# Metabolic hallmarks of metastasis formation

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Metastasis to distant organs is a predictor of poor prognosis. Therefore, it is of paramount importance to understand the mechanisms that impinge on the different steps of the metastatic cascade. Recent work has revealed that particular metabolic pathways are rewired in cancer cells to support their transition through the metastatic cascade resulting in the formation of secondary tumors in distant organs. Indeed, metabolic rewiring induces signaling pathways during initial cancer invasion, circulating cancer cells depend on enhanced antioxidant defenses, and cancer cells colonizing a distant organ require increased ATP production. Moreover, the local environment of the metastatic niche dictates the metabolic pathways secondary tumors rely on. Here we describe mechanisms of metabolic rewiring associated with distinct steps of metastasis formation.

## *Introduction*

Metastasis formation is the leading cause of death in cancer patients[1, 2]. The phenotypic complexity during the multi-step cascade of metastasis formation[3] strongly contributes to the difficulties of identifying effective inhibitors against this deadly process. First, cancer cells invade the surrounding tissue and enter into the

circulation. Upon survival in the circulation, they disseminate to distant organs. Often the pre-metastatic niche in a distant organ is primed, through factors secreted from the primary tumor, to facilitate the survival of disseminated cancer cells[4, 5]. While cancer cells in distant organs can undergo a state of dormancy, they eventually colonize the metastatic niche, resulting in micro-metastases. Micro-metastases gradually transition to established metastases (i.e. secondary tumors), which are defined, similar to the primary tumor, by proliferation. The importance of metabolic changes enabling cancer cells to transition through the different steps of metastasis formation is beginning to be realized, and connections to the local environment and the cancer cell phenotype are being established[6-10]. Thus, targeting metabolic changes that enable metastasis formation could open innovative possibilities to inhibit this deadly process[11]. In this review, we present the emerging picture on how metabolic rewiring supports the different steps of the metastatic cascade.

## *Cellular metabolism in (cancer) cells*

Cellular metabolism is a biochemical reaction network that allows cells to convert nutrients into various small molecules, known as metabolites. These conversions have several functions that include (but are not limited to) the following aspects[6]: First, they allow cells to maintain cellular homeostasis[12]. For example, the generation of ATP sustains energy-demanding cellular processes and glutathione is used to balance oxidative stress. ATP is produced by glycolysis in the cytosol and by oxidative phosphorylation in the mitochondria, which relies on the generation of the redox equivalents NADH and $FADH_2$ through metabolic pathways such as the tricarboxylic acid (TCA) cycle and proline catabolism. Glutathione reduction relies on the generation of the redox equivalent NADPH, which can occur through multiple metabolic pathways including the pentose phosphate pathway and the TCA cycle. Second, metabolic conversions allow cells to generate biomass[13], such that amino acids, nucleotides and fatty acids needed for protein, DNA/RNA and membrane synthesis, respectively, are generated. Third, they allow cells to link metabolism to signaling and epigenetic

events[14, 15]. Examples are acetyl-CoA, which is a substrate for histone modifications, and α-ketoglutarate, which supports the activation of mTORC1 and acts as a cofactor for enzymes involved in DNA demethylation. Cancer cells rewire these metabolic pathways to ensure increased survival, proliferation, invasion, motility and other cellular phenotypes enabling cancer initiation and progression. Depending on the phenotype of the cancer cell different essential metabolic functions emerge and the associated metabolic pathways can be targeted to impair them[1, 15]. In the following sections, we review metabolic pathways that are important for cancer cells during metastasis formation (i.e. in cancer cells of different phenotype) and link these, where possible, to different functions of cellular metabolism.

## *Metabolic rewiring supports cancer cell invasion by inducing signaling pathways*

The invasion of cancer cells into the surrounding tissue is the first step in the metastatic cascade. Extensive research, considering various biological processes including metabolism, has been performed to understand how cancer cells become invasive. Unfortunately, no overarching pattern of metabolic changes has been identified that discriminates invasive from non-invasive cancer. This suggests that, depending on the origin of the cancer, genetic drivers and environment, cancer cells can exploit multiple metabolic pathways to become invasive. Many of the metabolic changes supporting invasion result in the activation of signaling pathways. In turn, cancer cells can gain the ability to degrade the extracellular matrix (ECM; which reduces the physical barrier of invasion), remodel interactions (such as decreasing cell-cell/matrix contact, increasing focal adhesion turnover, increasing invadopodia formation[16]) and activate an epithelial-to-mesenchymal transition (EMT). Moreover, metabolites can stimulate motility of cancer cells by inducing chemoattraction. In the following, we summarize the current knowledge on metabolic pathways and enzymes involved in enabling cancer invasion (Figure 1).

