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The Warburg metabolism fuels tumor metastasis

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Abstract
The Warburg effect is prevalent in human cancer. Accordingly, most cancer cells display highly elevated glycolysis without proportionally increasing pyruvate oxidation. The metastatic process imposes strong selective pressure on cancer cells, and metastasizing cancer cells experience heightened oxidative stress. By constraining mitochondrial oxidative metabolism, the Warburg effect helps cancer cells to minimize oxidative stress, thereby facilitating metastatic dissemination. The PGC1α transcriptional coactivator is a central coordinator of oxidative metabolism. While promoting oxidative metabolism and reversing the Warburg effect, PGC1α critically activates antioxidant genes and protects cells against oxidative damage. Therefore, depending on the context, PGC1α may promote or suppress tumor metastasis. Cancer cells generally retain metabolic flexibility and can resist antiglycolysis treatment by undergoing metabolic reprogramming. Synthetic lethal combination therapies are thus essential to attack the liabilities of the Warburg metabolism for therapeutic benefit.

Keywords Cancer · Tumor metastasis · Warburg effect

1 Metastatic inefficiency

Cancer metastasis is a multi-step process by which malignant cells spread from the primary tumor to discontinuous organ sites in the body [1]. The development of therapy-resistant metastasis in vital organs is the leading cause of cancer mortality. To successfully colonize a distant organ, cancer cells must complete a sequential series of steps to become clinically detectable lesions. The hematogenous metastatic cascade of cancer cells includes detachment from the primary tumor, local invasive migration toward blood vessels, intravasation into the circulation, transport through the bloodstream as circulating tumor cells (CTCs), arrest in capillaries of target organs, extravasation, and growth into macrometastases.

It has been estimated that the majority of cancer patients have initiated the metastatic process by the time of diagnosis [2]. In fact, as part of the *liquid biopsy*, CTCs are detectable in blood in most malignancies, particularly at advanced stages [3]. In a mouse model of breast cancer, millions of cancer cells are shed from a tumor per gram mass into the bloodstream every day [4]. Cancer cells enter the blood circulation through active and/or passive mechanisms [5]. The invasive subpopulation of cancer cells in the primary tumor may actively invade blood vessels. Meanwhile, tumor vasculature is often unstable and leaky due to abnormal angiogenesis, thus facilitating the escape of cancer cells [6].

Although the entry of cancer cells into the blood circulation is rather common, the presence of CTCs in the circulation is not predictive of metastasis. Indeed, only a few of these cells actually give rise to metastatic tumors. In an experimental metastasis model, the vast majority of the CTCs in blood are rapidly eliminated. One day after intravenous injection into the circulation, < 0.1% of B16 melanoma cells were still viable, and < 0.01% of tumor cells within the circulation survived to develop lung metastases [7]. In a liver metastasis model [8], the majority of intraportally injected B16F1 melanoma cells survived in the liver microcirculation and extravasated by day 3. However, only a small subset (2.5%) of extravasated cells developed into micrometastases, and only a small subset (1%) of micrometastases progressed to form macrometastases by day 13 (most micrometastases disappeared). A substantial fraction of injected cancer cells remained by day 13 as solitary dormant cells. Overall, the process of metastasis is highly inefficient. Metastatic inefficiency seems to be principally attributed to the elimination of cancer cells in the bloodstream and/or failure of disseminated cancer cells to grow into macroscopic metastatic foci at the secondary sites. This explains why a large

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number of cancer cells are released from an established tumor into the blood circulation every day, but only a tiny minority of them will successfully form macrometastases. Signaling pathways, transcriptional programs, and metabolic changes that promote cell survival and/or proliferation will generally enhance the metastatic potential of cancer cells.

2 Warburg metabolism

Cellular metabolism converts nutrients into energy and biomass, thus critically fueling the survival and proliferation of cancer cells. Glucose is the main energy source for mammalian cells. Glucose is taken up into the cell and metabolized to pyruvate in the cytosol through glycolysis. The process yields two ATP molecules from one glucose molecule. In normal quiescent cells, glycolysis-derived pyruvate is predominantly imported into the mitochondria where it is oxidized to acetyl coenzyme A (CoA) by the pyruvate dehydrogenase complex (PDC). Acetyl-CoA enters the tricarboxylic acid (TCA) cycle (also known as the Krebs cycle or citric acid cycle), followed by oxidative phosphorylation (OXPHOS) for high-efficiency ATP generation. Through complete oxidation, one molecule of glucose produces 36 ATP molecules. It is nearly 100 years since Otto Warburg reported that cancer cells metabolize glucose in a manner distinct from that of normal tissues. Rapidly proliferating cancer cells dramatically increase glucose uptake and glycolytic rates. However, even in the presence of adequate tissue oxygen, increased pyruvate yield does not proportionally increase pyruvate oxidation in the mitochondria. Instead, most pyruvate is converted to lactate in the cytoplasm by lactate dehydrogenase (LDH), and the resulting abundant lactate is secreted into the tumor microenvironment. Therefore, glycolysis and the TCA cycle are uncoupled in cancer cells. This metabolic phenomenon is referred to as aerobic glycolysis or the "Warburg effect" [9]. The Warburg metabolic phenotype is a widespread cancer-associated trait.

It is postulated that Warburg metabolism satisfies the anabolic demand of dividing cells [10]. Increased glucose consumption generates large quantities of various intermediate glycolytic metabolites, which may partly be diverted to multiple biosynthetic pathways instead of giving rise to pyruvate. Thus, glycolytic intermediates can provide important building blocks for biomass synthesis (e.g., synthesis of amino acids, nucleic acids, and lipids) that is required for cancer cell growth and division (Fig. 1).

