further regulates nuclear stiffness via phosphorylation of inner nuclear membrane proteins (e.g., emerin) [45].

Volume regulation

Intracellular water content and cell volume during cell migration are regulated by ion transporters (e.g., Na^+/H^+ exchangers, $Na^+/K^+/2Cl^-$ cotransporters) and aquaporins (AQPs) which jointly control the intracellular water content [46,47]. ATP is consumed by ion channel pump activity and by phosphorylation to regulate AQP activity regulation through phosphorylation (e.g., the cAMP–PKA axis) (Figure 1E) [46,48,49]. AQPs cooperate with cytoskeletal proteins and support protrusion formation at the leading edge (e.g., AQP-1, -4, and -5) or local shrinkage and detachment at the cell rear [50].

Proteolytic tissue remodeling

Invasive cells can facilitate their movement through the ECM by its proteolytic degradation through matrix metalloproteinases (MMPs) and other proteases in an energy-dependent multistep process (Figure 1F) [51]. ATP and GTP are consumed for MMP expression, protein folding, transport of MMP-containing vesicles via microtubules and motor proteins [52], MMP activation [53], and exocytosis [54]. Cell surface-localized proteolysis depends on the delivery and recycling of transmembrane proteases (e.g., membrane-type I matrix metalloproteinase, MT1-MMP) as well as on phosphorylation of the cytoplasmic tail (Figure 1F) [55].

Metabolic pathways fueling cell migration

The metabolic pathways delivering energy for basic cell functions, including cytoskeletal dynamics and cell movement, depends on intracellular ATP, GTP, NAD(P)H, and flavin adenine dinucleotide (FADH). In response to energy deprivation, cells upregulate nutrient uptake and

Filopodia: thin, spindle-shaped, and dynamic actin-rich protrusions at the leading edge that probe and adhere to the environment during cell migration.
Glycolysis: a rapidly adaptive metabolic pathway (100-fold faster than OXPHOS), which yields 2 moles of ATP per mole of glucose and can occur under oxygen-dependent or -independent, aerobic, or non-aerobic conditions.
Hypoxia-inducible factor 1 (HIF-1): a central regulator of cell response to hypoxia, including transcription of genes involved in glucose metabolism, survival and release of pro-angiogenic cytokines.
Integrins: adhesion receptors that connect the actin cytoskeleton to extracellular ligands through affinity regulation and clustering, thereby forming transient extracellular matrix (ECM) interactions and generating traction forces required for migration.
Lamellipodia: flat membrane protrusions at the front of moving cells which extend by actin network dynamics, adhesion to substrate by integrins, and pull the cell forward by myosin II motors.
Macropinocytosis: internalization of extracellular proteins and necrotic cell debris (necrocytosis), often followed by degradation in phagolysosomes.
Mesenchymal migration: a mode of migration characterized by fibroblast-like morphology, focalized interactions with the extracellular matrix (ECM), and protease-dependent ECM degradation.
Non-muscle myosin II: an actin-binding protein involved in actin crosslinking and the contraction of actin filaments.
Oxidative phosphorylation (OXPHOS): an efficient energy-producing pathway in mitochondria that depends on oxygen and converts glucose via the tricarboxylic acid (TCA) cycle into ATP (36 ATP per glucose molecule).
Pentose phosphate pathway: an alternative pathway of glucose metabolism that provides metabolites for nucleotide synthesis, cell survival, and growth, including NADP and ribose 5-phosphate.
Pseudopodia: actin-rich short-lived membrane protrusions involved in cell migration and chemotaxis.
Rho-family GTPases: a family of signaling G proteins that function as regulators of cytoskeletal dynamics, cell polarity, adhesion, and migration.
Stress fibers: parallel bundles of filamentous actin that are contracted by myosin II motors in cooperation with actin-binding proteins (e.g., α-actinin).

