

Oxidative metabolism in cancer growth

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Purpose of review

Recent evidence suggests that oxidative metabolism may have a key role in controlling cancer growth. This review will provide an overview of the evidence accumulated so far. More than 80 years ago, Otto Warburg suggested that impaired oxidative metabolism may cause malignant growth. This assumption, later known as Warburg's hypothesis, has been experimentally addressed for many decades. It employs multiple approaches including cell lines, implanted xenografts and other animal models, by biochemical methods to quantify glycolytic and mitochondrial fluxes and signaling pathways including the rates of intermediate metabolism, respiration and oxidative phosphorylation.

Recent findings

The hallmarks of cancer growth, increased glycolysis and lactate production in tumors, have raised attention recently due to novel observations suggesting a wide spectrum of oxidative phosphorylation deficits and decreased availability of ATP associated with malignancies and tumor cell expansion. The most recent findings suggest that forcing cancer cells into mitochondrial metabolism efficiently suppresses cancer growth, and that impaired mitochondrial respiration may even have a role in metastatic processes.

Summary

This review summarizes published evidence on the essential interaction of tumor growth and mitochondrial metabolism, implicating novel approaches for the prevention and treatment of malignant disease.

Keywords

cancer, metabolism, mitochondria, oxidative phosphorylation

Introduction

Cancer is a major cause of premature death. Incidence of malignant disorders increases with age, whereas metabolic activity and resting energy expenditure resembling oxidative metabolism of humans decreases with age. Hence, an inverse relationship between mitochondrial capacity and induction as well as promotion of tumor growth in mammals may exist and even be of etiological importance. To investigate whether this apparent link is based on scientific evidence rather than being a mere coincidence, remarkable efforts have been undertaken in the past to elucidate this particular connection. The following review aims to summarize published evidence on mechanistic interdependencies between cancer biology and mitochondrial metabolism.

Oxidative versus nonoxidative metabolism

Nowadays, glucose represents a key component of the daily diet, and the standard nutritive sugar, sucrose, contains 50% of glucose and 50% of fructose. Glucose is actively transported into mammalian cells, and it is, as well as fructose, converted into pyruvate under normal physiological conditions. The latter is either converted into lactate precluding further immediate breakdown, or metabolized and decomposed by mitochondria (see below). Enzymatic breakdown of glucose into pyruvate is called glycolysis. Glycolysis takes place in the cytosol of eukaryotic cells, and may occur in the presence as well as the absence of oxygen; for this reason, many prokaryotes and lower eukaryotes including *Saccharomyces cerevisiae* survive perfectly in the absence of oxygen by performing anaerobic glycolysis only [1]. Higher eukaryotes, especially mammals, require a persistent supply of oxygen. Glycolysis in either state provides comparably little energy equivalents per mol of glucose, nevertheless anaerobic glycolysis in *S. cerevisiae* is sufficient to maintain viability for long periods of time. Since higher organisms tend to use nutritive energy equivalents more efficiently than lower organisms [2], mammals and their evolutionary precursors have obtained a significantly more efficient way to generate readily available energy equivalents than glycolysis: by oxidation of the ultimate metabolite of glycolysis, pyruvate, an approximately 14-fold amount of readily available energy equivalents are obtained. Hence the main advantage of glycolysis is the independency from oxygen availability, while the main disadvantage is a comparably low yield of readily available energy equivalents [3].

Curr Opin Clin Nutr Metab Care 9:339–345. © 2006 Lippincott Williams & Wilkins.

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Current Opinion in Clinical Nutrition and Metabolic Care 2006, 9:339–345

Abbreviations

mtDNA mitochondrial DNA
OXPPOS oxidative phosphorylation
Fe/S iron–sulfur cluster