In this regard, besides its vital role in energy metabolism, the TCA cycle also generates important metabolic intermediates that cancer cells need as precursors for various anabolic pathways to make key cellular components (e.g., amino acids, nucleotides, fatty acids) (Fig. 1). It is thus puzzling why most pyruvate is *discarded* as lactate by the glycolytic cells and is not fully used for bioenergetic and biosynthetic purposes. *First*, it is noteworthy that most cancer cells maintain functional mitochondria. The rate of mitochondrial respiration in cancer cells remains comparable to that of normal cells [9]. Despite increased contributions of glycolysis to ATP generation due to the Warburg effect, mitochondrial oxidative metabolism remains a significant source for ATP in cancer cells. It was estimated that depending on the cancer cells examined, 40% to 80% of the total cellular ATP is derived from OXPHOS [11, 12]. Some cancer cells rely on the TCA cycle for survival and proliferation [13–16]. Cancer cells may also use glutamine as a significant source of carbons to replenish TCA cycle intermediates. Therefore, mitochondria are metabolically active in most cancer cells, producing both ATP and intermediate metabolites to sustain cell proliferation. *Second*, oxidative metabolism is subjected to feedback inhibition. Glucose oxidative metabolism generates acetyl-CoA, NADH, and ultimately, ATP. The generation of acetyl-CoA from pyruvate by PDC is a major control point of the TCA cycle [17, 18]. High concentrations of acetyl-CoA and NADH not only allosterically inhibit PDC but also activate pyruvate dehydrogenase kinases (PDKs), which, in turn, phosphorylate and inactivate PDC. Moreover, key individual enzymes of the TCA cycle (e.g., citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase) are inhibited by ATP and other oxidative reaction products. Overall, when the energy charge is high and biosynthetic intermediates are abundant, the negative feedback regulation stifles the TCA cycle at the entry into the cycle and at the key reactions of the cycle. By uncoupling glycolysis from the TCA cycle, cancer cells can maximally consume glucose to saturate their biosynthetic and bioenergetic needs. *Third*, oncogenic signals inhibit PDC through covalent modifications (Fig. 1). Various oncogenic signaling pathways (e.g., hypoxia, myc, Wnt) upregulate PDKs to inactivate PDC through serine phosphorylation [18]. Oncogenic non-receptor tyrosine kinase Src and a few receptor tyrosine kinases (RTKs) suppress PDC via direct phosphorylation of tyrosine residues [19, 20]. Therefore, oxidative metabolism is retained but contained in cancer cells, while glycolysis is disproportionally elevated.

3 Metabolic heterogeneity and plasticity

Cancer is a disease with extraordinary heterogeneity at multiple levels, including cellular metabolism. Metabolism is governed by both oncogenes and tumor suppressor genes [21]. Different tumors result from diverse oncogenic alterations, thus exhibiting heterogeneous metabolic profiles. But, metabolism is not completely cell-autonomous. Tumor metabolic heterogeneity is further exacerbated by the heterogeneous composition of the tumor microenvironment. Cancer metabolism is impacted by cells' locations relative to blood vessels, the availability of oxygen and nutrients, pH, and signaling molecules [22]. Both the intrinsic and extrinsic factors determine metabolism in cancer cells.

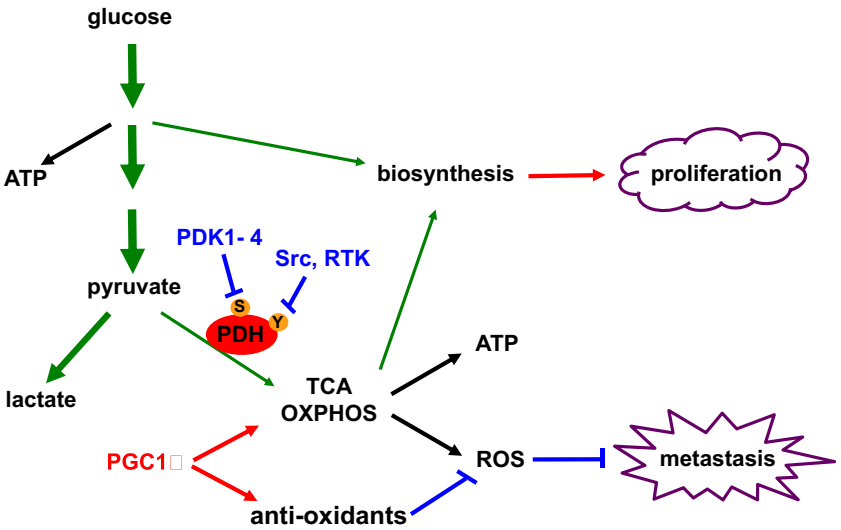

Fig. 1 Schematic illustration of glucose metabolism in cancer cells. Glucose is converted to pyruvate through glycolysis, and a fraction of glycolytic intermediates is diverted to biosynthetic pathways to fuel cell proliferation. Although oxidative metabolism persists, it is restrained (at least in part due to inhibition of PDC by PDKs and/or Src and receptor tyrosine kinases) and pyruvate is predominantly shunted to lactate production. The mitochondrial respiration by-products (ROS) exhibit antimetastasis activity, which may explain why cancer cells keep pyruvate oxidation in check. PGC1α coordinates oxidative metabolism and antioxidant capacity, thus impacting tumor metastasis. The flux of glucose carbon is indicated by green arrows. S and Y represent serine and tyrosine residues, respectively, in pyruvate dehydrogenase (PDH)