### Glucose and lactate metabolism

The enzymes hexokinase (HK) 2 and pyruvate kinase (PK) M2 define the start and end of glycolysis. HK2 and PKM2 catalyze the irreversible reaction of glucose to glucose-6-phosphatase and phosphoenolpyruvate to pyruvate, respectively. Both enzymes are expressed in many fetal or embryonic and proliferative (including cancer) tissues, while other isoforms of these enzymes are predominately found in differentiated tissues[1]. The hexokinase reaction is a first rate-limiting step of glycolysis, while the pyruvate kinase reaction connects glycolysis to lactate and amino acid metabolism[13, 17]. The influence of HK2 and PKM2 on metastasis formation has been intensively studied. Their expression correlates with motility and invasive capacity in cancer cells of different origin[18-24]. Knockdown of either HK2 or PKM2 results in reduced lactate production and impaired glycolysis[19, 24]. These metabolic changes could be causal for inducing motility and invasion of cancer cells because flux through glycolysis results in the generation of the metabolic byproduct methylglyoxal, which has been shown to activate YAP signaling in breast cancer cells[25]. Interestingly, YAP signaling induces EMT in breast cancer[26, 27]. Moreover, lactate promotes breast cancer progression by supporting chemoattraction, which stimulates cancer cell migration[28]. Additionally, changes in pH associated with increased lactate production stimulates, in part through NF-κB signaling, the secretion and activation of hydrolases such as cathepsins and matrix metallopeptidase 9 (also known as matrix metalloproteinase 9), which degrade ECM components; therefore, reducing the physical barrier permissive for invasion[7]. A lactate transporter MCT1-dependent activation of NF-κB signaling has also been identified in invasive cancer cells[29]. NF-κB signaling has not only been associated with ECM degradation, but also with an induction of EMT[7]. Thus, these observations suggest that glycolysis and associated lactate metabolism can induce signaling pathways that promote cancer invasion and motility by driving proteolytic activity and EMT programs.

## Mitochondrial metabolism

Several studies have shown that mitochondrial metabolism, in particular oxidation of various nutrients (such as glutamine, glucose, fatty acids) in the TCA cycle, is linked to cancer invasion. Yet, its role is controversial, since both mitochondrial overload and dysfunction, as well as activation and inhibition of mitochondrial biogenesis have been shown to promote invasiveness of cancer cells[30-32]. Indeed, mitochondrial overload and dysfunction results in reactive oxygen species (ROS) production. This in turn can activate the protein tyrosine kinases Src and Pyk2, which promote cancer cell motility by remodeling cell-cell and cell-matrix interactions[30]. Accordingly, treatment with a mitochondrial ROS scavenger or perturbation of mitochondrial redox homeostasis prevented cancer progression in different mouse models[30, 33]. Inhibition of the mitochondrial biogenesis regulator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) has shown opposing effects on cancer invasion, which might be linked to the cancer cell of origin and genetic drivers[31, 32, 34]. Supporting this role, PGC-1α expression prevents PTEN loss induced prostate cancer progression by activating catabolism via estrogen-related receptor alpha (ERRα)[31]. In breast cancer however, PGC-1α expression drives oxidative metabolism as well as mitochondrial biogenesis, and in the final consequence, invasion leading to metastasis formation[32]. Accordingly, mitochondrial metabolism, including oxidation of glutamine or fatty acids has been linked to breast, melanoma and ovarian cancer invasion and agressiveness[35-37] as well as stemness in chronic myeloid leukemia[38]. Functionally, these observations could be linked to cell motility programs because actin cytoskeleton remodeling and contraction are energy demanding processes, which could be fueled by mitochondrial produced ATP. In addition, PGC-1α silencing has been found to increase invasiveness of melanoma cells by modulating cell-cell and cell-matrix interactions via the transcription factor 4 (TCF4) – focal adhesion kinase (FAK) signaling axis[34].

Connected to mitochondrial metabolism is the conversion of glutamine to glutamate. Invasive breast cancer cells secrete glutamate to stimulate the recycling of transmembrane matrix metalloprotease (MT1-MMP) via a metabotropic glutamate receptor (GRM3)[39]. Moreover, asparagine, an amino acid that is produced from

aspartate via asparagine synthetase (ASNS), promotes breast cancer cell invasion[40]. ASNS expression in primary tumors was found to be correlated with metastatic relapse and ASNS knockdown as well as dietary reduction of asparagine impaired cancer progression. Mechanistically, asparagine availability increased the expression of EMT genes[40]. Thus, mitochondrial metabolism is anti- or pro-invasive depending on the tumor context and often connected to a change in the activation of signaling pathways known to promote cancer progression.

## Fatty acid metabolism

Fatty acid synthesis, uptake and processing has been linked to cancer progression. Upon anti-angiogenic treatment withdrawal, xenograft tumors of human breast and colon cancers elevate fatty acid synthesis, which allowed re-growth and metastasis formation[41]. Moreover, lipid accumulation and free fatty acid uptake increases the invasiveness, migration and progression of various cancers including breast and liver cancers[42-44], likely through the induction of EMT that was induced via transforming growth factor (TGF) β and Wnt family member (Wnt) signaling in liver cancers[43]. Furthermore, breast cancer cells downregulate the mitochondrial protein penicillin-binding/B-lactamase like protein (LACTB) resulting in elevated phospholipid metabolism, increased proliferation and EMT induction, via zinc finger E-box-binding homeobox (ZEB) 1[45]. In addition, increased fatty acid desaturation has been associated with stemness in ovarian cancer cells through induction of NF-κB signaling[46].

