Abstract

For decades, tumor cells have been considered defective in mitochondrial respiration due to their dominant glycolytic metabolism. However, a growing body of evidence is now challenging this assumption, and also implying that tumors are metabolically less homogeneous than previously supposed. A small subpopulation of slow-cycling cells endowed with tumorigenic potential and multidrug resistance has been isolated from different tumors. Deep metabolic characterizations of these tumorigenic cells revealed their dependency on mitochondrial respiration versus glycolysis, suggesting the existence of a common metabolic program active in slow-cycling cells across different tumors. These findings change our understanding of tumor metabolism and also highlight new vulnerabilities that can be exploited to eradicate cancer cells responsible for tumor relapse. Cancer Res; 75(18); 3687–91. ©2015 AACR.

Dysfunctional Mitochondria and Cancer

Speaking on the origins of cancer, Otto Warburg stated, "When the respiration of body cells has been irreversibly damaged, cancer cells by no means immediately result" (1). Warburg was Director of the Max Planck Institute for Cell Physiology when he gave this seminal lecture, and he was likely unaware of the profound impact his work on "aerobic glycolysis" would have had for the next several decades. Today mitochondria, or "grana" as they were named in Warburg's time, are much more than the bioenergetics "powerhouse" of the cell; rather, they are now regarded as important biosynthetic and signaling organelles (2). Despite a more comprehensive understanding of the complex physiology of mitochondria, the relationship between mitochondria and cancer cells continues to be perceived very simplistically: cancer cells have diminished capacity for oxidative phosphorylation (OXPHOS) due to dysfunctional mitochondria. However, the full extent of this impairment, as well as its significance, is not yet completely understood (3).

Mitochondrial DNA mutations and their significance

An obvious place to look for clues regarding mitochondrial defects in cancer is the mitochondrial genome. Despite the fact that mutations in mitochondrial DNA (mtDNA) genes encoding subunits of respiratory complexes have been found in the majority of tumors (3, 4), their high frequency and diversity have made it difficult to pinpoint their role in cancer. Moreover, the functional interpretation of many of the mutations remains ambiguous, and in many cases it remains unclear whether they represent gain of function mutations, neutral polymorphisms or even artifacts (4–6). To date, very few studies have demonstrated a direct relationship between recurrent mtDNA mutations and tumor maintenance (7, 8). However, it is generally accepted that mtDNA mutations are responsible for the induction of reactive oxygen species (ROS) at the expense of a less-efficient electron transfer chain (ETC). ROS, in turn, activate several signaling pathways critical for tumor growth and maintenance (9). Ultimately, OXPHOS activity is not suppressed in tumor cells and mtDNA mutations seem to be functional to the detailed bioenergetics and biosynthetic state of a cancer cell. Consistently, none of the pathogenic point mutations responsible for primary mitochondrial disease have never been associated with cancer (4).

Challenging Warburg hypothesis

An important advance in our understanding of the dependency of cancer cells on mitochondrial respiration came in the 1990s when experimental approaches to deplete mtDNA in mammalian cells became available (10). As originally described in yeast, long-term exposure of human cells to low concentrations of ethidium bromide depleted mitochondrial DNA and consequently reduced OXPHOS activity, a state known as rho-0. Surprisingly, when OXPHOS activity was suppressed, cancer cells changed their behavior and showed an impaired ability to grow in an anchorage-independent manner as a dramatic reduction in tumorigenic potential (11–13). Importantly, tumor cells with defective OXPHOS became extremely sensitive to cytotoxic drugs (12). These data demonstrated that tumors depend on mitochondrial respiration for the maintenance of their fully transformed phenotype, and were the first to challenge the Warburg hypothesis.

Such a conclusion is further supported by a recent study based on melanoma and breast cancer models of rho-0 cells. Tan and colleagues (14), consistent with previous studies, found that rho-0 tumor cells depleted of respiratory complexes were unable to accomplish lung seeding after mouse tail-vein injection and had increased tumor latency upon subcutaneous transplantation. Interestingly, cells derived from these delayed tumors showed
partial reactivation of OXPHOS due to the acquisition of host mtDNA. More importantly, the authors observed a further increase or fully restored respiration in circulating tumor cells and in metastatic lesions, respectively. These findings indicate that recovery of mitochondrial respiration by a subpopulation of cells undergoing intravasation and extravasation is essential to support tumor dissemination, which is consistent with other recent work demonstrating that OXPHOS activity and increased mitochondrial biogenesis are critical features of circulating tumor cells during the metastatic process (15).