Although the Warburg effect is prevalent, human cancers display a wide range of metabolic profiles, ranging from strict aerobic glycolysis to robust oxidative metabolism [23, 24]. Positron emission tomography (PET) imaging technology is powerful in detecting most human tumors in clinical setting, highlighting the prevalence of avid glucose uptake by cancer cells relative to adjacent tissues, a key feature of the Warburg effect. Mitochondrial respiratory gene expression is suppressed in many cancers [25]. Isotope tracing analysis *in vivo* revealed that glucose oxidation is suppressed in human clear cell renal cell carcinomas [26]. By contrast, enhanced glucose oxidation and glycolysis are commonly observed in human lung cancers, glioblastomas, and breast cancer brain metastases *in vivo* [27–30]. Subsets of diffuse large B cell lymphoma and melanoma cells exhibit increased mitochondrial energy metabolism *in vitro* [31, 32].

Metabolic heterogeneity exists even within the same tumor due to variability in genetic alterations, epigenetic regulation, and transcriptional programs. In a microfluidic single-cell assay of approximately 100 glioblastoma cells from the same cell line, 80% of cells display high glucose uptake and the other 20% display low glucose uptake, suggesting the existence of two distinct subpopulations [33]. Cytotoxic treatment of melanoma cells led to the enrichment of a drug-resistant, slow-cycling tumorigenic subpopulation of cells that show elevated oxidative phosphorylation and dependence on mitochondrial respiration [34]. In a mouse model of pancreatic cancer that is generally glycolytic, ablation of the oncogenic driver induces massive tumor regression, yet a small subpopulation of dormant cells survives and is responsible for tumor relapse. These cells display features of cancer stem cells, a strong reliance on oxidative phosphorylation, and a decreased dependence on glycolysis [16]. Breast cancer stem cells (BCSCs) display plasticity transitioning between quiescent mesenchymal-like (M) and proliferative epithelial-like (E) states, which rely on distinct metabolic pathways and display markedly different sensitivities to inhibitors of glycolysis and redox metabolism [35].

Most cancer cells are metabolically flexible and able to adapt to a changing microenvironment. Cancer cells typically have a substantial reserve capacity for energy production by OXPHOS when glycolysis is suppressed. Inhibiting glycolysis in glycolytic cancer cells restores mitochondrial OXPHOS [36, 37]. When glycolytic cancer cells are cultured in constant low-glucose conditions, they upregulate OXPHOS and rely on it for survival and proliferation [38]. When cancer cells are under lactic acidosis, they transition to a non-glycolytic phenotype, characterized by oxidative metabolism [39]. Therefore, lactate secreted by glycolytic cancer cells may be consumed by oxidative cancer cells [40, 41] or may cause metabolic reprogramming in glycolytic cells. Conversely, targeting mitochondrial oxidative metabolism in oxidative melanoma cells renders them highly glycolytic [42]. The metabolic flexibility enables cancer cells to compensate for deprivation of one fuel by utilization of other nutrients.

4 Metastatic suppression by oxidative metabolism

The metastatic cascade involves overcoming physical and physiological stress and incurs substantial energy demands,

thus imposing strong selective pressures. Metastasizing cancer cells indeed experience heightened oxidative stress [43]. Only a few malignant cells are able to survive the harsh stress during the migratory journey (esp. in the circulation) and adapt to a generally incompatible microenvironment in distant organs to form metastatic lesions.

Attachment to extracellular matrix provides pro-growth and pro-survival signals. Cell detachment from matrix induces robust production of reactive oxygen species (ROS), which, in excess, can lead to cell death known as *anoikis*, a barrier to metastasis [44–46]. An emerging concept is that there is a "sweet spot" for ROS, where too few suppress proliferation, and moderate ROS levels activate signaling pathways to support cell proliferation, survival, and migration, whereas excessive ROS accumulation causes severe damage to cellular components and triggers cell death [47]. Cancer cells usually display elevated levels of ROS. To maintain their viability, cancer cells develop an increased antioxidant capacity that keeps ROS levels below a critical cytotoxic threshold. We and others previously found that when detached from matrix, untransformed mammary epithelial cells shut off PDC and glucose oxidation [48, 49]. Mitochondrial oxidative metabolism is the main source of ROS. Our study further suggests that this metabolic switch enables detached cells to decrease mitochondrial ROS generation and extend anchorage-independent survival [49]. Forced activation of PDC in detached cells increases mitochondrial respiration, leading to production of excessive ROS and heightened anoikis. Conversely, inhibition of PDC prolongs survival of cells in suspension [49]. Therefore, in response to cell detachment that induces oxidative stress, cells rewire glucose metabolism to decrease ROS production to improve their viability. Furthermore, we found that cell detachment markedly upregulates manganese superoxide dismutase (MnSOD, or SOD2), a principal mitochondrial antioxidant enzyme that is induced by oxidative stress and detoxifies ROS [44]. This powerful antioxidant response allows cells to ameliorate the detrimental effects of ROS [44]. The metabolic and antioxidant responses help cells to maintain redox homeostasis and maximally survive the cellular oxidative stress.