Connected to fatty acid metabolism is the metabolite acetyl-CoA, which is an important donor for protein and histone acetylation. Inhibition of the fatty acid synthesis enzyme acetyl-CoA carboxylase (ACC) 1 through phosphorylation (that was induced by leptin signaling) resulted in acetyl-CoA accumulation[47]. This in turn, activated an EMT program via Smad2 transcription factor acetylation and consequently promoted breast cancer cell invasion[47]. An additional nutrient driving acetyl-CoA production is acetate. While several groups have shown that inhibition of cytosolic acetate metabolism inhibits growth of primary breast, prostate and liver

tumors through decreased biosynthesis of membrane phospholipids[48, 49], there is indication that it might promote hepatocellular carcinoma invasiveness and migration under hypoxia[50]. Mechanistically, it was suggested that inhibition of cytosolic acetate metabolism, by acetyl-coenzyme A synthetase (ACSS) 2 silencing, leads during hypoxia to deacetylation of HIF (hypoxia inducible factor) 2$\alpha$. This in turn results in HIF2$\alpha$ dependent EMT activation. In summary, fatty acid and acetyl-CoA metabolism can impact cancer invasiveness at least in part through the induction of an EMT.

A recent study has also suggested that patient-derived xenograft melanoma models that can undergo metastasis formation had increased levels of metabolites supporting histone methylation[51]. Accordingly, inhibiting histone methylation impaired invasiveness and metastatic spread in these models[51]. While the exact mechanism by which the histone methylation observed in this study promotes invasiveness remains unknown, it is tempting to speculate that it activates the expression of genes known to support cancer progression[15]. Thus, the methylation status of a tumor might be a useful predictor of cancer progression risk.

Taken together, the diversity of the metabolic pathways supporting cancer invasion limits the possibility of designing overarching treatment strategies. Moreover, the time window for a treatment that targets cancer invasiveness is narrow because the dissemination of cancer cells to distant organs can be a very early event that likely takes place before initial cancer diagnosis[52, 53]. Instead, metabolic changes in primary tumors could be predictors of metastasis risk.

### *Circulating tumor cells rely on antioxidant metabolism*

Metastasizing cancer cells need to survive in the circulation. During invasion cancer cells show altered and decreased cell-matrix interaction; this interaction is further reduced in the circulation. In non-transformed cells detachment from the matrix results in ROS accumulation induced anoikis[54]. Elevated antioxidant defenses allow

cancer cells to survive matrix detachment in vitro and as circulating tumor cells in vivo (Figure 2). For example, matrix detached fibrosarcoma cells showed reduced ROS induced anoikis[55]. This effect was mediated by activating transcription factor 4 (ATF4) and nuclear factor-erythroid 2-related factor 2 (Nrf2) induced expression of heme oxygenase 1 (HO-1)[55], which decreases the prooxidants heme and increases the antioxidants bilirubin[56]. Moreover, increased PGC1$\alpha$-dependency has been observed in circulating tumor cells[32], which can support ROS homeostasis[57]. Overexpression of oncogenes such as Erbb2 in non-transformed mammary epithelial cells results in increased survival upon matrix detachment that was dependent on ROS scavenging[58]. Erbb2 signaling activates the pentose phosphate pathway resulting in the production of NADPH, which is needed to recycle the ROS scavenger glutathione. Consequently, processes such as energy production that are impaired by ROS accumulation, are restored, which permits cancer cell survival during matrix detachment[58]. Accordingly, matrix detachment decrease glucose oxidation in breast cancer cells, which reduces mitochondrial ROS production[59]. In line, overexpression of the mitochondria pyruvate carrier (MPC), which is required for glucose oxidation, impairs matrix-detached colon cancer cells[60].

Matrix-detached cancer cells also rewire their metabolism to optimize ROS scavenging, particularly in mitochondria. Most matrix attached, normoxic and respiration competent cancer cells have reduced conversion of $\alpha$-ketoglutarate to citrate (reductive carboxylation)[61-64]. Yet, matrix detached lung, colon and breast cancer cells alter their metabolism and use a cross compartment $\alpha$-ketoglutarate – citrate cycle, i.e combining oxidative decarboxylation in the mitochondria with reductive carboxylation in the cytosol[65] (Figure 2). Thereby, $\alpha$-ketoglutarate and NADPH are reductively carboxylated in the cytosol to citrate and $NADP^+$. In turn, citrate is transported into the mitochondria and oxidized with concomitant NADPH production. Consequently, there is no net production of any metabolite or glutathione reduction capacity, but an enriched antioxidant defense in the mitochondria.