Tumors are metabolically heterogeneous

After two decades from the earlier experiments with \( \rho^0 \) cells, we can now speculate that depletion of respiration in tumors specifically affects features related to cancer stemness, such as anchorage-independent growth, tumorigenic potential, drug resistance, and invasion. If this were the case, one would expect tumors to be metabolically heterogeneous and characterized by at least two different metabolic programs: the bulk of tumor cells depending on aerobic glycolysis and proliferating normally in culture either in the \( \rho^0 \) state or upon pharmacologic or genetic downregulation of OXPHOS (14–16), and a subpopulation of cells endowed with tumorigenic and metastatic potential relying more heavily on mitochondrial respiration. Then, the fact that just a miniscule fraction of cancer cells is critically dependent on OXPHOS activity may be exactly the reason they have gone unnoticed for so long.

Tumorigenic cells are dependent on mitochondrial respiration

Recently, using an inducible mouse model of pancreatic cancer, we isolated and characterized a subset of tumor cells with features of cancer stem cells that are able to survive the genetic extinction of oncogenic signaling (16). Mutations of KRAS are driver events in pancreatic cancer, and continuous oncogenic signaling is indispensible for tumor maintenance (17, 18). Upon KRAS inactivation in our inducible mouse model, pancreatic tumors, as expected, underwent an apparently complete regression due to extinction of the oncogenic signaling in leukemias. Lagadinos and colleagues (20) recently demonstrated that quiescent acute myelogenous leukemia–initiating cells have a lower energetic level with respect to proliferating cells and rely mainly on OXPHOS for energy production. Similar to the surviving pancreatic tumor-initiating cells discussed above, leukemia-initiating cells lack the important compensatory mechanism responsible for increasing glycolysis in response to OXPHOS inhibition (20). These data mechanistically explain why tigecycline, a glycyclcline antibiotic, was identified in a chemical screen as an agent that selectively killed leukemic stem and progenitor cells (21). As elegantly demonstrated by the authors, tigecycline interfered with mitochondrial translation and depleted mitochondrial subunits of respiratory complexes, resulting in respiratory inhibition (21). In this context, interesting work was recently published highlighting the combinatorial effects exerted by oligomycin, a complex V mitochondrial inhibitor, and small-molecule tyrosine kinase inhibitors in BCR-ABL and FLT3\(^{ITD}\) leukemias (22). These data demonstrated synergistic effects between oligomycin and imatinib or quizartinib in eradicating leukemic cells, demonstrating that, as we showed in pancreatic cancer, OXPHOS inhibition is synthetically lethal with the extinction of the oncogenic signaling in leukemias.

Role of mitochondrial respiration in other tumors

But are leukemias and pancreatic cancers different from other tumors in their dependency on mitochondrial respiration? Or is this a general characteristic? A quick answer is provided by the early experiments using \( \rho^0 \) cells where a decrease in tumorigenic potential was described in several tumors of different origins, such as glioblastoma, breast, ovarian and cervical cancers, sarcoma, and melanoma (11–14). Especially in melanomas, several lines of evidence suggest that these aggressive and drug-resistant tumors also are metabolically heterogeneous and contain a subset of cells reliant on respiration. In 2013, two independent studies reported the importance of mitochondrial master regulator PGC1\(\alpha\) (PPARGC1A) in melanoma, proposing its use as a negative prognostic factor for patient stratification (23, 24). High expression levels of PGC1\(\alpha\) in melanoma cells have been associated with an increased dependency on mitochondrial respiration associated with a decreased glycolytic profile. Importantly, the tumorigenic potential of PGC1\(\alpha\)-expressing cells is strictly connected to the maintenance of their respiratory gene program, and tumor cells become highly sensitive to oxidative stress in its absence (23). Also, in melanoma, as demonstrated for other tumors, pharmacologic treatment with BRAF inhibitors selects for cells characterized by upregulated mitochondrial respiration and OXPHOS inhibition has been demonstrated to be synthetically lethal with extinction of oncogenic signaling (24).
Although cancer stem cells have never been isolated from melanoma, precluding the application of a classical hierarchical organization (25, 26), functional heterogeneity among cells has been described. The expression of H3K4 demethylase JARID1B has been shown to mark a subpopulation of slow-cycling melanoma cells endowed with multidrug resistance (27). This population of cells was confirmed to be required for continuous tumor growth and, due to its intrinsic resistance, is strongly enriched upon treatment with cytotoxic drugs or BRAF inhibitors. Deep characterization of JARID1B^high cells revealed a sustained increase in oxygen consumption due to the upregulation of genes involved in the ETC, whereas glycolysis was downregulated (28).