Because of the Warburg effect, cancer cells uncouple oxidative and fermentative glucose metabolism. As glucose oxidation is already constrained even in cells under attached conditions, Warburg cancer cells avoid the upsurge of ROS generation when detached from matrix, thus inherently resisting anoikis. Stimulation of PDC in metastatic breast cancer cells increases glucose oxidation, augments ROS levels upon cell detachment, restores susceptibility to anoikis, and suppresses metastasis [49]. Consistently, MnSOD expression is elevated in human breast cancer metastases compared with primary tumors and contributes to cancer cell's resistance to anoikis [44]. These results suggest that oxidative stress inhibits tumor metastasis (Fig. 1). The Warburg effect may not only limit mitochondrial ROS production, and increased glycolysis also enhances the branching pentose phosphate pathway to generate NADPH that is critical for antioxidant activity [50, 51]. Therefore, the Warburg effect minimizes the cellular oxidative stress. In addition, the proliferative Warburg metabolism is expected to be critical for metastatic growth at the secondary sites. Overall, the Warburg effect promotes metastasis [17].

Piskounova et al. [43] performed a metabolomics analysis on melanoma xenograft models. Melanoma cells isolated from the blood circulation and from metastatic sites display elevated levels of ROS compared with those in the primary tumors. The metastasizing melanoma cells apparently undergo metabolic changes to increase the endogenous antioxidants (glutathione and NADPH) to neutralize oxidative stress. Blocking NADPH generation inhibits distant metastasis without significantly affecting the growth of primary tumors in the same mice. Treatment with exogenous antioxidants increases CTCs and distant metastasis. The results suggest that cellular oxidative stress limits melanoma metastasis *in vivo* [43]. Only cells with increased antioxidant capacity are able to successfully withstand oxidative stress associated with traveling through the circulation and seeding at the metastatic sites. Consistently, another study showed that antioxidant supplementation markedly increases lymph node metastases but has no impact on primary tumors in an endogenous mouse model of melanoma [52].

The PGC1α transcriptional coactivator is a master positive regulator of mitochondrial biogenesis and oxidative metabolism [53]. PGC1α signal induces metabolic shift from glycolysis to OXPHOS in glioma cells, and this anti-Warburg effect promotes the differentiation of glioma cells into astrocytes and inhibits tumor growth [54]. While PGC1α suppresses metastasis in prostate cancer and melanoma models [55, 56], it promotes metastasis in breast cancer models [57, 58]. The role of PGC1α in tumor metastasis thus seems to be context-dependent. Although PGC1α-stimulated oxidative metabolism generates increased amounts of ROS, which are by-products of mitochondrial respiration, it should be noted that PGC1α is a critical transcriptional activator of key ROS-detoxifying enzymes including MnSOD and glutathione peroxidase 1 (GPX1) [59]. PGC1α promotes ROS detoxification and supports cell survival under oxidative stress conditions. Indeed, PGC1α overexpression protects cells from the deleterious effect of oxidative stress, and PGC1α-deficient cells accumulate ROS and are hypersensitive to death from oxidative stress [59]. Therefore, PGC1α is a powerful protector against ROS accumulation and damage [60], which may partly explain its pro-metastatic activity (Fig. 1).

5 Therapeutic targeting of the Warburg effect

Cancer metabolism is not just a by-product of cell transformation, but instead, it actively contributes to cell survival,

growth, proliferation, and metastasis. As such, cancer metabolism may present vulnerabilities that can be exploited therapeutically [61]. Since the Warburg effect is nearly universal, many tumors exhibit apparent glucose addiction and highly glycolytic phenotypes. However, given that metabolic flexibility is retained in most cancer cells, simply blocking glycolysis may activate alternative metabolic pathways and hence is ineffective in cancer therapy. Moreover, cancer stem cells are often slow-cycling and thus rely more on oxidative metabolism [62]. The antiglycolysis strategy may enrich such highly malignant cells.

Certain tumors may be metabolically less flexible due to specific genetic alterations. For example, the majority of sporadic clear cell renal cell carcinomas have lost and/or silenced the von Hippel-Lindau (VHL) tumor suppressor gene, which encodes the substrate-recognition component of an E3 ubiquitin ligase complex that targets the hypoxia-inducible factor (HIF) for destruction in the presence of oxygen [63]. HIF potently stimulates glucose uptake and glycolysis, while shutting down glucose oxidation [64]. Therefore, VHL-deficient tumors constitutively activate the HIF-driven metabolic program under normoxia and display the typical Warburg metabolism [26]. Such tumors are truly addicted to glucose and may not be able to freely switch to oxidative metabolism when glucose is deprived and thus may be hypersensitive to antiglycolysis treatment [65]. Alternatively, because glucose carbons are largely excluded from the TCA cycle, VHL-deficient cancer cells may probably depend on glutamine via reductive carboxylation to generate TCA intermediates for anabolic needs [66, 67]. Therefore, perturbation of the use of glutamine may also be selectively toxic to this type of cancer.

Most cancer cells are able to switch metabolic programs in response to the changing environment or treatments. Synthetic lethality approaches involving metabolic therapy may be more effective. Antiangiogenesis therapies block the formation of new blood vessels and cut off the blood supply to tumors. However, angiogenesis inhibitors have generally produced only modest survival benefits for cancer patients in clinical trials [68]. In preclinical models, potent angiogenesis inhibition does decrease tumor burden but concomitantly increases tumor invasion and metastasis [69–71]. As a result of pruning of the tumor microvasculature, intratumoral hypoxia is strongly induced [69–71], which may be responsible for the observed increase of tumor aggressiveness [72]. Hypoxia dictates glucose metabolism [64]. Under hypoxia, oxidative metabolism is largely prohibited, and cancer cells are forced to switch to glycolysis for proliferation and survival. Therefore, combining antiglycolysis and antiangiogenesis therapies may achieve improved therapeutic efficacy.

