Trends in Cell Biology

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Review

Fueling Cell Invasion through Extracellular Matrix

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Cell invasion through extracellular matrix (ECM) has pivotal roles in cell dispersal during development, immune cell trafficking, and cancer metastasis. Many elegant studies have revealed the specialized cellular protrusions, proteases, and distinct modes of migration invasive cells use to overcome ECM barriers. Less clear, however, is how invasive cells provide energy, specifically ATP, to power the energetically demanding membrane trafficking, F-actin polymerization, and actomyosin machinery that mediate break down, remodeling, and movement through ECMs. Here, we provide an overview of the challenges of examining ATP generation and delivery within invading cells and how recent studies using diverse invasion models, experimental approaches, and energy biosensors are revealing that energy metabolism is an integral component of cell invasive behavior that is dynamically tuned to overcome the ECM environment.

Highlights

Cell invasion through extracellular matrix (ECM) is an energetically demanding process that requires dynamic membrane trafficking, F-actin polymerization, and actomyosin contraction to degrade, remove, and migrate through ECM.

Experimental evidence examining actively invading cells shows that mitochondrial oxidative phosphorylation generates the ATP to fuel cell invasion.

Mitochondria traffic to energetically demanding focal adhesions, cortical actin, and invasive protrusions to supply ATP to the invasive machinery.

Invading cells dynamically adapt glucose uptake, ATP generation, and, when invading collectively, leader cell position, to overcome different ECM barriers during invasion.

Invasion through ECM Requires Energy Intensive Cellular Machinery

Animals are made of tissues comprising cells that are scaffolded, shaped, and confined by macromolecular assemblies of **extracellular matrix (ECM**, see Glossary). Several cell types acquire the specialized ability to invade through ECM. For example, vertebrate neural crest cells invade through ECM to disperse and form skeletal structures, nerves, and pigment cells [1], and leukocytes traverse ECM to reach sites of infection and injury [2]. Dysregulation of invasion occurs in human pathologies, such as rheumatoid arthritis [3], multiple sclerosis [4], and cancer [5]. Given its importance to human health, particularly in driving metastatic cancer, there has been significant interest in understanding how cells invade.

ECMs take several major forms, such as thin, dense, planar basement membrane matrices that surround tissues; 3D, fibrillar, type I collagen-rich interstitial matrices that sit between tissues; and rigid, dense, mineralized bone [6]. Each ECM offers unique obstacles for invading cells. Examining how invasive cells dynamically interact with, and overcome, ECM barriers has been a primary focus in the field and we know a great deal about the regulation and function of specialized F-actin-rich invasive protrusions, termed invadopodia and podosomes [7–9], and the dynamic trafficking of ECM-degrading **matrix metalloproteinases (MMPs)** [10–12]. Many studies have also elucidated the rapid and flexible conversion between migration modes (mesenchymal, amoeboid, lobopodial, and collective) that invasive cells undergo [13–15]. Although these aspects of invasion are crucial in breaching ECM, force-producing F-actin polymerization and actomyosin contraction as well as MMP delivery and membrane trafficking are energetically demanding processes [16–20], and less is known about how cells power **cell invasion**. Investigations of how cells fuel invasion have primarily studied invasive cancer cell lines and the *Caenorhabditis elegans* anchor cell (AC), which carries out a normal invasion event that shares many features with cancer cells [21–24]. In this review, we outline these studies that inform current knowledge of this key, but understudied, aspect of cell invasion.

Oxidative Phosphorylation and Glycolysis Are the Primary Sources of ATP

ATP is the main energy currency in cells, and the formation, hydrolysis, or transfer of the high-energy terminal phosphate bond in ATP is the link between energy-producing and energy-

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demanding processes [25]. The primary fuel source catabolized to produce ATP is glucose, although fatty acids and glutamine are also commonly used [26]. Most ATP in cells is derived from oxidation of glucose through **oxidative phosphorylation (OXPHOS)** in the mitochondria [27]. During **glycolysis**, the initial step of glucose catabolism, glucose is converted into two pyruvate molecules in the cytoplasm, which generates two ATP molecules (Figure 1). In most cells under aerobic conditions, pyruvate is then transported into the mitochondria and enters the **tricarboxylic acid (TCA) cycle** for complete oxidation to CO_2, which generates approximately 32 ATP molecules through OXPHOS [25]. However, under anaerobic conditions, and even in aerobic conditions through a process termed the Warburg Effect or **aerobic glycolysis**, pyruvate is reduced to lactate, which can be converted back into pyruvate within the cell or excreted [28]. Although seemingly inefficient, aerobic glycolysis can generate ATP as rapidly as complete oxidation of glucose through OXPHOS and may have benefits, such as providing a carbon source for the construction of nucleotides, lipids, and proteins (Figure 1 for overview) [29].

