

# Mitochondrial Uncoupling and the Warburg Effect: Molecular Basis for the Reprogramming of Cancer Cell Metabolism

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## Abstract

**The precise mitochondrial alterations that underlie the increased dependence of cancer cells on aerobic glycolysis for energy generation have remained a mystery. Recent evidence suggests that mitochondrial uncoupling—the abrogation of ATP synthesis in response to mitochondrial membrane potential—promotes the Warburg effect in leukemia cells, and may contribute to chemoresistance. Intriguingly, leukemia cells cultured on bone marrow–derived stromal feeder layers are more resistant to chemotherapy, increase the expression of uncoupling protein 2, and decrease the entry of pyruvate into the Krebs cycle—without compromising the consumption of oxygen, suggesting a shift to the oxidation of nonglucose carbon sources to maintain mitochondrial integrity and function. Because fatty acid oxidation has been linked to chemoresistance and mitochondrial uncoupling, it is tempting to speculate that Warburg’s observations may indeed be the result of the preferential oxidation of fatty acids by cancer cell mitochondria. Therefore, targeting fatty acid oxidation or anaplerotic pathways that support fatty acid oxidation may provide additional therapeutic tools for the treatment of hematopoietic malignancies.** [Cancer Res 2009;69(6):2163–6]

## The Warburg Effect and Mitochondrial Uncoupling

More than half a century ago, Otto Warburg (1) proposed that cancer cells originated from non-neoplastic cells acquired a permanent respiratory defect that bypassed the Pasteur effect, i.e., the inhibition of fermentation by oxygen. This hypothesis was based on results of extensive characterization of the fermentation and oxygen consumption capacity of normal and malignant tissues—including mouse ascites and Earle’s cells of different malignancies but same genetic origin—that conclusively showed a higher ratio of fermentation to respiration in the neoplastic cells. Moreover, the data indicated that the more malignant Earle’s cancer cells displayed a higher ratio of fermentation to respiration than their less malignant counterparts, suggesting to Warburg and his colleagues that a gradual and cumulative decrease in mitochondrial activity was associated with malignant transformation. Interestingly, the precise nature of these gradual and cumulative changes has eluded investigators for nearly 80 years, albeit Warburg’s observations of an increased rate of aerobic

glycolysis in cancer cells have been reproduced countless times—not to mention the wealth of positron emission tomography images that support an increased uptake of radiolabeled glucose in tumor tissues.

It is noteworthy that although Warburg’s hypothesis remains a topic of discussion among cancer biologists, Otto Warburg himself had alluded to an alternative hypothesis put forth by Feodor Lynen—one which did not necessitate permanent or transmissible alterations to the oxidative capacity of mitochondria—that suggested the possibility that the increased dependence of cancer cells on glycolysis stemmed not from their inability to reduce oxygen, but rather from their inability to synthesize ATP in response to the mitochondrial proton gradient ( $\Delta\Psi$ M; ref. 1). Lynen’s hypothesis was partly based on his work (2) and the previous work of Ronzoni and Ehrenfest (3) using the prototypical protonophore 2,4-dinitrophenol, which causes a “short circuit” in the electrochemical gradient that abolishes the mitochondrial synthesis of ATP, and decreases the entry of pyruvate into the Krebs cycle. Subsequent work showed that mitochondrial uncoupling (i.e., the abrogation of ATP synthesis in response to  $\Delta\Psi$ M) results in a metabolic shift to the use of nonglucose carbon sources to maintain mitochondrial function (4, 5). Given the elusiveness of permanent transmissible alterations to the oxidative capacity of cancer cells that could broadly support Warburg’s hypothesis, could Lynen’s hypothesis better explain the dependence of cancer cells on glycolysis for ATP generation?