Treatment of glycolytic cancer cells with antiglycolysis agents or the PDK inhibitor dichloroacetate (DCA) shifts cellular metabolism from glycolysis to OXPHOS and increases mitochondrial ROS production [37, 73–76]. Presumably depending on cancer cell's antioxidant capacity, oxidative stress generated from this metabolic shift is sometimes sufficient to cause apoptosis or may sensitize cancer cells to further oxidative stress. Ionizing radiation and many chemotherapeutic drugs currently used in clinical practice kill cancer cells in large part through the induction of high levels of free radicals [47]. Therefore, combinations of pro-OXPHOS metabolic therapies (e.g., glycolysis inhibitors or DCA) with pro-oxidant radiation/chemotherapies may lead to excessive production of oxidative stress, which would exceed the cellular antioxidant capacity of cancer cells and effectively induce cell death.

6 Summary

The Warburg effect not only provides an ample supply of metabolites for the anabolic needs of proliferating cancer cells but also mitigates oxidative stress to help cancer cells to survive the stringent metastatic process. Although Warburg metabolism is common, cancer cells typically maintain functional mitochondria and can switch between aerobic glycolysis and oxidative metabolism in response to single metabolic treatments. Therefore, combination therapies are required to effectively target the liabilities of Warburg cancer cells.

References

1. Talmadge, J. E., & Fidler, I. J. (2010). AACR centennial series: The biology of cancer metastasis: historical perspective. *Cancer Research, 70*, 5649–5669.
2. Schmidt-Kittler, O., Ragg, T., Daskalakis, A., Granzow, M., Ahr, A., Blankenstein, T. J., Kaufmann, M., Diebold, J., Arnholdt, H., Muller, P., et al. (2003). From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 7737–7742.
3. Caixeiro, N. J., Kienzle, N., Lim, S. H., Spring, K. J., Tognela, A., Scott, K. F., de Souza, P., & Becker, T. M. (2014). Circulating tumour cells—a bona fide cause of metastatic cancer. *Cancer Metastasis Reviews, 33*, 747–756.
4. Butler, T. P., & Gullino, P. M. (1975). Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. *Cancer Research, 35*, 512–516.
5. Bockhorn, M., Jain, R. K., & Munn, L. L. (2007). Active versus passive mechanisms in metastasis: do cancer cells crawl into vessels, or are they pushed? *The Lancet Oncology, 8*, 444–448.
6. Nagy, J. A., Chang, S. H., Dvorak, A. M., & Dvorak, H. F. (2009). Why are tumour blood vessels abnormal and why is it important to know? *British Journal of Cancer, 100*, 865–869.
7. Fidler, I. J. (1970). Metastasis: quantitative analysis of distribution and fate of tumor emboli labeled with 125 I-5-iodo-2′-deoxyuridine. *Journal of the National Cancer Institute, 45*, 773–782.
8. Luzzi, K. J., MacDonald, I. C., Schmidt, E. E., Kerkvliet, N., Morris, V. L., Chambers, A. F., & Groom, A. C. (1998).

Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *The American Journal of Pathology, 153*, 865–873.