The Challenge of Understanding How Cells Power Invasion

The cell metabolism underlying ATP generation is adaptable and complex. One experimental hurdle in discerning the main source of ATP for invasive cells is that glycolysis provides the primary fuel source for OXPHOS and, thus, perturbing glycolysis can affect both glycolysis and OXPHOS. In addition, some cells can flexibly switch between, and even combine outputs of, the aerobic glycolytic pathway and OXPHOS in response to environmental cues, cell differentiation states, and metabolic pathway perturbations [26,30–33]. Glycolytic enzymes and mitochondria can also be directed to specific subregions of the cell to provide localized ATP production for energy-dependent processes [34–36] and, thus, their subcellular location should ideally be known to determine whether they contribute ATP to invasion. Furthermore, although cells primarily use glucose to fuel OXPHOS, in some cells, if glucose is limited, the amino acid glutamine and fatty acids can be funneled into the TCA cycle for ATP production through mitochondrial OXPHOS [26]. Another challenge in experimentally understanding the role of glycolysis and OXPHOS in cell invasion is that, in addition to generating ATP, glycolysis and the TCA cycle generate metabolites that make amino acids, lipids, and nucleotides [29,37], which may also be needed for invasive ability. Glycolysis and OXPHOS also contribute to reactive oxygen species (ROS) signaling and chromatin

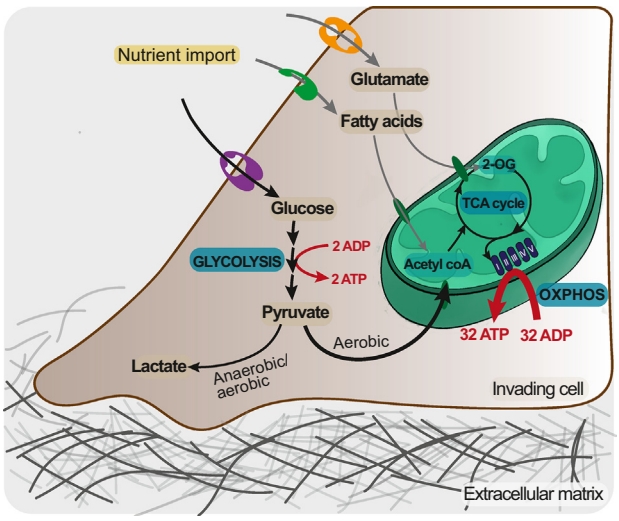

Figure 1. Schematic Diagram of Energy Metabolism that Can Lead to ATP Production within an Invading Cell. One molecule of glucose (6C) is broken down into two molecules of pyruvate (3C) by glycolysis to produce two molecules of ATP. The pyruvate can be converted into lactate under aerobic and anaerobic conditions or be transported into mitochondria to be converted into acetyl coA (2C), which is fed into the tricarboxylic acid (TCA) cycle to generate a proton gradient and produce 32 molecules of ATP via electron transport chain complex V. In some cases, glutamate and fatty acids can also be incorporated into mitochondrial metabolism to generate ATP (gray arrows). Abbreviations: 2-OG, 2-oxoglutarate; OXPHOS, oxidative phosphorylation.

Glossary

Aerobic glycolysis: when, in the presence of oxygen, pyruvate is reduced to lactate, and is the primary generator of ATP in the cell.
Cell invasion: cellular behavior where specialized cells move through ECM barriers using proteolytic matrix degradation, physical displacement of the matrix, and deformation of the cell.
Collective cell migration: when groups of cells maintain cell–cell contacts and invade through ECM guided by a leader cell(s).
Extracellular matrix (ECM): a noncellular 3D assembly of macromolecules comprising collagens, proteoglycans, and glycoproteins that provides cells with a structural scaffolding as well as biochemical and biomechanical cues.
Genetically encoded fluorescent biosensor: chimeric proteins comprising a sensing unit and fluorescent reporting unit that provide a fluorescence-based readout for levels of a metabolic compound or activity of another protein.
Glycolysis: a ten-step enzymatic cytoplasmic pathway that breaks down one glucose molecule (six carbons) into two pyruvate molecules (three carbons) and generates two net molecules of ATP and two molecules of NADH.
***In vitro* matrices:** synthetic extracellular matrices made to mimic or modulate matrix stiffness and confinement experienced by cells invading through ECM *in vivo*. These can be made of Matrigel (solubilized basement membrane proteins), collagen gels (acid-solubilized rat tail tendon type I collagen), or hydrogels (polymers that are coated with collagen or fibronectin to mimic matrix stiffness).
Matrix metalloproteinases (MMPs): a family of zinc-based endopeptidases that broadly degrade ECM proteins.
Mechanosensitive: when cells sense the mechanical signals in their microenvironment, such as force, stress, and rigidity, and respond to mechanical cues to adapt to the changing environment.
Oxidative phosphorylation (OXPHOS): process that occurs across the inner mitochondrial membrane by which the electrons from reduced NADH and $FADH_2$ move through the electron transport chain to reduce oxygen to water. The energy from electron transfer is harnessed to pump protons across

modifications [29,37], which may influence invasion. Lastly, calcium is taken up into mitochondria and promotes increased ATP production. It has been proposed that mitochondria may in turn buffer cytosolic calcium levels, a key signaling molecule that could also modulate invasion [38]. Thus, understanding how cells power invasion requires the ability to specifically perturb glycolysis and OXPHOS, to examine the output of these metabolic pathways at the cellular and subcellular levels, and a deep understanding of how mitochondria function within invasive cells. Importantly, numerous tools are now available, including **genetically encoded fluorescent biosensors**, to study metabolism and ATP production within invasive cells more holistically (Table 1].

the inner membrane and generates a proton gradient, which is used by ATP synthase to produce approximately 32 molecules of ATP.