Over the past several decades, it has become increasingly clear that mitochondrial uncoupling occurs under physiologic conditions, such as during cold acclimation in mammals, and is mediated, at least in part, by uncoupling proteins (UCP; ref. 6, 7). UCP1 was the first UCP identified, and was shown to play a role in energy dissipation as heat in mammalian brown fat (6). During cold acclimation, UCP1 accumulates in the inner mitochondrial membrane and short circuits  $\Delta\Psi$ M (created by the mitochondrial respiratory chain) by sustaining an inducible proton conductance (7). Other UCPs have been identified in humans (UCP2-4), although their functions may be unrelated to the maintenance of core body temperature, and instead involved in the reprogramming of metabolic pathways. For instance, recent work shows that UCP2 is necessary for efficient oxidation of glutamine (8), and may promote the metabolic shift from glucose oxidation to fatty acid oxidation (4). Likewise, UCP3 has also been shown to promote fatty acid oxidation in muscle tissue via, in part, an increased flux of fatty acid anions (9); however, such as for UCP2, the nature of its proton conductance remains controversial (reviewed in ref. 10). More interesting, perhaps, are recent observations that UCP2 is overexpressed in various chemoresistant cancer cell lines and primary human colon cancer, and that overexpression of this UCP leads to an increased apoptotic threshold (11), suggesting that in addition to metabolic reprogramming, UCPs may *ipso facto* provide a prosurvival advantage to malignant cells.

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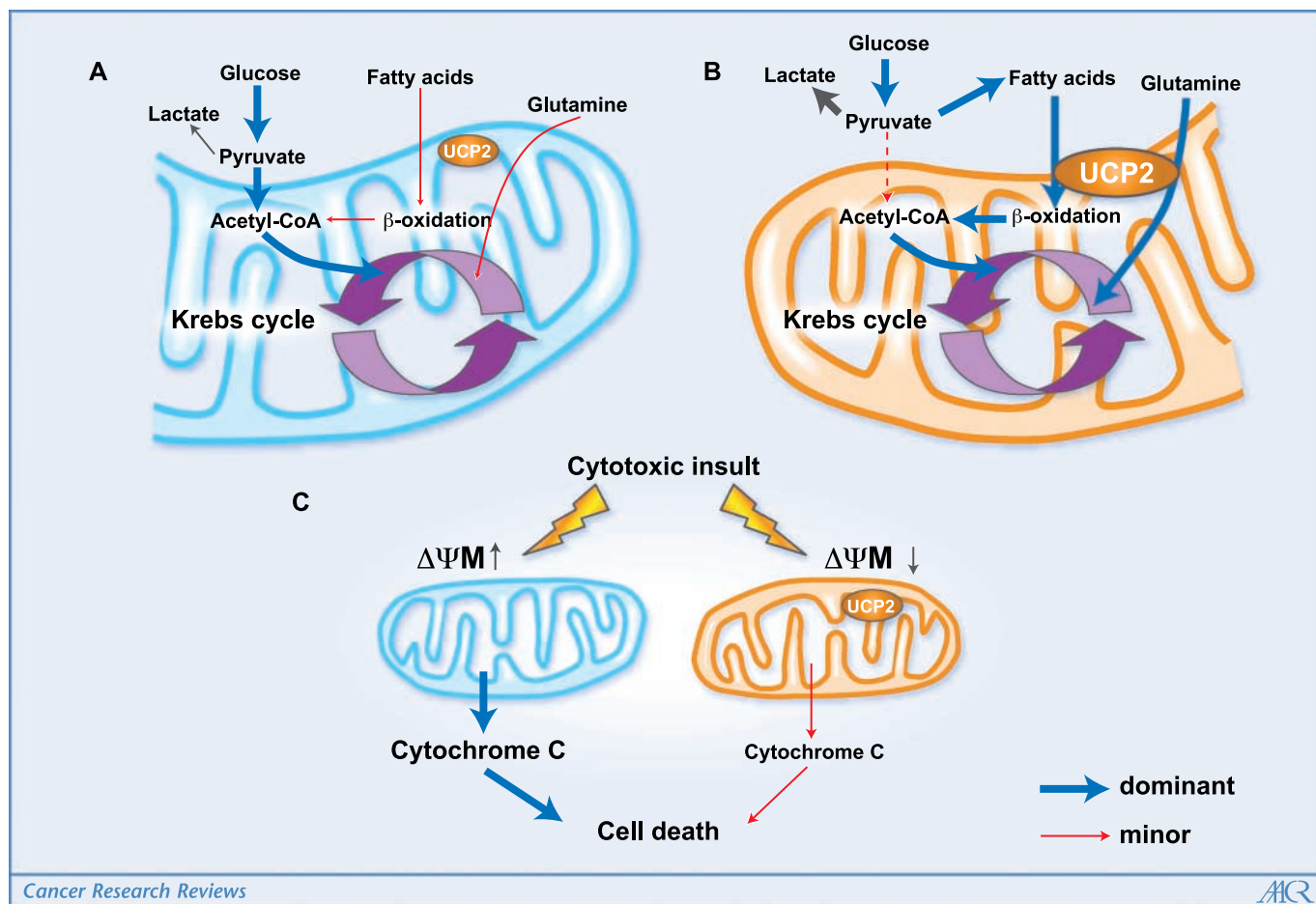
It is important to point out that physiologic fatty acid oxidation has been shown to be associated with an uncoupling and/or thermogenic phenotype in various cell types (reviewed in ref. 12). In addition, it is also significant that glycolysis-derived pyruvate, as well as  $\alpha$ -ketoglutarate derived from glutaminolysis, may be necessary to provide anaplerotic substrates (i.e., those that replenish intermediates in metabolic cycles) for efficient Krebs cycle use of fatty acid-derived acetyl CoA (13), suggesting the possibility that in certain cell types, high rates of aerobic glycolysis may be necessary for efficient mitochondrial oxidation of fatty acids (“fats burn in the fire of carbohydrates”). The above support the concept—and indirectly, Lynen’s hypothesis—that the Warburg effect may, in fact, be the result of fatty acid and/or glutamine oxidation in favor of pyruvate use.