9. Koppenol, W. H., Bounds, P. L., & Dang, C. V. (2011). Otto Warburg's contributions to current concepts of cancer metabolism. *Nature Reviews. Cancer, 11*, 325–337.
10. Vander Heiden, M. G., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science, 324*, 1029–1033.
11. Guppy, M., Leedman, P., Zu, X., & Russell, V. (2002). Contribution by different fuels and metabolic pathways to the total ATP turnover of proliferating MCF-7 breast cancer cells. *The Biochemical Journal, 364*, 309–315.
12. Nakashima, R. A., Paggi, M. G., & Pedersen, P. L. (1984). Contributions of glycolysis and oxidative phosphorylation to adenosine 5′-triphosphate production in AS-30D hepatoma cells. *Cancer Research, 44*, 5702–5706.
13. Birsoy, K., Wang, T., Chen, W. W., Freinkman, E., Abu-Remaileh, M., & Sabatini, D. M. (2015). An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis. *Cell, 162*, 540–551.
14. Molina, J. R., Sun, Y., Protopopova, M., Gera, S., Bandi, M., Bristow, C., McAfoos, T., Morlacchi, P., Ackroyd, J., Agip, A. A., et al. (2018). An inhibitor of oxidative phosphorylation exploits cancer vulnerability. *Nature Medicine, 24*, 1036–1046.
15. Sullivan, L. B., Gui, D. Y., Hosios, A. M., Bush, L. N., Freinkman, E., & Vander Heiden, M. G. (2015). Supporting aspartate biosynthesis is an essential function of respiration in proliferating cells. *Cell, 162*, 552–563.
16. Viale, A., Pettazzoni, P., Lyssiotis, C. A., Ying, H., Sanchez, N., Marchesini, M., Carugo, A., Green, T., Seth, S., Giuliani, V., et al. (2014). Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature, 514*, 628–632.
17. Lu, J., Tan, M., & Cai, Q. (2015). The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Letters, 356*, 156–164.
18. Stacpoole, P. W. (2017). Therapeutic targeting of the pyruvate dehydrogenase complex/pyruvate dehydrogenase kinase (PDC/PDK) axis in cancer. *Journal of the National Cancer Institute, 109*.
19. Fan, J., Kang, H. B., Shan, C., Elf, S., Lin, R., Xie, J., Gu, T. L., Aguiar, M., Lonning, S., Chung, T. W., Arellano, M., Khoury, H. J., Shin, D. M., Khuri, F. R., Boggon, T. J., Kang, S., & Chen, J. (2014). Tyr-301 phosphorylation inhibits pyruvate dehydrogenase by blocking substrate binding and promotes the Warburg effect. *The Journal of Biological Chemistry, 289*, 26533–26541.
20. Jin, Y., Cai, Q., Shenoy, A. K., Lim, S., Zhang, Y., Charles, S., Tarrash, M., Fu, X., Kamarajugadda, S., Trevino, J. G., Tan, M., & Lu, J. (2016). Src drives the Warburg effect and therapy resistance by inactivating pyruvate dehydrogenase through tyrosine-289 phosphorylation. *Oncotarget, 7*, 25113–25124.
21. Levine, A. J., & Puzio-Kuter, A. M. (2010). The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science, 330*, 1340–1344.
22. Gatenby, R. A., & Gillies, R. J. (2004). Why do cancers have high aerobic glycolysis? *Nature Reviews. Cancer, 4*, 891–899.
23. Hu, J., Locasale, J. W., Bielas, J. H., O'Sullivan, J., Sheahan, K., Cantley, L. C., Vander Heiden, M. G., & Vitkup, D. (2013). Heterogeneity of tumor-induced gene expression changes in the human metabolic network. *Nature Biotechnology, 31*, 522–529.
24. Jose, C., Bellance, N., & Rossignol, R. (2011). Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? *Biochimica et Biophysica Acta, 1807*, 552–561.
25. Reznik, E., Wang, Q., La, K., Schultz, N., & Sander, C. (2017). Mitochondrial respiratory gene expression is suppressed in many cancers. *Elife, 6*.
26. Courtney, K. D., Bezwada, D., Mashimo, T., Pichumani, K., Vemireddy, V., Funk, A. M., Wimberly, J., McNeil, S. S., Kapur, P., Lotan, Y., Margulis, V., Cadeddu, J. A., Pedrosa, I., DeBerardinis, R. J., Malloy, C. R., Bachoo, R. M., & Maher, E. A. (2018). Isotope tracing of human clear cell renal cell carcinomas demonstrates suppressed glucose oxidation *in vivo*. *Cell Metabolism, 28*, 793–800.e792.
27. Fan, T. W., Lane, A. N., Higashi, R. M., Farag, M. A., Gao, H., Bousamra, M., & Miller, D. M. (2009). Altered regulation of metabolic pathways in human lung cancer discerned by (13)C stable isotope-resolved metabolomics (SIRM). *Molecular Cancer, 8*, 41.
28. Hensley, C. T., Faubert, B., Yuan, Q., Lev-Cohain, N., Jin, E., Kim, J., Jiang, L., Ko, B., Skelton, R., Loudat, L., Wodzak, M., Klimko, C., McMillan, E., Butt, Y., Ni, M., Oliver, D., Torrealba, J., Malloy, C. R., Kernstine, K., Lenkinski, R. E., & DeBerardinis, R. J. (2016). Metabolic heterogeneity in human lung tumors. *Cell, 164*, 681–694.
29. Maher, E. A., Marin-Valencia, I., Bachoo, R. M., Mashimo, T., Raisanen, J., Hatanpaa, K. J., Jindal, A., Jeffrey, F. M., Choi, C., Madden, C., Mathews, D., Pascual, J. M., Mickey, B. E., Malloy, C. R., & DeBerardinis, R. J. (2012). Metabolism of [U-13 C]glucose in human brain tumors *in vivo*. *NMR in Biomedicine, 25*, 1234–1244.
30. Marin-Valencia, I., Yang, C., Mashimo, T., Cho, S., Baek, H., Yang, X. L., Rajagopalan, K. N., Maddie, M., Vemireddy, V., Zhao, Z., Cai, L., Good, L., Tu, B. P., Hatanpaa, K. J., Mickey, B. E., Matés, J. M., Pascual, J. M., Maher, E. A., Malloy, C. R., DeBerardinis, R. J., & Bachoo, R. M. (2012). Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain *in vivo*. *Cell Metabolism, 15*, 827–837.
31. Caro, P., Kishan, A. U., Norberg, E., Stanley, I. A., Chapuy, B., Ficarro, S. B., Polak, K., Tondera, D., Gounarides, J., Yin, H., Zhou, F., Green, M. R., Chen, L., Monti, S., Marto, J. A., Shipp, M. A., & Danial, N. N. (2012). Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. *Cancer Cell, 22*, 547–560.
32. Vazquez, F., Lim, J. H., Chim, H., Bhalla, K., Girnun, G., Pierce, K., Clish, C. B., Granter, S. R., Widlund, H. R., Spiegelman, B. M., & Puigserver, P. (2013). PGC1alpha expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. *Cancer Cell, 23*, 287–301.
33. Xue, M., Wei, W., Su, Y., Kim, J., Shin, Y. S., Mai, W. X., Nathanson, D. A., & Heath, J. R. (2015). Chemical methods for the simultaneous quantitation of metabolites and proteins from single cells. *Journal of the American Chemical Society, 137*, 4066–4069.
34. Roesch, A., Vultur, A., Bogeski, I., Wang, H., Zimmermann, K. M., Speicher, D., Korbel, C., Laschke, M. W., Gimotty, P. A., Philipp, S. E., et al. (2013). Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. *Cancer Cell, 23*, 811–825.
35. Luo, M., Shang, L., Brooks, M. D., Jiagge, E., Zhu, Y., Buschhaus, J. M., Conley, S., Fath, M. A., Davis, A., Gheordunescu, E., Wang, Y., Harouaka, R., Lozier, A., Triner, D., McDermott, S., Merajver, S. D., Luker, G. D., Spitz, D. R., & Wicha, M. S. (2018). Targeting breast cancer stem cell state equilibrium through modulation of redox signaling. *Cell Metabolism, 28*, 69–86.e66.
36. Fantin, V. R., St-Pierre, J., & Leder, P. (2006). Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell, 9*, 425–434.
37. Le, A., Cooper, C. R., Gouw, A. M., Dinavahi, R., Maitra, A., Deck, L. M., Royer, R. E., Vander Jagt, D. L., Semenza, G. L., & Dang, C. V. (2010). Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proceedings of the National*