The importance of ROS scavenging for the survival of circulating tumor cells and metastasis formation was demonstrated by treating different mouse models harboring melanoma with the antioxidants N-acetyl-cysteine, which enriched the number of melanoma cells in circulation and resulted in lymph node metastasis[66, 67]. Thus, it emerges that cancer cells survive matrix detachment by upregulating various antioxidant pathways, reducing ROS production, and redirecting scavenging capacity to the mitochondria.

ROS scavenging not only reduces anoikis, but also increases the stemness of cancer cells, which in turn can support metastasis formation[68]. Unlike non-transformed mammary epithelial cells, transformed breast cancer cells have been shown to increase EMT gene expression patterns upon matrix detachment[69]. This can inhibit the gluconeogenic enzyme fructose bisphosphatase (FBP) 1, reducing oxidative metabolism, through a largely unknown mechanism[70]. Consequently, ROS production by mitochondria is reduced, and supports the interaction of β-catenin with TCF4, which can induce stemness through the Wnt signaling cascade[70]. Thus, decreased ROS production can support cancer cell stemness. Finally, increased fatty acid uptake has been identified in circulating tumor cells[44, 71]. Whether this is associated with ROS reduction remains to be determined.

In summary, it has emerged that antioxidant defense is essential for circulating tumor cells to evade cell death. Targeting this metabolic vulnerability might be particularly interesting in combination with primary cancer surgery, which has the potential to increase the number of circulating tumor cells depending on the tumor location and the surgical technique applied[72-74].

### *The pre-metastatic niche is primed for glucose availability*

Secreted factors from the primary tumor prepare a permissive pre-metastatic niche, which supports metastatic seeding by altering the stromal, immune cell and/or matrix

composition of the niche[75]. Very little is known about the metabolic priming of the pre-metastatic niche. Yet, it was recently shown that primary tumors can contribute to a permissive pre-metastatic niche that facilitates the adaptation of metastasizing breast cancer cells upon arrival in the lung and the brain. Specifically, primary breast tumors have the capacity to release exosomes containing miR-122, which suppresses glucose metabolism of resident cells in the pre-metastatic niche by downregulating PKM and glucose transporter 1 (GLUT1)[76]. The increased availability of glucose allows metastasizing breast cancer cells to accommodate their nutrient requirements and facilitated metastatic seeding. Accordingly, treatment with anti-miR-122 significantly reduced metastasis formation in xenograft mouse models[76]. Whether this priming of the pre-metastatic niche is especially important for metastasizing cancer cells arising from highly glycolytic primary breast cancers[1] remains to be determined. Moreover, whether the pre-metastatic niche is also primed for other nutrients is not known. Thus, the translational potential of interfering with the metabolic priming of the pre-metastatic niche remains to be determined.

### *Metastatic colonization depends on increased ATP production*

Once cancer cells have reached a distant organ they need to colonize the new environment. This process, which will ultimately result in the formation of micro-metastases, includes survival, seeding, establishment of cell-matrix interactions, ECM remodeling and finally outgrowth. It has emerged that the metabolic pathways that allow cancer cells to undergo metastatic colonization differ depending on the organ. It is tempting to speculate that this is a result of the particularities of the environment (such as nutrients, matrix and stromal cells) that cancer cells encounter within different organs. While different pathways are identified to be important for (early) metastatic colonization, they all converge to fuel ATP production (Figure 3). Accordingly, breast cancer cells that colonize the lung rely on the proline cycle[69]. In this cycle, proline and FAD are converted to 1-pyrroline-5-carboxylic acid (P5C) and $FADH_2$. Next, P5C and NADPH are converted back to proline and $NADP^+$. The $FADH_2$

production in this cycle can be coupled to the mitochondrial electron transport chain, thereby producing ATP at the expense of NADPH. Inhibiting the proline cycle enzyme proline dehydrogenase (PRODH) is sufficient to decrease the number of arising metastases in aggressive breast cancer mouse models[69]. In addition, breast cancer cells that metastasize to the lung rely on increased PGC-1α activity[77]. Mechanistically, PGC-1α allowed metastasizing cancer cells to increase their bioenergetic flexibility, i.e. ATP production from both glycolysis and mitochondrial oxidative phosphorylation. Similarly, oral squamous cell carcinoma cells with metastatic seeding capacity to the lymph node expressed CD36, which allows them to take up fatty acids and oxidize them in the mitochondria[71], potentially to produce ATP. Thus, in environments such as the lung and the lymph node, colonizing cancer cells rely on mitochondria to fuel their increased energy needs.