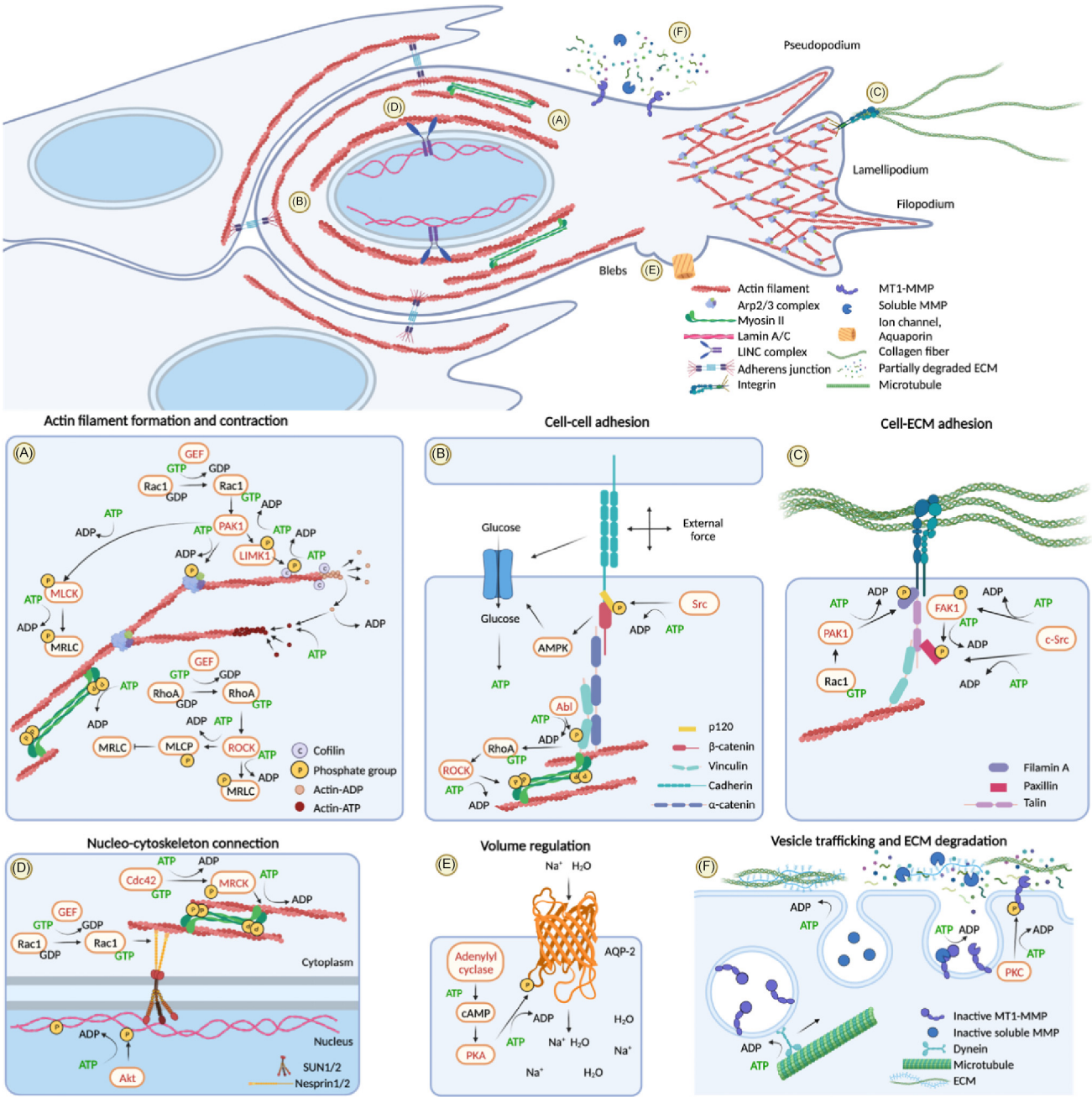

Figure 1. Energy-consuming processes during migration. Overview (top panel) and individual ATP-consuming steps involved in cell movement (bottom panels, A–F). (A) Actin–ATP monomers polymerize to filaments. The dynamics of the filaments is controlled by actin-binding proteins, including cofilin, under the control of LIMK and phosphatases, that are under the control by Rac1. Rac 1 further controls PAK1–Arp2/3 engagement for filament branching. Non-muscle myosin II (NM II) mediates actin filaments contraction, under the control of MRLC phosphorylation regulated by MLCK and MLCP controlled by Rac1 and RhoA, respectively. (B) ATP consumption is involved in cadherin adaptor molecules regulation via phosphorylation (e.g., of p120-catenin) under Src kinase control. In response to external forces, E-cadherin stimulates AMPK signaling. AMPK stimulates increased glucose uptake and its conversion into ATP. AMPK further acts on kinases (Abl) to phosphorylate vinculin and the RhoA–ROCK–myosin II axis. (C) Integrin activity, clustering, and turnover require energy because they are regulated by cycles of phosphorylation and dephosphorylation of their cytoplasmatic tail and adaptor proteins. The examples show paxillin phosphorylation by FAK and Src kinases, and filamin A controlled by the

(Figure legend continued at the bottom of the next page.)

Box 1. Analysis of energy metabolism with single-cell and subcellular resolution

A range of classical methods allow analysis of energy production and consumption in cell populations in bulk culture, including detection of oxygen consumption, production of lactate, ATP concentration (luciferase assay), and the rate of extracellular acidification [139]. However, linking mechanisms of cell migration to cell metabolism is technically challenging because it depends on coregistering cell migration and energy fluxes in live-cell culture over time followed by image analysis of individual cells in cell populations to account for interindividual heterogeneity during mixed responses.