Tricarboxylic acid (TCA) cycle: a biochemical sequence that occurs in the mitochondrial matrix and extracts high-energy electrons in the form of reduced electron carriers NADH and $FADH_2$ from carbon fuels (glucose, glutamate, and fatty acids) via oxidation of acetyl coA into two molecules of CO_2.

Experimental Evidence for OXPHOS Generating the ATP That Fuels Cell Invasion

Most studies on cell invasion have examined cultured cancer cells. Surprisingly, although upregulation of aerobic glycolysis and suppression of mitochondrial and OXPHOS gene expression are associated with increased metastatic potential and reduced patient survival in many cancers [39], experimental analysis has not yet supported a clear role for glycolysis in providing ATP to power invasion. For example, a mouse mammary carcinoma cell line (4T1), which metastasizes to several tissues, undergoes metabolic reprogramming such that OXPHOS is reduced and glycolysis is increased [33]. While inhibition of reprogramming reduces both the size and number of metastatic lesions in mice, careful *in vivo* analysis indicated that invasion of 4T1 cells from the vasculature into the liver (extravasation) is not affected, suggesting that glycolysis is required for proliferation of the metastatic cells after they have invaded. Instead, isolation of 4T1 cells within mice found that invasive circulating cancer cells (CCCs) specifically upregulate genes associated with OXPHOS and mitochondrial biogenesis [40]. Furthermore, one of these genes, *PGC1a*, an inducer of mitochondrial biogenesis, appears to help drive this dynamic switch to OXPHOS within invasive cells, because it is markedly upregulated in CCCs from several different cancers and promotes increased mitochondrial DNA content, oxygen consumption rate (mitochondrial activity), and ATP production as measured by a bioluminescence assay (Table 1). Supporting a functional role for OXPHOS in fueling invasion, depletion of the PGC-1α protein as well as blocking OXPHOS with rotenone, an inhibitor of mitochondrial electron transport chain Complex I (Table 1), dramatically decreased breast and melanoma cancer cells invasion through polycarbonate membranes coated with Matrigel [40]. In addition, downregulation of PGC-1α suppressed metastasis of both breast and melanoma cancer cells in mice [40]. The reliance of invasive cells on OXPHOS is also seen in **collective cell migration** of lung cancer cells, which is a form of invasion where groups of cells are led by a leader cell that bears the energetically demanding role of degrading and remodeling ECM. Measurement of the rates of OXPHOS and glycolysis in leader and follower cells using Seahorse Extracellular Flux Analyzer (Table 1) showed that leader cells use OXPHOS whereas follower cells rely on glycolysis to meet energy needs [41]. Furthermore, impairing OXPHOS by pharmacologically inhibiting the enzyme linking glycolysis to the TCA cycle in mitochondria (pyruvate dehydrogenase) shifted leader cells into aerobic glycolysis, which reduced their ability to invade through 3D collagen gels [41].

Additional studies using pharmacological inhibitors and metabolic profiling have shown requirements for OXPHOS in promoting invasiveness in many types of cancer, including ovarian [42], prostate [43], pancreatic [44], and melanoma [45]. AC invasion in *C. elegans* is an *in vivo* developmental model of cell invasion. The AC invades through basement membrane using dynamic F-actin-rich invadopodia that are enriched in MMPs, similar to invasive cancer cells [22]. Furthermore, regulators of invadopodia formation and turnover, such as cofilin and integrins, are also conserved between the AC and cancer cells, as are transcriptional regulators of invasion, such as Fos [46,47]. RNAi knockdown experiments revealed that OXPHOS is also required for AC invasion [22]. Together, these findings have established the crucial role of OXPHOS in