Cancer cells metastasizing to other organs, such as the liver, seem to depend on non-mitochondrial ATP production (Figure 3). Accordingly, colon cancer cells that metastasize to the liver rely on extracellular metabolic energetics[78]. In particular, they release creatine kinase brain-type (CKB) to the extracellular space, which scavenges available extracellular ATP by converting creatine into phosphocreatine[78]. This metabolite can be taken up by colonizing cancer cells and be used to phosphorylate ADP to ATP. It is tempting to speculate that the reliance of metastasizing cancer cells on extracellular ATP is a transient process, bridging the time needed to activate cell intrinsic ATP production in the liver environment. Indeed, breast cancer cells that colonize the liver depend on pyruvate dehydrogenase kinase (PDK) 1, which is activated by hypoxic signaling and shifts energy production from the mitochondria to glycolysis[79].

Taken together, cancer cells colonizing a distant organ have increased energy needs that they fuel in an environment-dependent manner. The reasons for this striking dependence remain elusive, but targeting this metabolic requirement is of

translational relevance because many cancer patients already present with disseminated cancer cells upon diagnosis.

### *Environment dictates metabolic rewiring in secondary tumors*

Cancer cells within an established secondary tumor (i.e. macro-metastases) have a similar cellular phenotype as cancer cells within a primary tumor, i.e. many of them proliferate. Despite this similar phenotype, it has been shown that the metabolism of secondary tumors from human melanoma patients converge toward a state of low oxidative metabolism[80] and that breast cancer-derived lymph node metastases display increased transketolase (enzyme of the pentose phosphate pathway) expression[81] in comparison to their respective primary tumors. While cell intrinsic changes between primary and secondary tumors contribute to the differential metabolism of secondary tumors, cell extrinsic changes in the environment (such as nutrients) can be an important determinant.

Nutrients are known to dictate the activity of metabolic pathways[15, 82-85]. As the nutrient environment of a secondary tumor differs from that of the primary tumor, it seems likely that secondary tumors rely on different metabolic pathways depending on the organ in which they grow (Figure 4). Accordingly, breast cancer-derived lung metastases switch to pyruvate carboxylase dependent refilling of the TCA cycle (anaplerosis) due to an enriched availability of pyruvate in the lung environment[85]. Similarly, brain metastases originating from different tissues use acetate rather than glucose or glutamine to drive an oxidative TCA cycle[86, 87]. Indeed, cancer cells isolated from brain metastases gain glucose independent growth capacity by relying, in part, on gluconeogenic enzymes such as FBP1[88]. Thus, it emerges that nutrients available in distant organs can determine the metabolism of arising metastases.

Beyond nutrients, additional aspects of the local environment may also shape the metabolism of secondary tumors (Figure 4). There is evidence that the brain microenvironment requires the activity of the pentose phosphate pathway and

glutathione synthesis[89-91], which could be connected to the production of ROS by high oxygen tension in the brain. Moreover, increased activity of the pentose phosphate pathway can enable epigenetic changes that allow pancreatic cancer cells to proliferate as liver and lung metastases[92].

In conclusion, the local environment dictates the metabolic vulnerabilities of established metastases proliferating in distant organs. While current knowledge on the metabolism of secondary tumors is largely descriptive, it suggests that the treatment of metastatic cancers needs to be adapted to the organ hosting the metastasis rather than to the primary tumor type[85].

## *Concluding remarks*

The collective evidence presented here supports the notion that the metastatic cascade triggers different metabolic requirements in cancer cells that can be targeted for therapy (Figure 4). Yet, many questions remain (see also outstanding questions), including how the epigenetic state of the primary tumor can be exploited to predict metastasis formation risk, what is the importance and extent of metabolic priming in the pre-metastatic niche, how disseminated cancer cells transition from a dormancy state to an active proliferating state, how increased energy production supports colonization of a distant organ, and how cancer cells shape their metastatic niche to support metastatic outgrowth in distant organs. Moreover, it will be important to determine to which extent the presence of different stromal or immune cells in the metastatic site rewires the metabolism of cancer cells during metastatic seeding and growth. Despite a large number of outstanding questions, conclusions that may be relevant to the clinical management of metastases are beginning to emerge. As such, metabolic hallmarks of early events of metastasis could be translated to biomarkers predicting metastasis formation risk. Moreover, metabolic rewiring that supports metastatic colonization could be combined or eventually replace preventative chemotherapy after, for example, breast cancer surgery. Chemotherapy targets

primarily proliferating cells[93], but cancer cells that have disseminated into distant organs (and are not yet proliferating as established secondary tumors) can evade this treatment because they often display reduced proliferation. Consequently, targeting the specific metabolic requirements of these cancer cells and in particular their need for ATP could prevent their survival and outgrowth in the metastatic niche. Finally, it is of utmost importance to consider the organ environment when treating secondary tumors, because certain metabolism targeting drugs might only work in specific organs.