At the single-cell level, fluorescent reporters allow detection of the oxidative state of mitochondria (e.g., JC-1 [140]), the oscillation of glycolysis [141], the ATP:ADP ratio (PercevalHR), glucose uptake (2-NBDG probe), NADH:NAD^+ redox state (Peredox probe), and H_2O_2 gradients (e.g., HyPer 7) [142]. To link the metabolic state to cytoskeletal action, energy flux reporting needs to be linked to molecular intervention of defined pathways, and recording of the effects on metabolism caused by the intervention. For example, inhibition of RhoA reduces the oscillating activity of glycolysis in adherent endothelial cells, indicating that glycolysis fuels RhoA-mediated actomyosin contractility [141]. However, mapping the role of metabolic pathways for the generation of individual actin-based structures, such as protrusion types or cortical versus perinuclear actin filaments, and their respective energy needs will require spatially defined live-cell measurements at a subcellular resolution. This will allow reporting locally produced and consumed ATP equivalents or local enzyme activity in metabolic pathways. In addition, the coregistration of several metabolic pathways in the same cell is currently limited due to the spectral overlap of available fluorescent reporters. Thus, in-depth information linking the relative weight and cooperation of energy pathways to migration modes and individual actin-based structures will require the development of functionally and spectrally complementary single-cell reporter systems. In addition, combining spectrally unmixed multichannel recordings of energy states with molecular-based *in silico* modeling on ATP consumption will enable predictions of the energy needs of individual cellular substructures and the changes associated with plasticity responses [49].

adapt the metabolic pathways (reviewed in [56]). We briefly summarize key metabolic pathways and their interdependence with cell migration.

Energy production under normal conditions

When oxygen and glucose supply is unperturbed, invading cancer cells balance their energy homeostasis mostly between **oxidative phosphorylation (OXPHOS)** and glycolysis to maintain migration activity in response to mechanical and chemical cues in the microenvironment [57]. OXPHOS provides localized energy production to the most energy-demanding regions of the cell. Mitochondrial trafficking to the leading edge of the cell supports cytoskeletal dynamics, membrane protrusion, and focal adhesion assembly [19–22]. Localized glycolysis occurs near sites of cytoskeletal activity and supports migration dynamics by ATP production [58,59]. For example, phosphofructokinase-1 (PFK-1), the rate-limiting enzyme of glycolysis, binds to actin in its active form, thus controlling glycolysis near cytoskeletal activity [17]. Other glycolytic enzymes, including aldolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), bind to actin filaments as inactive enzymes which, after release, undergo activation near sites of cytoskeletal dynamics [17]. Under both normal conditions and metabolic deprivation, the bioenergetic functions of OXPHOS and glycolysis are complementary, cooperative, and respond to nutrient availability [60]. Glycolysis can occur without oxygen involvement but is rapidly adaptive in delivering ATP, whereas OXPHOS depends upon oxygen availability and constitutively produces high amounts of ATP [60].

Energy deprivation

Under conditions of metabolic stress, including acidosis, hypoxia, and nutrient deprivation, additional mechanisms for ATP production become activated, including **autophagy**, amino acid and

Rac1–PAK1 axis. (D) ATP is engaged in MRCK activity and regulation by the Rho GTPase Cdc42, which leads to myosin contractility around the nucleus. ATP is further required for the activity of the LINC (linker of nucleoskeleton and cytoskeleton) complex and dynamic actin filaments connecting to the nucleus. For example, Rac1 interacts with Nesprin-2 to connect the LINC complex to actin. Src-mediated phosphorylation of lamin A causes lamin A disassembly from the inner nuclear lamina. (E) ATP-dependent phosphorylation and regulation of ion channel pumping into the cytoplasm and aquaporin activity. For example, AQP-2 is phosphorylated by PKA, which is activated by cAMP. (F) ATP-consuming steps during extracellular matrix (ECM) degradation, including kinesin and dynein-mediated vesicle transport of proteases, endo/exocytic protease transport, autocatalytic activation of the zymogen, and zymogen cleavage by activating protease. MT1–MMP activity can further be regulated through LIMK-mediated phosphorylation of the cytoplasmic tail. Figure created with BioRender.com.

creatine metabolism, and lipid oxidation [61,62]. Within minutes after energy deprivation, cancer cells can adapt ATP production by activating pathways regulating cell metabolism, including **AMP-activated protein kinase (AMPK)**, **hypoxia-inducible factor (HIF-1)**, and calpain, which enable an acute bioenergetic response [63,64]. AMPK stimulates glucose uptake (through the glucose transporters Glut1 and Glut4) and ATP production through glycolysis [64]. AMPK further promotes the use of alternative energy sources, including lipid import into mitochondria for **fatty acid oxidation** and **autophagy** [64]. In addition, AMPK reduces energy expenditure by inhibiting the mTOR pathway, which delays RNA translation and cell-cycle progression [65]. HIF-1 mediates the transition from oxidative to glycolytic metabolism [66] as well as autophagy [67] to maintain ATP levels. In response to hypoxia, HIF signaling upregulates the expression of glycolytic proteins (e.g., GLUT1, PFKFB, lactate dehydrogenase A) [66,68] and inactivates the tricarboxylic acid (TCA) cycle [66]. In addition to adapting their energy metabolism, tumor cells further broaden the spectrum of sources of rate-limiting metabolites and molecules to fuel the TCA cycle by degradation products that are typically present in the metabolically perturbed microenvironment (Box 2). These include proline from degraded ECM sources [69], amino acids (e.g., glutamine, glutamate) by intracellular biosynthesis or from the extracellular space [70], extracellular creatine [71], and lactate which is produced by glycolysis in metabolically perturbed tumors [72]. Extracellular nutrients become internalized via molecular transporters (GLUT1, MCT1, LAT1), whereas multi-molecular aggregated proteins and lipids as well as cell fragments become internalized via **macropinocytosis** [62,73].