Academy of Sciences of the United States of America, 107, 2037–2042.
38. Birsoy, K., Possemato, R., Lorbeer, F. K., Bayraktar, E. C., Thiru, P., Yucel, B., Wang, T., Chen, W. W., Clish, C. B., & Sabatini, D. M. (2014). Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature, 508*, 108–112.
39. Xie, J., Wu, H., Dai, C., Pan, Q., Ding, Z., Hu, D., Ji, B., Luo, Y., & Hu, X. (2014). Beyond Warburg effect—dual metabolic nature of cancer cells. *Scientific Reports, 4*, 4927.
40. Brooks, G. A. (2018). The science and translation of lactate shuttle theory. *Cell Metabolism, 27*, 757–785.
41. Feron, O. (2009). Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiotherapy and Oncology, 92*, 329–333.
42. Lim, J. H., Luo, C., Vazquez, F., & Puigserver, P. (2014). Targeting mitochondrial oxidative metabolism in melanoma causes metabolic compensation through glucose and glutamine utilization. *Cancer Research, 74*, 3535–3545.
43. Piskounova, E., Agathocleous, M., Murphy, M. M., Hu, Z., Huddlestun, S. E., Zhao, Z., Leitch, A. M., Johnson, T. M., DeBerardinis, R. J., & Morrison, S. J. (2015). Oxidative stress inhibits distant metastasis by human melanoma cells. *Nature, 527*, 186–191.
44. Kamarajugadda, S., Cai, Q., Chen, H., Nayak, S., Zhu, J., He, M., Jin, Y., Zhang, Y., Ai, L., Martin, S. S., Tan, M., & Lu, J. (2013). Manganese superoxide dismutase promotes anoikis resistance and tumor metastasis. *Cell Death & Disease, 4*, e504.
45. Li, A. E., Ito, H., Rovira, I. I., Kim, K. S., Takeda, K., Yu, Z. Y., Ferrans, V. J., & Finkel, T. (1999). A role for reactive oxygen species in endothelial cell anoikis. *Circulation Research, 85*, 304–310.
46. Schafer, Z. T., Grassian, A. R., Song, L., Jiang, Z., Gerhart-Hines, Z., Irie, H. Y., Gao, S., Puigserver, P., & Brugge, J. S. (2009). Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. *Nature, 461*, 109–113.
47. Gorrini, C., Harris, I. S., & Mak, T. W. (2013). Modulation of oxidative stress as an anticancer strategy. *Nature Reviews. Drug Discovery, 12*, 931–947.
48. Grassian, A. R., Metallo, C. M., Coloff, J. L., Stephanopoulos, G., & Brugge, J. S. (2011). Erk regulation of pyruvate dehydrogenase flux through PDK4 modulates cell proliferation. *Genes & Development, 25*, 1716–1733.
49. Kamarajugadda, S., Stemboroski, L., Cai, Q., Simpson, N. E., Nayak, S., Tan, M., & Lu, J. (2012). Glucose oxidation modulates anoikis and tumor metastasis. *Molecular and Cellular Biology, 32*, 1893–1907.
50. Brand, K. A., & Hermfisse, U. (1997). Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. *The FASEB Journal, 11*, 388–395.
51. Cairns, R. A., Harris, I. S., & Mak, T. W. (2011). Regulation of cancer cell metabolism. *Nature Reviews. Cancer, 11*, 85–95.
52. Le Gal, K., Ibrahim, M. X., Wiel, C., Sayin, V. I., Akula, M. K., Karlsson, C., Dalin, M. G., Akyurek, L. M., Lindahl, P., Nilsson, J., et al. (2015). Antioxidants can increase melanoma metastasis in mice. *Science Translational Medicine, 7*, 308re308.
53. Handschin, C., & Spiegelman, B. M. (2006). Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocrine Reviews, 27*, 728–735.
54. Xing, F., Luan, Y., Cai, J., Wu, S., Mai, J., Gu, J., Zhang, H., Li, K., Lin, Y., Xiao, X., Liang, J., Li, Y., Chen, W., Tan, Y., Sheng, L., Lu, B., Lu, W., Gao, M., Qiu, P., Su, X., Yin, W., Hu, J., Chen, Z., Sai, K., Wang, J., Chen, F., Chen, Y., Zhu, S., Liu, D., Cheng, S., Xie, Z., Zhu, W., & Yan, G. (2017). The anti-Warburg effect elicited by the cAMP-PGC1alpha pathway drives differentiation of glioblastoma cells into astrocytes. *Cell Reports, 18*, 468–481.
55. Luo, C., Lim, J. H., Lee, Y., Granter, S. R., Thomas, A., Vazquez, F., Widlund, H. R., & Puigserver, P. (2016). A PGC1alpha-mediated transcriptional axis suppresses melanoma metastasis. *Nature, 537*, 422–426.
56. Torrano, V., Valcarcel-Jimenez, L., Cortazar, A. R., Liu, X., Urosevic, J., Castillo-Martin, M., Fernandez-Ruiz, S., Morciano, G., Caro-Maldonado, A., Guiu, M., et al. (2016). The metabolic co-regulator PGC1alpha suppresses prostate cancer metastasis. *Nature Cell Biology, 18*, 645–656.
57. Andrzejewski, S., Klimcakova, E., Johnson, R. M., Tabaries, S., Annis, M. G., McGuirk, S., Northey, J. J., Chenard, V., Sriram, U., Papadopoli, D. J., et al. (2017). PGC-1alpha promotes breast cancer metastasis and confers bioenergetic flexibility against metabolic drugs. *Cell Metabolism, 26*, 778–787 e775.
58. LeBleu, V. S., O'Connell, J. T., Gonzalez Herrera, K. N., Wikman, H., Pantel, K., Haigis, M. C., de Carvalho, F. M., Damascena, A., Domingos Chinen, L. T., Rocha, R. M., et al. (2014). PGC-1alpha mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. *Nature Cell Biology, 16*, 992–1003 1001–1015.
59. St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jager, S., Handschin, C., Zheng, K., Lin, J., Yang, W., et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell, 127*, 397–408.
60. Austin, S., & St-Pierre, J. (2012). PGC1alpha and mitochondrial metabolism—emerging concepts and relevance in ageing and neurodegenerative disorders. *Journal of Cell Science, 125*, 4963–4971.
61. Butler, E. B., Zhao, Y., Munoz-Pinedo, C., Lu, J., & Tan, M. (2013). Stalling the engine of resistance: targeting cancer metabolism to overcome therapeutic resistance. *Cancer Research, 73*, 2709–2717.
62. Batlle, E., & Clevers, H. (2017). Cancer stem cells revisited. *Nature Medicine, 23*, 1124–1134.
63. Kaelin, W. G., Jr. (2007). The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. *Clinical Cancer Research, 13*, 680s–684s.
64. Semenza, G. L. (2013). HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *The Journal of Clinical Investigation, 123*, 3664–3671.
65. Chan, D. A., Sutphin, P. D., Nguyen, P., Turcotte, S., Lai, E. W., Banh, A., Reynolds, G. E., Chi, J. T., Wu, J., Solow-Cordero, D. E., et al. (2011). Targeting GLUT1 and the Warburg effect in renal cell carcinoma by chemical synthetic lethality. *Science Translational Medicine, 3*, 94ra70.
66. Metallo, C. M., Gameiro, P. A., Bell, E. L., Mattaini, K. R., Yang, J., Hiller, K., Jewell, C. M., Johnson, Z. R., Irvine, D. J., Guarente, L., Kelleher, J. K., Vander Heiden, M. G., Iliopoulos, O., & Stephanopoulos, G. (2011). Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature, 481*, 380–384.
67. Sun, R. C., & Denko, N. C. (2014). Hypoxic regulation of glutamine metabolism through HIF1 and SIAH2 supports lipid synthesis that is necessary for tumor growth. *Cell Metabolism, 19*, 285–292.
68. Ye, W. (2016). The complexity of translating anti-angiogenesis therapy from basic science to the clinic. *Developmental Cell, 37*, 114–125.
69. De Bock, K., Mazzone, M., & Carmeliet, P. (2011). Antiangiogenic therapy, hypoxia, and metastasis: risky liaisons, or not? *Nature Reviews. Clinical Oncology, 8*, 393–404.
70. Sennino, B., & McDonald, D. M. (2012). Controlling escape from angiogenesis inhibitors. *Nature Reviews. Cancer, 12*, 699–709.
71. Sennino, B., Ishiguro-Oonuma, T., Wei, Y., Naylor, R. M., Williamson, C. W., Bhagwandin, V., Tabruyn, S. P., You, W. K., Chapman, H. A., Christensen, J. G., Aftab, D. T., & McDonald, D. M. (2012). Suppression of tumor invasion and metastasis by concurrent inhibition of c-Met and VEGF signaling in pancreatic neuroendocrine tumors. *Cancer Discovery, 2*, 270–287.