Table 1. Tools and Techniques to Study ATP Metabolism

Tool/technique	What it measures	How it works	Refs
Biochemical bulk measurements			
Seahorse Extracellular Flux Analyzer (Agilent)	Glycolysis	Extracellular acidification rate (ECAR) measures change in extracellular pH	[67]
	Mitochondrial respiration rate	Oxygen consumption rate (OCR) measures change in oxygen concentration	[40]
ATP bioluminescence assay	ATP	Luciferase chemiluminescence proportional to ATP concentration	[40]
^{13}C metabolic flux analysis	Metabolic flux	Stable ^{13}C-glucose or ^{13}C-glutamine incorporation analysis by mass spectrometry or NMR	[68]
Inhibitors			
2-Deoxyglucose	Glycolysis	Hexokinase inhibitor	[67]
3-Bromopyruvate	Glycolysis	Hexokinase II inhibitor	[69]
Rotenone	OXPHOS	Mitochondrial complex I inhibitor	[40]
Thenoyltrifluoroacetone (TTFA)	OXPHOS	Mitochondrial complex II inhibitor and OXPHOS uncoupler	[70]
3-Nitropropionic acid	OXPHOS	Mitochondrial complex II inhibitor	[71]
Malonate	OXPHOS	Mitochondrial complex II inhibitor (reversible)	[72]
Antimycin A	OXPHOS	Mitochondrial complex III inhibitor	[72]
Sodium azide	OXPHOS	Mitochondrial complex IV inhibitor	[72]
Oligomycin	OXPHOS	Mitochondrial complex V inhibitor	[49]
Carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazon (FCCP)	ATP synthesis	Uncoupler of mitochondrial OXPHOS by disrupting proton gradient	[40]
Phloretin	Glucose import	GLUT inhibitor	[67]
Phlorizin	Glucose import	SGLT inhibitor	[67]
Fluorescent dyes (excitation/emission wavelength)			
Mitotracker	Mitochondrial morphology	Cell permeable, mitochondrion-specific dyes. Various spectra available	[73]
MitoView	Mitochondrial morphology	Cell permeable, mitochondrion specific dyes. Various spectra available	[74]
MitoSOX	Mitochondrial superoxides	Red mitochondrial superoxide indicator (510/580 nm)	[75]
TMRE/TMRM	Mitochondrial membrane potential	Red fluorescence based on mitochondrial membrane potential (549/574 nm)	[73]
MitoProbe DiIC$_1$(5)	Mitochondria membrane potential	Far-red fluorescence signal increases with increased mitochondrial activity (638/658 nm)	[76]
2-NBDG/6-NBDG	Glucose	Green fluorescent glucose analog (465/540 nm)	[56]
Genetically encoded biosensors (excitation/emission wavelength)			
PyronicSF	Pyruvate	Intensiometric (488/525 nm)	[77]
Laconic	Lactate	FRET ratiometric (430/485, 528 nm)	[78]
OGsor	2-Oxaloglutarate	FRET ratiometric (440/478, 528 nm)	[78]
PercevalHR	ATP:ADP	Ratiometric (405, 490/530 nm)	[79]
ATeams	ATP	FRET ratiometric (435/475, 527 nm)	[80]
BTeam	ATP	BRET ratiometric (no excitation required/460, 527nm)	[81]
iATPsnFR	ATP	Intensiometric (490/512 nm)	[66]
NAD+ biosensor	NAD+	Intensiometric (488/520 nm)	[82]
Peredox	NADH–NAD+	Intensiometric (488/520 nm)	[83]
Green Glifon 50, 600, 4000	Glucose	Intensiometric (488/525 nm)	[84]
FGBP	Glucose	Intensiometric (485, 400/528 nm)	[85]
iGlucoSnFR	Glucose	Intensiometric (488/495–575 nm)	[86]

supporting cell invasion and highlight the dynamic nature of metabolism and the importance of analyzing actively invading cells to determine the source of ATP that drives invasion.

Mitochondria Localize to Energetically Demanding Invasive Machinery

ATP is not stored or kept at high levels in cells and its diffusion is limited by the dense and structured intracellular space [48]. Within neurons, mitochondria traffic to energy-demanding synapses and growth cones and use OXPHOS to locally supply ATP [36]. Similarly, recent work found that mitochondria localize to the energy-demanding leading edge and protrusions of many different cancer cells, fibroblasts, the *C. elegans* AC, as well as the uropod of lymphocytes (Figure 2A) [22,43,44,49–51]. Here, mitochondria are thought to locally provide ATP to fuel membrane dynamics, actomyosin contractility, focal adhesion turnover, and F-actin polymerization [22,44,51,52]. Similar to neurons, localization depends upon microtubules, the microtubule motor protein kinesin, and the mitochondrial adaptor proteins Miro1/2 [49,52,53]. Mitochondrial trafficking is also influenced by mitochondrial fusion and fission [54] and requires mitochondria to be energetically active [49,51]. Consistent with trafficked mitochondria providing localized ATP for invasion, visualization of ATeam, an ATP biosensor (Table 1), revealed that high levels of ATP localize with mitochondria to the invasive front of the AC in *C. elegans* (Figure 2A) [22]. In collectively migrating packs of lung cancer cells, leader cells also have more peripherally located mitochondria in contrast to follower cells, the mitochondria of which remain perinuclear [41]. Measurements comparing glycolysis, OXPHOS, and ATP levels in ovarian cancer cell bodies and protrusions using a Transwell cell culture chamber (Figure 2B), showed that pseudopodial protrusions have higher levels of ATP compared with cell bodies and that ATP within protrusions is derived by mitochondrial OXPHOS