The type and extent of adaptation of energy metabolism depend on the severity of oxygen and energy deprivation, and take place in a cell- and tumor type-dependent manner, to secure intracellular glucose, ATP, and NADPH production for cell survival and migration [56].

Interdependence of energy consumption and migration strategy

Cells can migrate individually, without cell–cell adhesion, or collectively when cell–cell adhesions are retained [74,75]. The ATP consumption involved in individual or collective migration depends on the engagement of the adhesive, cytoskeletal, and proteolytic activities, resulting in differing energy demands.

Collective migration

Collective movement depends on actin dynamics in coordination with cadherin-based cell–cell adhesion and gap junction intercellular communication, in concert with integrin-mediated

Box 2. Intersection of energy deprivation, metabolic stress, and toxic waste

In the metabolically perturbed tumor microenvironment, adaptations of energy production often coincide with metabolic stress responses induced by non-toxic and toxic extracellular metabolites and inflammation, as well as stress responses to therapy. Oxygen deprivation increases the intracellular production of reactive oxygen species (ROS) by mitochondrial complexes I and III. In addition, extracellular ROS is produced by activated neutrophils and macrophages in the tumor microenvironment [143]. ROS can oxidize protein thiols, lipids, and DNA, and can directly perturb cell integrity [144] and activate pathways of cell adhesion and migration by cysteine oxidation of signaling proteins [e.g., mitogen-activated protein kinase (MAPK) and NF-kB], ABPs, actin [145] or upstream receptors (e.g., EGFR) [144]. Ultimately, excessive ROS production can impair cancer cell migration and survival, and limit metastatic spread [146]. Non-toxic metabolites including purine nucleotides (e.g., adenosine) can activate G protein-coupled adenosine receptors (ADORAs) which promote cytoskeletal activation and invasion, as well as proliferation and angiogenesis [4]. Intracellular and extracellular products which accumulate in metabolically stressed tissue, including H^+, lactate, and ammonia, can perturb the metabolism, viability, and migration of cells. For example, extracellular acidosis leads to the activation of RhoA, downregulates cell–cell adhesions, and upregulates MMP expression, ultimately favoring epithelial-to-mesenchymal transition (EMT) and invasive properties [67]. Lactate, in addition to lowering the extracellular pH, can also act as a signaling metabolite to increase the HIF-1α-dependent hypoxia response leading to proliferation, dissemination, and escape from the immune system mediated by the lactate-activated G protein-coupled receptor GPR81 [72]. In concert, stress pathways and bioenergetic adaptation mediate integrated metabolic stress responses that favor tumor cell invasion and metastatic escape.

mechano-coupling to the ECM and proteolytic ECM remodeling [74,76]. Owing to its mechano-chemical complexity, the energy demands of collective migration in cancer cells are high (Figure 2) [16,77].

Leader cells directing and paving the way for collective invasion require a higher level of intracellular ATP/ADP compared to follower cells [77]. High ATP demands may result from