### Mitochondrial Uncoupling in Leukemia Cells

We have recently reported that leukemia cells cultured on bone marrow-derived mesenchymal stromal cells (MSC) show increased aerobic glycolysis and reduced  $\Delta\Psi$  (14). *A priori* we hypothesized that MSC decreased mitochondrial function in leukemia cells; however, our experiments revealed that the oxygen consumption capacity of leukemia cells was not affected and, in fact, displayed a

transient (~6–8 h) increase after exposure to MSC. In addition, leukemia cells cultured on MSC were less sensitive to the  $\Delta\Psi$ -dissipating effects of oligomycin and, as previously reported (15, 16), more resistant to apoptosis induced by a variety of chemotherapeutic agents, suggesting that leukemia cells cultured on MSC feeder layers were displaying a prosurvival mitochondrial metabolic shift, rather than a compromised mitochondrial function. Additionally, it was observed that in contrast to hypoxia (~6% oxygen), which markedly increased the uptake of glucose, and a fluorescent glucose derivative from the medium, MSC feeder layers did not increase the uptake of glucose in leukemia cells, further supporting the notion that the increased accumulation of lactate in the medium of MSC-leukemia cocultures is indicative of reduced entry of pyruvate into the Krebs cycle of leukemia cells.

Because the above observations supported the possibility that MSC may induce mitochondrial uncoupling in leukemia cells, we investigated whether MSC feeder layers were modulating the expression of UCPs (UCP1–4). We observed that leukemia cells only expressed UCP2 and that MSC induced pronounced accumulation of this UCP. Surprisingly, siRNA silencing of UCP2 expression did not completely overcome the dissipation of  $\Delta\Psi$  induced by MSC, albeit decreased expression of this protein markedly decreased the



**Figure 1.** Mitochondrial uncoupling mediates the metabolic shift to aerobic glycolysis in cancer cells. *A*, coupled mitochondria (blue) oxidize pyruvate through the Krebs cycle. *B*, uncoupled mitochondria (orange) display a metabolic shift to the oxidation of other carbon sources, supported, in part, by fatty acid and glutamine metabolism that may depend on UCP2 expression. *C*, uncoupled mitochondria are more resistant to cytotoxic insults and oppose the activation of the intrinsic apoptotic pathway.

accumulation of lactate in the medium of MSC-leukemia cocultures. Moreover, although leukemia cells rapidly lost  $\Delta\Psi\text{M}$  when exposed to MSC feeder layers (~30 minutes), maximal expression of UCP2 did not occur until 24 to 48 hours after coculture, and conversely, the rapid dissipation of  $\Delta\Psi\text{M}$  was insensitive to inhibition of protein synthesis with cycloheximide. Taken together, the above results suggest that although UCP2 expression may contribute to the observed loss of  $\Delta\Psi\text{M}$ , it is likely that other factor(s) may initiate the dissipation of the electrochemical gradient; however, the data reported support the notion that UCP2 is indeed involved in metabolic reprogramming away from the oxidation of pyruvate, a phenomenon that may, in turn, facilitate the maintenance of a reduced  $\Delta\Psi\text{M}$ .