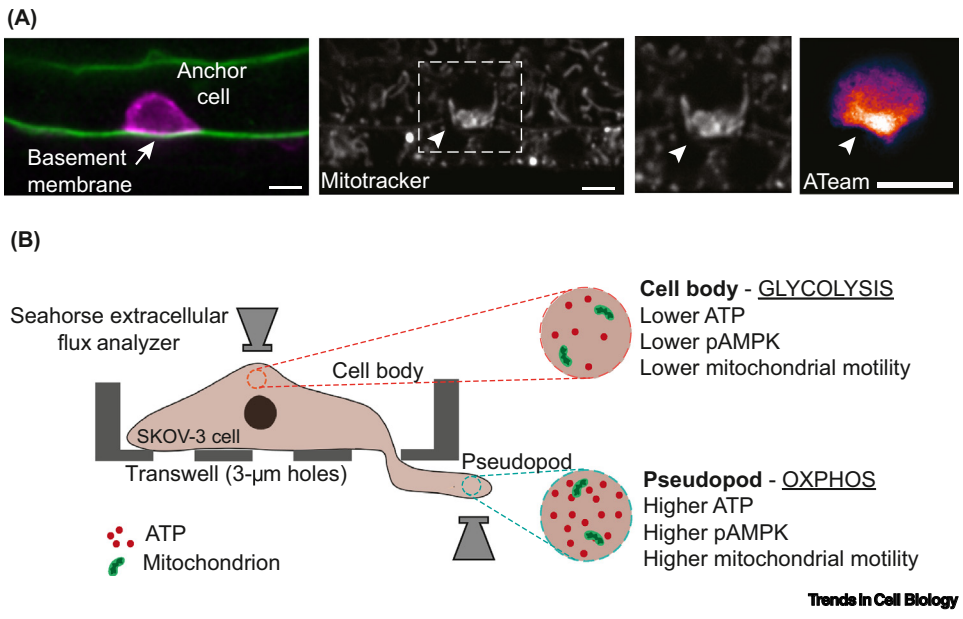

Figure 2. Mitochondria Provide ATP via Oxidative Phosphorylation (OXPHOS) at Invasion Sites. (A) The *Caenorhabditis elegans* anchor cell (left panel, magenta) is in contact with an intact basement membrane (green, arrow, visualized with laminin::GFP) just before invasion. Before and during invasion through the basement membrane (middle and right panels), mitochondria enrich within the anchor cell at the site of invasion (visualized by Mitotracker, arrowheads) as do elevated levels of ATP (visualized by ATeam, arrowhead). Scale bar: 5 μm. (B) A custom Transwell assay was used with a Seahorse XF analyzer to determine the energy metabolism in the cell body versus the pseudopod of SKOV-3 ovarian cancer cells. The Transwell also allowed for biochemical isolation of the cell body and pseudopod. This analysis revealed the pseudopod contains higher phosphorylated AMP kinase (pAMPK) and ATP levels and that OXPHOS generates the ATP within the pseudopod. By contrast, in the cell body, there are lower ATP and pAMPK levels and the ATP is primarily generated by aerobic glycolysis. Adapted, with permission, from [22] (A).

[49]. By contrast, these studies found that the ATP generated in the cell body is derived primarily by glycolysis, thus demonstrating that, even within cells, there can be heterogeneity in the source of ATP [49]. These studies also showed that mitochondrial localization to the leading edge of ovarian cancer cells depends on activated AMP kinase (AMPK), a critical cellular energy sensor and metabolic regulator that is phosphorylated and activated (pAMPK) in response to elevated ADP and AMP. Targeted delivery of an AMPK activity stimulator (AICAR) led to migration of mitochondria to the treated area of the cell. Conversely, photo-activated AMPK inhibition showed the opposite result, inhibition of mitochondrial flux into the leading edge of migrating cells in both 2D and 3D matrices [49]. While these results do not exclude other functions for localized mitochondria within invasive cells, they suggest that the ATP-hydrolyzing machinery is sensed by invasive cells (at least in some cancer cells by AMPK), which mediates mitochondrial trafficking and localized ATP production to sustain or complete invasion. AMPK signaling has many inputs that allow it to sense metabolic flux in cells, and many outputs that instigate specific responses to metabolic stimuli. The effectors of AMPK signaling that direct mitochondrial recruitment to the leading edge are unknown. Furthermore, while mitochondria localize to the energetically demanding leading edge of many different cancer cell types, fibroblasts, and the *C. elegans* AC, a role for AMPK has thus far only been established in ovarian cancer cells.

Nutrient Uptake by Invasive Cells

To fuel OXPHOS and ATP production, animal cells primarily draw upon a few abundant nutrients, including glucose, glutamine, and fatty acids, and use a variety of nutrient transporters, such as the GLUT/SGLT family, SLC1/SLC6/SLC7/SLC38 families, and CD36/FATP/FABP proteins, respectively, to bring these nutrients into the cell [26]. Glucose appears to be a common carbon source to fuel invasion because glucose uptake is associated with cancer invasiveness [55]. In addition, dynamic glucose uptake has been visualized within invasive breast cancer cells with a fluorescent glucose analog 2-NBDG (Table 1) and levels of exogenous glucose affect invasive behavior [56,57]. Glutamine is taken up at a higher rate in more invasive ovarian cancer cells, and can compensate for lack of glucose to maintain ATP levels in cells, suggesting that it is used as a fuel source [58]. Fatty acid uptake is also linked to cell invasion, because metastasis-initiating human oral carcinoma cells express high levels of the fatty acid receptor CD36 [59], and adipocyte lipids are transferred to melanoma and breast cancer cells through fatty acid transporters, which promotes their invasive ability [60,61]. However, it is not yet clear whether lipid uptake by cancer cells is used to fuel ATP production or used for one of the many other functions that lipids could have in cell invasion, such as signaling and membrane formation [12]. The large number of nutrient transporters and flexibility of nutrient uptake in cells has made studying nutrient uptake challenging. In addition, it is unclear how nutrient uptake to fuel OXPHOS (Figure 1) is coordinated with localized mitochondria at energetically demanding invasion sites within cells.