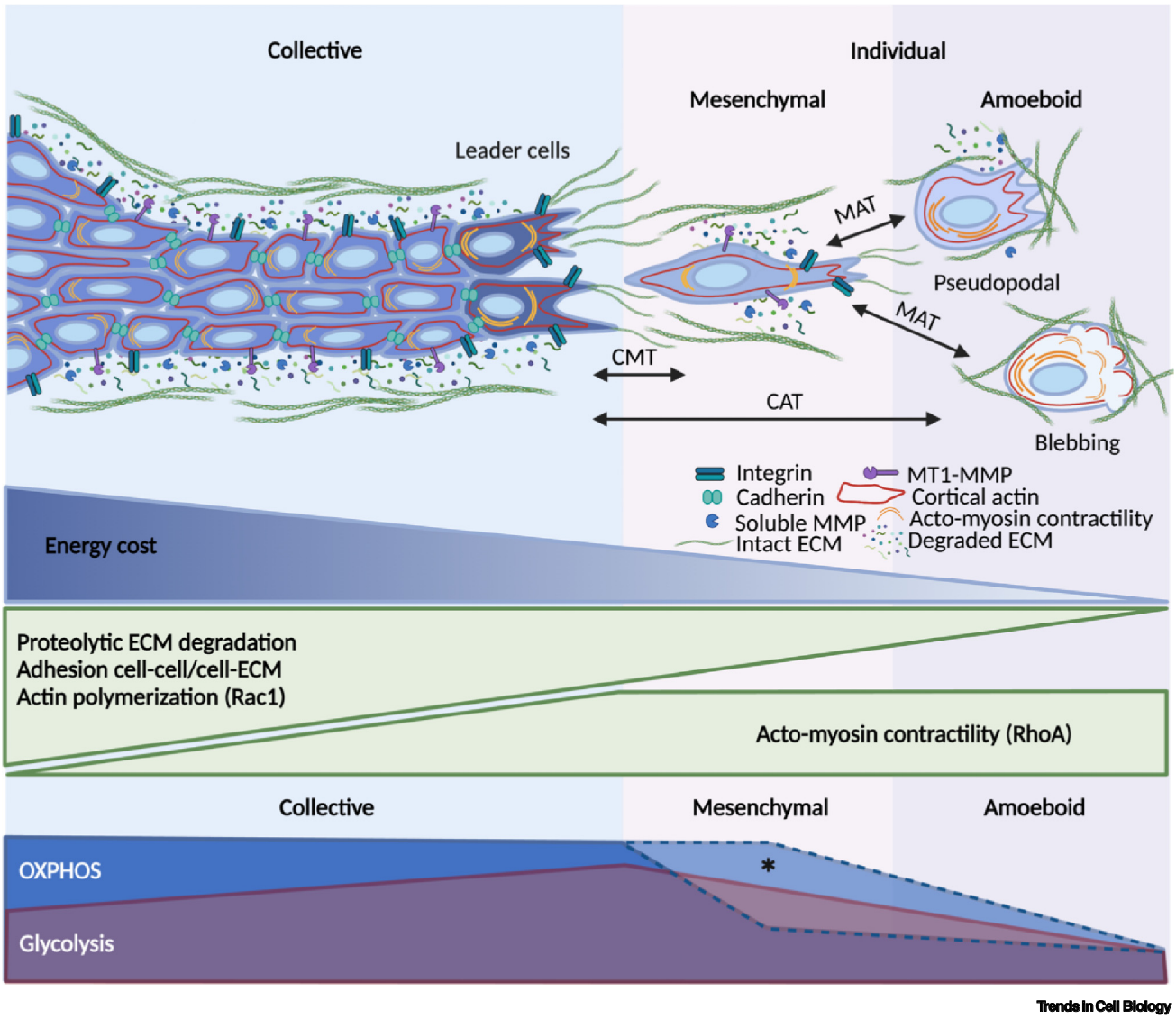

Figure 2. Interdependence of energy consumption and migration strategy. Collective migration depends on cell–cell adhesion, Rac1-mediated actin dynamics, Rho-A-mediated contractility, and integrin-mediated extracellular matrix (ECM) adhesion and deformation together with pericellular proteolysis. Because of its molecular and mechanical complexity, collective migration is energetically costly, particularly for the leader cells that must overcome substrate resistance. Collective-to-amoeboid transition (CAT) single-cell transition is mediated by the downregulation of intercellular adhesions. Losing cell–cell junctions allows mesenchymal single cells to save some energy, even though their elongated morphology still requires actin activity at the leading edge, cytoskeletal contractility, ECM adhesion, and proteolysis. Mesenchymal-to-amoeboid transition (MAT) results from lowering adhesion to the substrate and pericellular proteolysis and variable activity of glycolysis. The pseudopodal amoeboid mode retains actin-rich protrusions whereas the blebbing mode predominantly relies on Rho-mediated actomyosin contractility. By lowering most of the ATP-consuming steps of motility, the amoeboid mode seems to minimize the energy demands of migration. The lower panel shows the hypothetical coupling of migration modes and metabolic reprogramming. Figure created with BioRender.com.

Rac1-mediated protrusion formation, high activity of integrins, adhesion-regulating kinases, RhoA-mediated actomyosin contractility, and pericellular proteolysis (e.g., MMP-14 and cathepsin B) [78]. Leader cells further depend upon connexin-43-dependent extracellular release of purine derivatives, including ATP, ADP, and adenosine, which activate the adenosine receptor 1 (ADORA1), Akt, and leader cell function in an autocrine manner [4]. To remove tissue barriers, leader cells perform MMP-mediated proteolysis and realign ECM structures to create trails of least resistance [79]. In concert, these mechanical and molecular activities of leader cells result in high energy demands.

Follower cells maintain actin-based connections with the leader and the neighboring cells through cadherins [80] and simultaneously generate force transmission via integrins to the ECM substrate by lateral lamellipodia [3]. They further reinforce cadherin-mediated junctions in response to pulling forces [81] and contribute to proteolytic ECM degradation [79,82]. Follower cells maintain moderately reduced ATP levels, possibly because reduced mechanical work is necessary to move along a path initially built by leader cells [77].

Leader and follower cells are interconvertible. As leader cells invade, their energy gradually depletes, leading to leader–follower cell transition that allows a follower cell to become a new leader cell [78]. Metabolic shifts, detectable as increased mitochondrial respiration or upregulation of the glucose transporter 1 (GLUT1), support the energy required during collective migration in both leader and follower cells [77,83]. Thus, cell positioning and function in moving cell groups are reflected by differing energy consumption, although the mechanochemical activities and subcellular structures underlying the correlation between metabolic programs and cell positioning during collective migration remain to be identified (Box 1).