Our data using the protonophore CCCP also supported the notion that, at least in leukemia cells, dissipation of the proton gradient *per se* opposed the onset of apoptosis. Likewise, MSC feeder layers protected OCI-AML3 cells from apoptosis, but not the growth inhibitory effects of mitoxantrone, AraC, and vincristine. It is noteworthy that leukemia cells that did not increase the expression of UCP2 when cultured with MSC feeder layers did not increase lactate generation, did not dissipate  $\Delta\Psi\text{M}$ , and were not protected from the cytotoxic effects of chemotherapy when cultured with MSC, suggesting that the observed metabolic reprogramming in OCI-AML3 cells is associated with chemoresistance. It is thus provoking to speculate that targeting UCP2, as well as the metabolic reprogramming involved in initiating and maintaining the dissipation of  $\Delta\Psi\text{M}$  (increased glutamine and/or fatty acid metabolism, etc.), could be exploited therapeutically to overcome microenvironment-induced chemoresistance.

## Implications of Mitochondrial Uncoupling

The metabolic shift from the oxidation pyruvate to the uncoupled oxidation of glutamine or fatty acids highlights two critical concepts. First, glycolysis remains the critical pathway by which cancer cells meet their energy demands, not because of permanent transmissible alterations to the oxidative capacity of cells, but rather because of the inability of uncoupled mitochondria to generate ATP. Second, the continued reduction of oxygen, in the absence of pyruvate oxidation, suggests that anaplerotic reactions from nonglucose carbon skeletons must be replenishing critical intermediates from the Krebs cycle—reactions that may be amenable to therapeutic intervention, and that may critically depend on highly conserved UCPs—to in turn support the oxidation of fatty acids or glutamine (Fig. 1). Curiously, anaplerotic reactions have recently been reported to support the activity of the Krebs cycle in glioma cells (17), which use most of their glutamine carbon skeletons to regenerate  $\alpha$ -ketoglutarate, while at the same time using glucose carbon skeletons to synthesize fatty acids. Moreover, the required NADPH (the biosynthetic reducing

equivalent) for fatty acid synthesis was provided by conversion of glutamate-derived malate to pyruvate and, to a lesser extent, from the activity of the pentose phosphate shunt, further highlighting the importance of glutamine metabolism via the Krebs cycle (17). In the above study, it was evident that the metabolism of glucose was largely anaerobic, although the cells maintained the ability to consume oxygen, as well as an active Krebs cycle, suggesting the possibility that mitochondrial uncoupling and UCPs may promote the observed metabolic pattern.

Notably, a recent report showed that the entry of pyruvate into the Krebs cycle, via pyruvate dehydrogenase, is suppressed in cancer cells, and that the reactivation of pyruvate dehydrogenase activity by dichloroacetate induced cell death in several solid tumor cell lines and xenografts (18), supporting the notion that mitochondrial glucose oxidation may be incompatible with cancer cell survival. Likewise, it is interesting that pharmacologic inhibition of fatty acid oxidation has been shown to potentiate apoptosis induced by a variety of chemotherapeutics in cancer cell lines (19), as well as palmitate-induced apoptosis in hematopoietic cells (20), suggesting *a priori* that the metabolism of fatty acids in the mitochondria may be linked to cell survival. In light of the above, it is intriguing to propose that targeting the mitochondrial metabolism of fatty acids and/or glutamine may hold therapeutic promise for the treatment of human malignancies. Conversely, given the important role of UCPs in the metabolic shift associated with increased fatty acid and glutamine metabolism in favor of glucose oxidation, it would be of great interest to develop therapeutic strategies that targeted these proteins.

In conclusion, recent investigations into the mechanisms that underlie the Warburg effect suggest that (a) mitochondrial uncoupling can promote aerobic glycolysis in the absence of permanent and transmissible alterations to the oxidative capacity of cells, (b) aerobic glycolysis may represent a shift to the oxidative metabolism of nonglucose carbon sources, and (c) mitochondrial uncoupling may be associated with increased resistance to chemotherapeutic insults. The above suggest the importance of understanding the mechanisms of mitochondrial uncoupling and their relation to metabolic alterations observed in cancer cells.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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