Cells Use Adaptive Energy Mechanisms to Overcome Distinct ECM Barriers

Through use of live-cell imaging, fluorescent energy probes, *in vivo* genetic analysis, and cell culture studies in several different cancer cells using ***in vitro* matrices**, recent findings revealed that nutrient uptake, ATP delivery, and invasion strategies undergo remarkable alterations to fuel invasion through ECM barriers. For example, the ATP:ADP ratio, which often indicates greater ATP production [52,57], increases in metastatic breast cancer cells cultured in denser 3D collagen matrices, as does glucose uptake and ATP hydrolysis [56]. Confining cells in microfabricated 3D collagen microtracks, which mimic the complex architecture of the ECM, also leads to an increase in the ATP:ADP ratio, glucose uptake, and ATP hydrolysis [62]. Together, these results suggest that breast cancer cells ramp up nutrient acquisition and ATP production to overcome dense and confining ECM environments.

Pancreatic ductal carcinoma is a highly aggressive cancer and patients have poor prognosis. These invasive pancreatic cancer cells also have a robust, adaptable energy response to breaching ECM. By culturing cells on fibronectin-coated hydrogels of increasing stiffness and performing wounding assays on these cells embedded within Matrigel matrix, it was discovered that, in response to stiff matrices, pancreatic cancer cell mitochondria fuse, increase in mass, generate more ATP, and move into protrusions that invade Matrigel [44]. In addition, an unbiased metabolomic analysis of pancreatic cancer cells cultured on substrates of varying stiffness revealed that the phosphocreatine–creatine kinase system contributes to invasion in response to rigid substrates [44]. Phosphocreatine is a phosphagen, which is an energy storage molecule that diffuses more readily than ATP [63]. Creatine kinase can use ATP to create phosphocreatine and then later catalyze the recovery of ATP from phosphocreatine (Figure 3A). It was found that stiff matrices promote increased creatine production in pancreatic cancer cells and trigger a **mechanosensitive** integrin-mediated nuclear translocation of the cotranscriptional activator YAP, which increases cytoplasmic creatine kinase expression. Disruption of the phosphocreatine system impaired F-actin turnover, actomyosin traction-force generation, cell invasive ability, and metastasis to the liver in a mouse model [44], demonstrating a crucial role for this ATP storage and transport system in supporting invasion. Interestingly, earlier studies showed that colorectal cancer cells that metastasize to the liver in mice also express elevated levels of creatine kinase [64]. However, the disseminated colorectal cancer cells secrete creatine kinase into the extracellular space of the liver, the main site in the body for creatine synthesis, which uses extracellular ATP to convert creatine into phosphocreatine. The metastasizing colorectal cancer cells then import this phosphocreatine and use it to generate ATP. Inhibition of creatine kinase in colorectal cancer cell lines with short hairpin (sh)RNA or cyclocreatine, an inhibitor of creatine kinase, blocked their metastatic colonization of the liver, as did depletion of extracellular ATP. Conversely, overexpression of creatine kinase enhanced metastasis, and supplementation of phosphocreatine into the extracellular space dramatically increased metastasis of creatine kinase-depleted colorectal cells [64]. These studies suggest creatine kinase might be a common adaptive mechanism for invasive cancer cells; however, the role of creatine kinase in specifically enhancing colorectal cell invasion has not yet been determined.

Another fascinating energy adaptation occurs during collective cell invasion in breast cancer. Using spheroid and organoid breast cancer cell cultures in 3D collagen gels, it was discovered that leader cells take up more glucose than do follower cells [57]. Intriguingly, when the leader cell ATP:ADP ratio drops to a low level, the leader cell stalls in its invasion, and is often replaced by a follower cell with a high ATP:ADP ratio that resumes invasion (Figure 3B) [57]. Increasing ATP production in leader cells extended leader cell lifetime, while glucose starvation (lowered ATP:ADP ratio) decreased leader cell lifetime. Leader–follower switching frequency also increased in denser collagen gels, where invasion is more energetically taxing. Thus, not only does energy adaptation occur within individual invasive cells, but it is also coordinated between collectively invading cells to overcome the ECM environment.

Understanding adaptive energy delivery may also help to therapeutically target invasive cells. One example of this was discovered by studying AC invasion through basement membrane in *C. elegans* [46]. While MMPs promote cell invasion by degrading basement membrane and other ECMs, they have failed as anti-invasive targets in clinical trials [65]. Recent studies in *C. elegans* discovered that the invasive AC alters its invasion program after MMP removal to break through the basement membrane [22]. During normal AC invasion, invadopodia armed with MMPs breach the basement membrane using a combination of force and basement membrane degradation [23]. After genetic removal of the MMPs, more mitochondria trafficked to the invasive front of the AC and provided increased levels of localized ATP to fuel the formation