Individually migrating cells

Depending on the adhesive strength of cell–matrix interaction and the extent of proteolytic remodeling of the ECM, individually moving cells deploy mesenchymal or amoeboid migration strategies that have different energy demands. Compared to collective-migrating cells, single cells lack cell–cell adhesions and cadherin-mediated responses to forces and signals, and accordingly move with reduced energy demands.

Mesenchymal single cells resemble leader cells during collective migration [6,77], although their energy demands are lower owing to the lack of cell–cell junctions. ATP consumption secures protrusive actin polymerization at the leading edge, strong adhesive interaction, spindle-shaped cell extension, and deformation of ECM by substantial actomyosin contraction (Figure 2) [16,84]. During migration, mesenchymal cells further remodel the ECM by proteolytic degradation and the deposition of ECM molecules [6]. The energy demands reflect the amount of actin-mediated cell protrusion and mechanical work executed by the cell. For example, lamellipodia and filopodia in moving cancer cells are disabled after the inhibition of OXPHOS or glucose metabolism [16]. Furthermore, glucose uptake and the ATP:ADP ratio (an indicator of energy production) are increased when cells exert force on the matrix and/or interact with denser matrices [84,85]. Likewise, when confronted with ECM substrates of high stiffness, moving cells upregulate integrin engagement, F-actin bundling, and stress fiber formation, and concomitantly maintain high levels of glycolysis through tripartite motif-containing protein 21 (TRIM21)-mediated upregulation of PFK-1 [59]. Thus, in mesenchymal cells, adhesion and contractility are coregulated with energy metabolism (Figure 2).

Amoeboid-moving cells develop weak adhesion to the ECM substrate and move via small **pseudopodia** or lamellipodia formed by protrusive actin polymerization or bleb-induced hydrostatic pressure towards the front [86,87]. The pseudopodal amoeboid type of migration occurs

in cells with actin-rich protrusions at the leading edge which generate weak adhesion to the ECM [88–90]. Amoeboid-moving cancer cells are sustained by low levels of mitochondrial activity, and are hence considered to be energetically efficient [16,91]. The amoeboid movement relies on rear-polarized myosin II activity controlled by the Rho–ROCK pathway [92], which drives the retrograde flow of the actin cytoskeletal cortex and generates frictional forces as well as non-adhesive mechanical intercalation with the substrate [93,94]. Depending on the cell type and the environmental conditions, amoeboid migration may or may not cause proteolytic modification of the ECM [95–98]. Although proteolytic activity consumes energy, it creates a path of least resistance that may reduce the need for actin-mediated cell deformation, thus, arguably, resulting in reduced net energy demands. Actin flow and actomyosin contractility are therefore retained in amoeboid-moving cells, but energy demands resulting from strong adhesions and force transmission, stress fibers, cell–cell interactions, and proteolytic ECM remodeling are reduced.

Reprogramming of cancer cell invasion by bioenergetic stress

Invading tumor cells, when confronted with metabolic challenges, can undergo a bioenergetic adaptation response which secures cell survival and persisting migration. Plasticity of invasion programs can be induced by hypoxia and nutrient deprivation, and result in **epithelial-to-mesenchymal transition (EMT)**, mesenchymal-to-amoeboid transition, and collective-to-amoeboid transition [16,99].

Intersection of energy metabolism and migration programs

Bioenergetic programs and mechanisms of invasion are interconnected. In parallel, energy metabolism programs cooperate with cellular responses to metabolic stress evoked by toxic metabolic products to secure survival and migration (Box 2). Deprivation of oxygen and/or nutrients can directly impact on the efficiency and/or mode of cell migration, and the ability of cancer cells to rewire their metabolism and exploit different energy sources is crucial to sustain migration. Pharmacological interference with either OXPHOS or glycolysis results in the conversion of collective to single-cell migration (discussed below) [16]. Hypoxia and HIF signaling support Rho GTPase-mediated actomyosin contractility and cell migration through the activation of glycolysis [100]. Restriction of glutamate availability inhibits pseudopodia formation and the migration of tumor cells [101]. Likewise, inhibition of glutaminase – which catalyzes the hydrolysis of glutamine to glutamate – has been shown to block the oncogenic transformation induced by at least three different Rho GTPases (Cdc42, Rac1, and RhoC) in fibroblasts [102], the invasion of cancer and lymphoma cells [102], and the expression and activity of metalloproteinases (e.g., MMP2 and MMP9) [103].

Whereas adaptive nutrient uptake secures energy fueling for migration activity, autophagy additionally impacts on the migration machinery directly by degrading proteins involved in cell adhesion and cytoskeletal dynamics. In moving fibroblasts, autophagosomes become polarized toward the cell front [104], where they degrade Rho guanine nucleotide exchange factors (e.g., guanine nucleotide exchange factor H1, GEF-H1) [125]. This in turn reduces RhoA activity and favors mesenchymal migration [125]. Autophagy further degrades cytoskeletal adapter proteins, including paxillin and talin, leading to disassembly of focal adhesions [105,106,107] and adherens junction proteins [108], which weakens cell–cell cohesion [109]. In cancer cells, autophagy either inhibits RhoA and migration in 2D culture [110] or activates RhoA and enhances cell migration through transwell filters [111]; the contribution of autophagy to cancer invasion may therefore depend on the cell type and migration model. The impact of other energy sources (e.g., creatine) and macropinocytosis [62,73] remains to be established.