In agreement with the models described above, OXPHOS inhibitors used in combination with cytotoxic drugs or BRAF inhibitors extended survival in vivo models by selectively targeting the slow-cycling population of cells and overcoming their intrinsic multidrug resistance (28).

Mitochondrial respiration as a metabolic trait of tumor dormancy

Independently from their tissue of origin, tumor cells relying on OXPHOS share a common feature: they are slow cycling. Several lines of evidence suggest that quiescence and reliance on mitochondrial respiration represent two sides of the same coin, although a direct link remains to be found. Dormancy seems to be a low energetic state in which cells have minimal biosynthetic needs relative to highly proliferative cells (16, 20). Although counterintuitive, OXPHOS dependency might confer to quiescent cells the ability to survive in hypoxic and nutrient-depleted environments that would limit the growth of actively proliferating cells. Indeed, studies in intact cells using high-resolution respirometry demonstrated that oxygen concentration is not a limiting factor for mitochondrial respiration until it drops below 1.0 μmol/L, a very extreme hypoxia (~0.1% pO2; K_M for oxygen at 0.05 ± 0.01 kPa; 29). These data are compatible with a model in which quiescent tumor cells could take advantage of their ability to completely oxidize carbon skeletons entering the tricarboxylic acid cycle to produce sufficient amount of ATP in hostile micro-environments where glucose and oxygen are limiting.

Interestingly, one of the major regulators of dormancy in tumor cells is the stress-activated protein kinase p38. Indeed, activation of p38 MAPK signaling has been documented to promote survival and suppress the growth of solitary, disseminated cancer stem-like cells in several cancer models (30–33). Recently, p38 has been demonstrated to activate a transcription factor network responsible for induction of quiescence in cancer cells (34). Although, to our knowledge, no reports have been published linking p38 activation to metabolic reprogramming in cancer, the role of p38 in regulating oxidative metabolism in normal muscle, liver, and brown adipose tissue is well established. Namely, PGC1α, the master regulator of mitochondrial biogenesis, is a transcriptional target of p38 through the activation of myocyte enhancer factor 2 (MEF2) and activating transcription factor 2 (ATF2; refs. 35, 36). Moreover PGC1α is directly phosphorylated by p38 at different residues (37), and these modifications modulate its stability, increasing PGC1α half-life and activity (38, 39). Because PGC1α is upregulated in quiescent, resistant tumor cells and is responsible for the maintenance of oxidative metabolism in this context (23, 24, 16), it would be extremely interesting to understand whether p38 MAPK signaling is the unifying element sustaining mitochondrial respiration and quiescence in treatment-resistant cancer cells.

Mitochondrial respiration as a new target for cancer therapy

Tumors are abnormal tissues made up of heterogeneous cells endowed with different functions, varying proliferative potentials, and in diverse states of differentiation, making it no surprise that they are heterogeneous at the metabolic level as well. Even if tumors appear primarily glycolytic due to the prevalence of cells bearing glycolytic features, other metabolic programs may coexist and can become evident after perturbations such as pharmacologic treatment. In light of the abundance of experimental
evidence from different models and different tumor types, it is reasonable to assume that a common metabolic program active in a small subset of quiescent/slow-cycling tumorigenic cells may exist across different tumor types (see Fig. 1). This program, relying more on mitochondrial respiration than on glycolysis, would functionally respond to the lower anabolic needs of dormant cells. On the contrary, in actively proliferating cancer cells, both mitochondria and glycolysis would be required to continuously supply the high-energy molecules and intermediate metabolites required to sustain and facilitate the biosynthetic pathways that enable proliferation. The hypothesis that a critical subpopulation of cells responsible for tumor maintenance, metastasis, and tumor relapse upon treatment shares the same metabolic program in different tumors is extremely appealing, as it may present new vulnerabilities that can be exploited to eradicate cancer (16, 40, 41). Obviously, additional studies are needed to corroborate previous findings and to identify more specific and less toxic inhibitors of mitochondrial respiration.

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References


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Tumors and Mitochondrial Respiration: A Neglected Connection

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