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## *Figures*

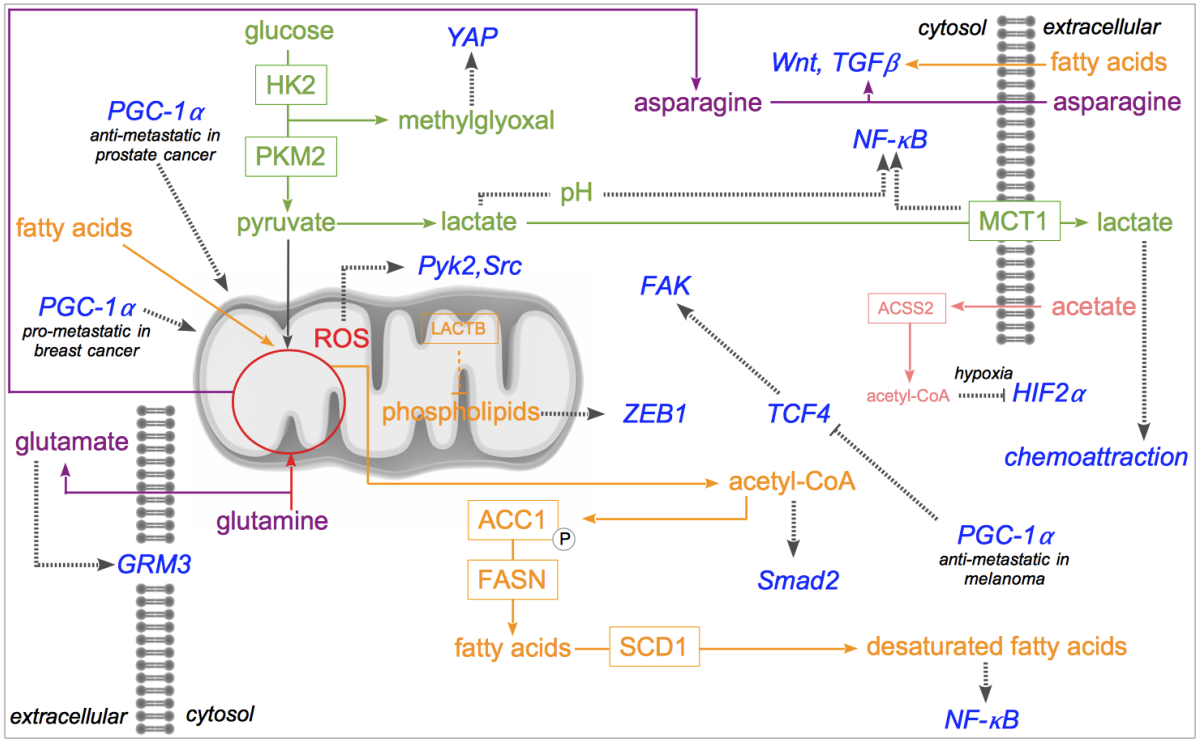


**Figure 1: Metabolic rewiring supports cancer cell invasion by inducing signaling pathways.**

These signaling pathways activate cellular program such as EMT and ECM degradation that are known to support invasion and motility. Enzymes are depicted in boxes. Green indicates glycolysis, red indicates oxidative mitochondrial metabolism, magenta indicates glutamine and asparagine metabolism, yellow indicates fatty acid metabolism, and rose indicates acetate metabolism. Signaling molecules/pathways are depicted in italics and bright blue. Bold dashed lines indicate regulation, while fine dashed lines depict downregulated metabolic pathways. ACC1 refers to acetyl-CoA carboxylase 1, ACSS2 refers to acyl-coenzyme A synthetase 2, FAK refers to focal adhesion kinase, FASN refers to fatty acid synthase, GRM3 refers to metabotropic glutamate receptor 3, HIF2α refers to hypoxia inducible factor 2α, HK2 refers to hexokinase 2, LACTB refers to penicillin-binding/B-lactamase like protein, MCT1 refers to monocarboxylate transporter 1, NF-κB refers to nuclear factor kappa B, P refers to phosphorylation, PGC-1α refers to peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PKM2 refers to pyruvate kinase M2, Pyk2 refers to protein-

tyrosine kinase 2, ROS refers to reactive oxygen species, SCD1 refers to stearyl-CoA desaturase 1, Smad 2 refers to Smad family member 2, Src refers to proto-oncogene tyrosine-protein kinase, TCF4 refers to transcription factor 4, TGFβ refers to transforming growth factor, Wnt refers to Wnt family member, YAP refers to yes-associated protein 1, ZEB1 refers to zinc finger E-box binding homeobox 1.