72. Rankin, E. B., & Giaccia, A. J. (2016). Hypoxic control of metastasis. *Science, 352*, 175–180.
73. Bonnet, S., Archer, S. L., Allalunis-Turner, J., Haromy, A., Beaulieu, C., Thompson, R., Lee, C. T., Lopaschuk, G. D., Puttagunta, L., Harry, G., et al. (2007). A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell, 11*, 37–51.
74. Michelakis, E. D., Sutendra, G., Dromparis, P., Webster, L., Haromy, A., Niven, E., Maguire, C., Gammer, T. L., Mackey, J. R., Fulton, D., et al. (2010). Metabolic modulation of glioblastoma with dichloroacetate. *Science Translational Medicine, 2*, 31ra34.
75. Sottnik, J. L., Lori, J. C., Rose, B. J., & Thamm, D. H. (2011). Glycolysis inhibition by 2-deoxy-D-glucose reverts the metastatic phenotype *in vitro* and *in vivo*. *Clinical & Experimental Metastasis, 28*, 865–875.
76. Sun, R. C., Fadia, M., Dahlstrom, J. E., Parish, C. R., Board, P. G., & Blackburn, A. C. (2010). Reversal of the glycolytic phenotype by dichloroacetate inhibits metastatic breast cancer cell growth *in vitro* and *in vivo*. *Breast Cancer Research and Treatment, 120*, 253–260.