EMT is induced by microenvironmental cytokine and growth factor signaling, as well as by hypoxia, acidosis, and nutrient deprivation [67,112–114]. By transcriptional control, EMT downregulates cell–

cell adhesions and includes cell elongation by cytoskeletal reorganization such that cells can detach from the epithelium and move individually [115]. This transition enhances invasion as well as changes in energy consumption and production, including a switch from OXPHOS to glycolytic energy metabolism [114,116]. The degree of EMT and the type of metabolic reprogramming are connected. *In vitro* and *in vivo* evidence [117,118], further confirmed by *in silico* modeling [119], shows that tumor cells undergoing partial EMT increase glycolysis levels and reach a hybrid state that has both epithelial and mesenchymal traits as well as high activity of both glycolysis and OXPHOS. This hybrid state may give rise to a fully mesenchymal phenotype with decreased glycolytic levels (Figure 2, asterisk) or, when glycolysis and OXPHOS are both decreased, to a transition into a quiescent mesenchymal-like state [114]. Additional bioenergetic programs implicated in EMT induction or the maintenance of EMT include the **pentose phosphate pathway** to support gluconeogenesis [120], and also proline and glutamine metabolism [69,121].

Amoeboid plasticity

When challenged by metabolic stress, including severe hypoxia or experimental induction of HIF signaling, collectively invading cancer cells abandon cell–cell interactions and transit to amoeboid movement, a change termed the 'collective-to-amoeboid transition' [15,122]. This plasticity response differs from the EMT because cells deactivate integrin-mediated cell–matrix adhesion and develop low-adhesion bleb-mediated movement [16]. This adaptation of the mode of migration depends on activation of the cysteine protease calpain-2 which cleaves talin and thereby weakens adhesion to ECM [16,123] (Figure 3). The amoeboid transition concurs with repression of oxidative respiration and glycolysis to very low levels (Figure 2), indicating that amoeboid dissemination of cancer cell movement can occur with very low energy consumption [124]. Amoeboid plasticity can further be induced when autophagy is inhibited, which leads to RhoA activation, actomyosin contractility, and rounding of otherwise mesenchymal fibroblastic cells [125]. Likewise, pharmacologic inhibition of OXPHOS or glycolysis causes both collective-to-amoeboid and mesenchymal-to-amoeboid transitions in cancer cells [16,126]. After culture in hypoxia, amoeboid-migrating cytotoxic T cells retain the full capability to accumulate in tumors

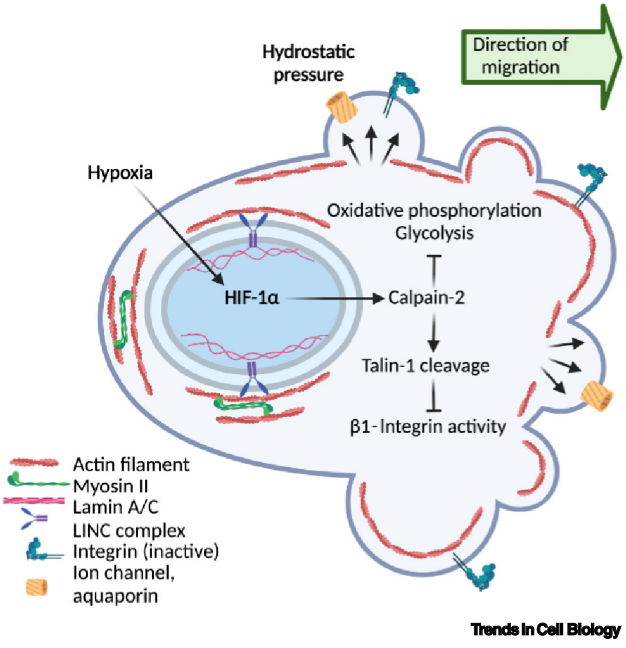

Figure 3. Amoeboid cancer cell migration: an 'eco-mode'. Hypoxic stress triggers the collective-to-amoeboid transition. This switch in migration mode relies on HIF-1α-mediated activation of calpain-2, a protease that cleaves Talin-1 and therefore decreases β1-integrin activity. This weakening of interactions with the extracellular matrix (ECM) causes cell rounding and the formation of polarized membrane blebs. This transition to an amoeboid and more cost-effective type of migration might secure cell evasion from challenging microenvironments. Figure created with BioRender.com. Abbreviation: LINC complex, linker of nucleoskeleton and cytoskeleton complex.

as well as their antitumor effector functions [127], indicating remarkable metabolic tolerance of amoeboid movement in leukocytes. Preconditioning of tumor cells by hypoxia is sufficient to strongly enhance experimental lung metastasis [16,128]. This may indicate that the metabolic programs are sufficiently sustained during the phase of circulation and impact on early organ colonization. However, it remains to be established how long bioenergetic reprogramming remains active at the metastatic site.