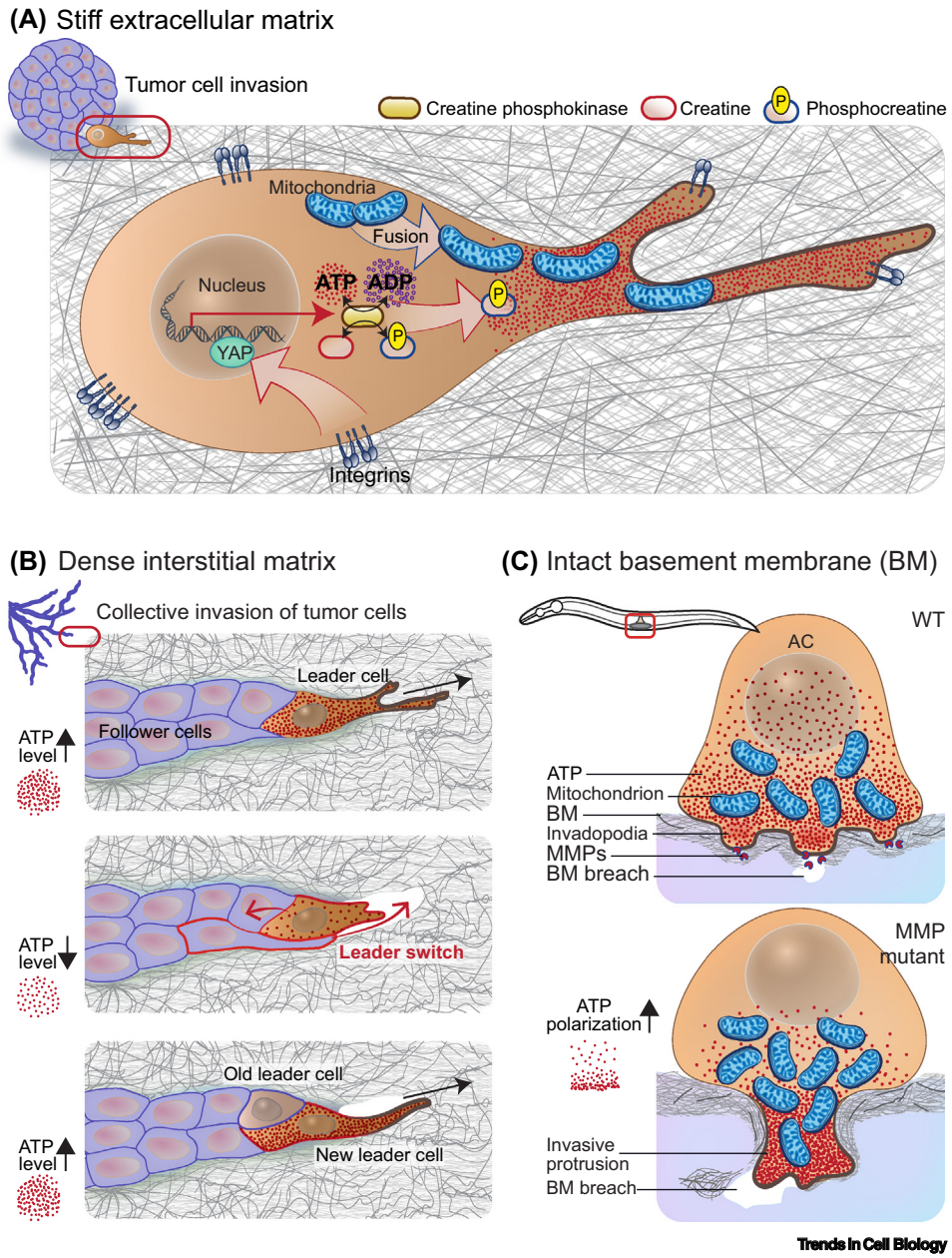

Figure 3. Invading Cells Adapt ATP Metabolism to Overcome Distinct Extracellular Matrix (ECM) Barriers. (A) In response to stiff matrices, invading pancreatic cancer cells localize fused mitochondria in invading protrusions and increase ATP production. In addition, through an integrin-mediated mechanosensitive mechanism, YAP enters the nucleus and leads to the transcription of creatine kinase, which mediates the transfer of phosphate onto creatine, an energy storage molecule that diffuses more readily than ATP. Creatine kinase can then later catalyze the recovery of ATP from phosphocreatine. (B) During collective migration, leader cells bear the energetically demanding role of degrading and remodeling ECM. When the ATP:ADP ratio of the leader cell drops to a low level, it halts in its invasion and is then replaced by a follower cell with a high ATP:ADP ratio that resumes invasion. (C) During *Caenorhabditis elegans* anchor cell (AC) invasion, small invadopodia depress and breach the basement membrane (BM) through a combination of matrix metalloproteinase (MMP)-mediated BM degradation and physical displacement. In the absence of MMPs, more mitochondria localize to the invasive front and deliver high levels of ATP to fuel the formation of a large F-actin-based protrusion that breaches the BM solely through physical displacement. Abbreviation: WT, wild type.

of a large F-actin protrusion that breaks through the basement membrane through physical force alone (Figure 3C). Highlighting the therapeutic potential of targeting this adaptive energy response, reduction of ANT-1.1, an adenine nucleotide translocator that helps shuttle ATP out of the mitochondria, in combination with the loss of MMPs, completely halted AC invasion, yet left the animals otherwise healthy [22]. Adaptive energy delivery mechanisms might also be triggered by pharmacological inhibition of key signaling pathways in tumor progression. For instance, phosphatidylinositol 3-kinase (PI3K) inhibitors currently in cancer clinical trials cause cultured glioblastoma cells to reposition mitochondria to the cortical cytoskeleton where they promote increased invasive behavior through Matrigel [51]. This unexpected metabolic response might explain, in part, the limited clinical efficacy of targeting PI3K.

These recent findings show that invasive cells adapt to different ECM barriers using diverse strategies. Yet, there are important similarities. Primarily, all known adaptations increase ATP within invading cells: metastatic breast cancer cells take up more glucose to fuel additional ATP production when faced with increased ECM density; pancreatic and colon cancer cells rely on the creatine phosphagen system to produce extra ATP; and the invading AC increases ATP production at the site of invasion in the absence of MMPs [22,44,56,62]. Further in pancreatic cancer cells, collectively migrating lung cancer cells, and the AC, mitochondria localize to the invasive front [57].