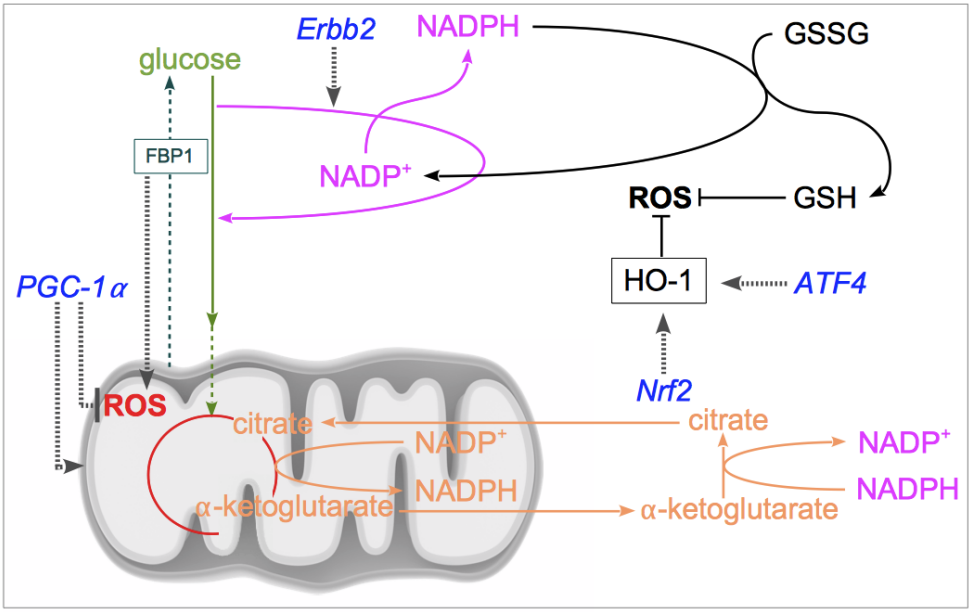


**Figure 2: Circulating tumor cells rely on enhanced antioxidant metabolism.**

Enzymes are depicted in boxes. Green indicates glycolysis, red indicates oxidative mitochondrial metabolism, orange indicates the α-ketoglutarate – citrate cycle, pink indicates pentose phosphate pathway, and dark green indicates gluconeogenesis. Signaling molecules are depicted in italics and bright blue. Bold dashed lines indicate regulation, while fine dashed lines depict downregulated metabolic pathways. ATF4 refers to activating transcription factor 4, Erbb2 refers to Erb-B2 receptor tyrosine kinase 2, FBP1 refers to fructose bisphosphatase 1, GSH refers to reduced glutathione, GSSG refers to oxidized glutathione, HO-1 refers to heme oxygenase, Nrf2 refers to nuclear factor like 2, PGC-1α refers to peroxisome proliferator-activated receptor gamma coactivator 1-alpha, ROS refers to reactive oxygen species.