Arguably, resulting from the constitutive lack of adherens junctions and low cell–matrix adhesion, amoeboid movement may represent an energetically low-demand 'eco-mode' of cell migration which is maintained by actin flow and hydrostatic regulation, but lacks energy-consuming cell–cell interactions and occurs with minimal ECM deformation and remodeling [16,129]. Because of its low mechanical and bioenergetic complexity, amoeboid movement may be particularly suited to securing evasion from perturbed tissue sites with limited nutrient requirements, and this may increase cell fitness for enhanced metastasis [7,16,99]. The bioenergetic pathways which support either integrin-mediated adhesion and actin-based treadmilling or poorly adhesive, ion- and water-channel-dependent migration modes in 3D environments remain to be clarified [16,49].

Concluding remarks

Understanding the intersection of cancer energy metabolism and adaptive cancer invasion programs is necessary to categorize the types, plasticity, and vulnerability of cancer metastasis (see Outstanding questions). Metabolic stress-induced EMT and amoeboid programs may occur independently or as overlapping programs in favor of local dissemination, intra- and extravasation, and organ colonization [7]. Consequently, discriminating between cell-intrinsic and microenvironmental mechanisms of amoeboid cancer cell dissemination and metastasis may be important to tailor suitable interference strategies. Targeting options may include upstream regulators that control migration mode switching, including mechanical stress and cytokine networks [130,131], as well as metastasis-enhancing pathways engaged by energy deprivation.

Therapeutic interference with the transition to migration modes with lowered energy consumption may (i) reduce the migration speed and cell dissemination through the tissue, and/or (ii) increase the energy deficit and hence compromise tumor cell survival. Calpain may emerge as a master regulator of cell migration plasticity in different contexts. Pharmacological inhibition of calpain and thereby limiting the calpain-mediated release of cancer cells from adhesive interactions with the ECM, abrogates the metastatic ability of cancer cells in response to hypoxia in experimental metastasis [16]. In addition, interfering with energy uptake and broadening of energy sources may delay invasion [56]. Pharmacological interference with AMPK, which secures energy production by glycolysis and other programs during periods of metabolic stress, may reduce the ability of tumor cells to adapt their metabolism at any step of the stressful metastatic cascade [64,132]. This is in line with recent *in silico* simulations which predict that AMPK inhibitors will only have therapeutic efficacy when tumor cells maintain metabolic stress signaling [132]. Preventing the export of lactate derived from glycolysis, by inhibiting monocarboxylate transporters (MCTs), limits extracellular lactate as an alternative source of energy, prevents EMT development, and reduces the efficacy of invasion [72].

Interference with other overlapping pathways supporting tumor cells in both survival and migration, including the heat-shock response, EMT pathways [e.g., transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6)], interference with Rho/ROCK pathways activated in amoeboid movement [133], and targeted reversion of autophagy induction [134] may allow further sensitization of tumor cells to metabolic stress and thus decrease metastatic escape and metastatic organ colonization [147].

Outstanding questions

How are the programs of energy production and migration modes jointly regulated to optimize both energy demands and cell movement?

Which energy-conserving mechanisms are relevant for metastatic dissemination and can be detected in circulating tumor cells in cancer patients?

Which metabolic vulnerabilities are particularly suited for molecular intervention and in which phase during the metastatic cascade?

Which biomarkers and samples are best suited to identify and monitor patient subsets with an adaptive metabolic stress response?

Which epigenetic alterations result from short- and/or long-lived metabolic stress and how do these alterations affect metastatic programs including amoeboid behaviors?

How do metabolic stress programs cooperate with other programs of cancer progression, including EMT and stemness?

To identify patient subsets and define personalized targets, biomarkers in liquid biopsies and/or circulating tumor cells that indicate upregulation of metabolic stress signaling in combination with a high load of circulating tumor cells may be used to identify engaged metabolic stress programs in a tumor type- and stage-dependent manner. Biomarkers indicating metabolic stress may be based on transcriptomic analysis in circulating tumor cells indicative of a metabolically silent state (e.g., AXL, GLUT1), autophagy (e.g., repression of miR-205), and/or hypoxia response (e.g., HIF-1) [135–137], as well as on metabolomic analysis reflecting the balance of oxidative and glycolytic programs [137].

Future avenues may include the identification of minimal metabolic deprivation stresses which can elicit reprogramming of metastasis. Likewise, the duration and mechanisms of persistence of metabolic stress signaling after evasion from the perturbed microenvironment remain to be identified, including epigenetic reprogramming involved in EMT and amoeboid programs. Both elongated and amoeboid-rounded migratory modes can contribute to the EMT spectrum [7,138], consistent with potentially broad adaptability of both metabolic pathways and migration strategies in response to nutrient deprivation and stress caused by toxic metabolites. Molecular intervention in migration programs alone may not suffice to combat metastasis. Instead, combined intervention in pathways supporting migration, cancer cell survival, and, as discussed here, the response to energy deprivation may require targeting by combined approaches [148].

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Declaration of interests

The authors declare no competing interests.

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