However, important gaps in our understanding of energy adaption during invasion remain. For example, during collective cell migration, it is not understood how leader cell energy depletion is sensed by the follower cells. Furthermore, it is unclear whether the integrin-mediated mechanotransduction observed in pancreatic cancer cells is commonly used by invasive cells to assess and tune metabolism to different ECMs. Lastly, the creatine kinase–phosphocreatine system has been minimally studied during cell invasion, and it is unknown whether it is widely used as an adaptive mechanism to provide additional ATP to fuel invasion.

Concluding Remarks

Recent studies have begun to reveal how invasive cells fuel the energetically demanding process of moving through ECM barriers. Key findings include the crucial role of localized mitochondrial OXPHOS in providing ATP to power the invasive machinery, as well as adaptable energy acquisition and delivery to overcome distinct ECM barriers. Experimental approaches to monitor energy metabolism within invasive cells have been crucial to advancing the field (Table 1) but have limitations. For example, biochemical techniques, such as measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse Extracellular Flux Analyzer, have allowed for bulk determination of the energy state of cell lines [40], demonstrating that invasive cells rely primarily on OXPHOS to fuel invasion. However, these biochemical techniques generally lack single-cell or spatial resolution. Pseudopodial isolation techniques using custom Transwell devices with small pores have helped overcome some biochemical limitations and provided important insight into subcellular energy metabolism. For example, biochemical pseudopodial isolation has shown that the leading-edge of cells relies on OXPHOS to generate ATP while the cell body uses glycolysis [49]. Notably, this subcellular isolation fails to capture the temporal dynamics of energy metabolism during invasion. Genetically encoded metabolism biosensors have helped address this gap and increased spatial and temporal resolution. For example, the ratiometric ATP:ADP sensor PercevalHR revealed the dynamic energy state of leader cells during collective cell migration [57]. However, these sensors are limited by the narrow range of metabolites that they can currently measure. Furthermore, Förster resonance energy transfer (FRET) sensors (ATeam) and ratiometric sensors (PercevalHR) require imaging in multiple wavelengths, which limits the visualization of other genetically encoded fluorescent proteins concurrently with the energy

Outstanding Questions

Mitochondrial localization to energetically demanding sites of invasion is observed across many invasive cells. While the energy sensor AMPK is required to localize mitochondria in ovarian cancer cells, it is unclear how it localizes mitochondria and whether AMPK coordinates energy sensing to localize mitochondria in other invading cells.

With the dizzying number of nutrient transporters, it is unclear how glucose and possibly other fuels are imported into invasive cells and how they provide localized mitochondria adequate carbon sources for oxidation to generate high levels of ATP.

While integrin-mediated mechanosensing is used to modulate energy metabolism in response to ECM in pancreatic cancer cells, the mechanism(s) other invasive cells use to sense the status of matrix to tune their metabolic output is unknown.

The phosphocreatine–creatine kinase system is used as an adaptive ATP generation mechanism in pancreatic and colorectal cancer. It is unclear whether this system is widely used by invasive cells or whether it is a specific adaptation used by only a few cancer types.

sensors. Single-wavelength fluorescent biosensors, such as ATP sensor, iATPSnFR and glucose sensor, Green Glifon (Table 1), utilize the fluorophore circularly permuted GFP (cpGFP) to provide a concentration-dependent fluorescent intensity change and use a single fluorescent wavelength. Recently, these sensors have also been targeted to specific subcellular regions, providing even finer subcellular resolution [66]. These single-wavelength intensiometric biosensors have yet to be used in studies of cell invasion and will provide key insights into how invading cell metabolism could interact with cellular morphology or proteins of interest.

With the continued development of precise energy sensors, live cell imaging, and expanded studies using diverse *in vitro* and *in vivo* models, we expect rapid advances in our understanding of how cells acquire and use energy during cell invasion. As highlighted in our review, we believe that it will be particularly important to determine whether AMPK is widely used to sense energy needs to direct mitochondria and ATP to sites of high energy use within invasive cells, how nutrient transport is coordinated with dynamic mitochondrial localization, whether all invasive cells adjust energy metabolism by sensing the matrix environment using integrin-mediated mechanotransduction, and whether the creatine kinase–phosphocreatine system is widely used as an adaptive ATP generation mechanism (see Outstanding Questions). Answering these questions will be important in developing a more unified understanding of the conserved and perhaps unique energy metabolism mechanisms that invasive cells use to overcome ECM barriers. As shown by the specific disruption of cell invasion after targeting energy metabolism in combination with MMPs in *C. elegans*, we anticipate that a deeper understanding of energy metabolism will lead to more effective therapeutic approaches to halt invasion in human diseases such as cancer and immune disorders, where unchecked invasive behavior leads to tissue destruction and often lethality.

Acknowledgements

We thank I. Kenny and C. Gianakas for comments on the manuscript and A. Kawska (info@illuscientia) for her work on Figure 3. D.R.S. and A.G. were supported by R35GM118049 and R21OD028766.

Declaration of Interests

The authors declare no competing interests.

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