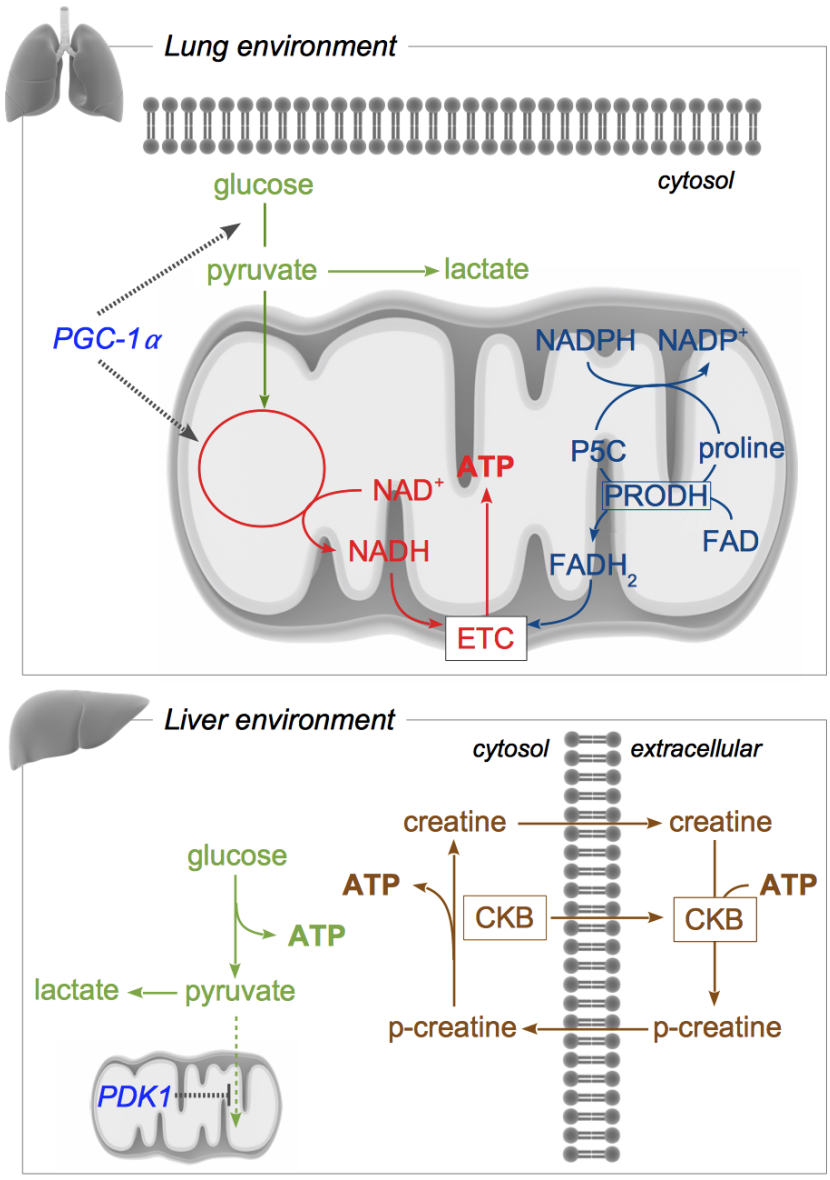


**Figure 3: Metastatic colonization depends on increased ATP production.**

Enzymes are depicted in boxes. Green indicates glycolysis, red indicates oxidative mitochondrial metabolism, dark blue indicates the proline cycle, brown indicates the creatine cycle. Signaling molecules are depicted in italics and bright blue. Bold dashed lines indicate regulation, while fine dashed lines depict downregulated metabolic pathways. CKB refers to creatine kinase brain-type, ETC refers to electron transport chain, PDK1 refers to pyruvate dehydrogenase kinase 1, PGC-1α refers to peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PRODH refers to proline dehydrogenase.

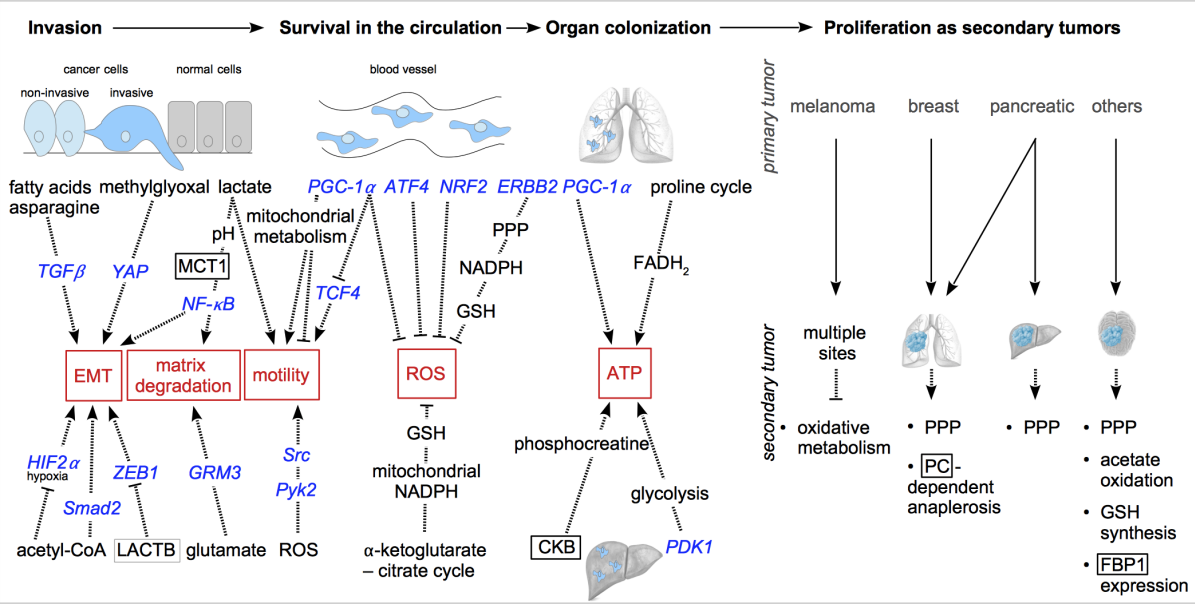


**Figure 4: The metastatic cascade is associated with metabolic rewiring**.

Normal cells are depicted in gray, while cancer cells are indicated in blue (light blue depicts non-invasive cancer cells, while dark blue depicts invasive cancer cells). Signaling molecules are depicted in italics and bright blue. Dashed lines indicate regulated pathways/processes, while black lines indicate the metastasis formation cascade. Enzymes (black) or processes important for the different steps of metastases formation (red) are highlighted in boxes. Acetyl-CoA refers to acetyl coenzyme A, ATF4 refers to activating transcription factor 4, CKB refers to creatine kinase brain-type, Erbb2 refers to Erb-B2 receptor tyrosine kinase 2, FBP1 refers to fructose bisphosphatase 1, GRM3 refers to metabotropic glutamate receptor 3, GSH refers to reduced glutathione, LACTB refers to penicillin-binding/B-lactamase like protein, NF-κB refers to nuclear factor kappa B, Nrf2 refers to nuclear factor-erythroid 2-related factor 2, PC refers to pyruvate carboxylase, PGC-1α refers to peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PPP refers to penthose phosphate pathway, Pyk2 refers to protein-tyrosine kinase 2, ROS refers to reactive oxygen species, Smad2 refers to small mother against decapentaplegic family member 2, Src refers to proto-oncogene tyrosine-protein kinase, TCF4 refers to transcription factor 4, YAP refers to yes-associated protein 1, ZEB1 refers to zinc finger E-box binding homeobox 1.