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Mitochondria

Historically, mitochondria were mainly considered important for the generation of organic intermediates to integrate metabolism of macronutrients, namely carbohydrates, fatty acids, and amino acids. Conversion of amino acids into carbohydrate precursors, carbohydrate derivatives (i.e. pyruvate) into fatty acids, and others are integrated by intermediate steps of the Krebs cycle. In parallel, reducing equivalents derived from the Krebs cycle, namely nicotinamide adenine dinucleotide phosphate (NADH) and FADH₂, are fueled into the respiratory chain where they become oxidized by an oxygen-dependent process called respiration. Oxidation means loss of electrons – these are passed on within the respiratory chain to generate an electrochemical gradient across the inner mitochondrial membrane; this proton gradient is named mitochondrial membrane potential. The proton gradient drives generation of ATP from ADP and inorganic phosphate ions, a process called oxidative phosphorylation (OXPHOS). While intermediate metabolism and OXPHOS were thought to be the primary roles of mitochondria for several decades, recent findings suggest an additional function: *Giardia lamblia*, an ancient protist, has been shown to carry mitochondrial remnant organelles which are unable to provide ATP by OXPHOS, but rather provide iron–sulfur (Fe/S) clusters [4]. These are essential components of several mitochondrial enzyme complexes, including aconitase (the enzyme converting citrate into isocitrate), and complexes I, II and III of the respiratory chain [5^{**}]. Briefly, Fe/S clusters contain non-heme iron which can both accept and donate electrons due to the variable redox state of iron [6]. Taken together, mitochondria are responsible for both the production of Fe/S clusters and the interconversion of primary energy substrates into each other, as well as into readily available energy equivalents, namely ATP. The main advantage of oxidative metabolism is the comparably high energy yield (i.e. moles of ATP per mol of glucose), while the main disadvantage is the evident dependency on cellular respiration, and hence availability of oxygen [3].

Warburg's hypothesis

The biochemist Otto Warburg hypothesized in 1924 that cancers may be caused by increased glycolysis and impaired respiration [7–10]. This primary assumption was based on his observations that tumor tissue specimens exhibit a comparably low respiratory rate, especially in comparison to adjacent healthy, that is nonmalignant, tissues. After becoming the Nobel-laureate for medicine and physiology in 1931 (for a different area of interest) [11], his cancer hypothesis obtained significant attention amongst scientists worldwide, and initiated endless controversies on the particular topic between supporters and opponents [12–16]. Warburg died in 1970, his hypothesis still being unconfirmed and becoming almost forgotten

due to the discovery of oncogenes, tumor suppressor genes, and other advances of tumor biology research.

Mitochondrial structure and morphology within cancer tissues

After both respiration and OXPHOS had been localized to the mitochondria, high-resolution microscopy-based techniques emerged to study the morphology of these organelles in cancer tissues and tumor cell lines. To briefly summarize a wide body of evidence, mitochondria from rapidly growing tumors are generally smaller and show less *cristae* than mitochondria from well differentiated tumors or normal tissues [17]. In his extensive review, Pedersen [17] further emphasized that tumors have a significantly reduced number of mitochondria. As predicted earlier [18,19], recent studies suggest increased prevalence of mutations of mitochondrial DNA (mtDNA) in various human cancers [20,21] (Fig. 1A). Recently, these findings have, at least in part, been questioned due to proposed methodological problems [22]. Nevertheless, functional studies by depletion-replenishment studies of mutated mtDNA clearly indicate that there is functional relevance of mutated mtDNA in proliferative processes [23^{*}].

In a series of publications, Cuezva and colleagues [24] determined expression levels of OXPHOS enzymes, most importantly the β -F1 subunit of ATP(synth)ase in human tumor samples (Fig. 1B). The authors showed that expression levels and distribution patterns of such enzymes are downregulated in cancer specimens of very different origin, including colon, esophagus, kidney, liver, mammary gland, and stomach [24,25,26^{*}]. These findings are complemented by earlier observations describing a repression of ATP(synth)ase activity in rodent models of liver carcinogenesis [27] (Fig. 1B). Furthermore, Racker and colleagues [28,29] have repeatedly focussed on the role of ATP(synth)ase in the control of cancer growth (Fig. 1B). Recent unbiased gene expression profiling in leukaemias also suggests differential regulation of ATP(synth)ases [30] (Fig. 1B).

Metabolic activity of cancers

While the metabolic alterations observed by Warburg were confirmed by the majority of scientists studying this particular problem, numerous mechanisms were proposed to cause increased glycolysis and impaired mitochondrial activity in cancer tissues.

Promotion of glucose transport in cancer

Metabolism requires uptake of macronutrients, specifically glucose. Since glucose has to be actively transported through the plasma membrane, early studies have focused on the potential induction of glucose transport (Fig. 1C). Following transformation with Fujinami sarcoma virus, increased glucose transport was detected [31].

c-Myc has been shown to increase lactate production and lactate dehydrogenase activity in cultured cells, resembling the glycolytic phenotype typically found in cancers. Lastly, the oncogene Akt (also termed protein kinase B (PKB)) has been shown to induce glycolysis in cultured cells [37] (Fig. 1E); in parallel, Akt/PKB activity has been associated with de-novo synthesis of fatty acids [38] (Fig. 1F), a process likely to be associated with promotion of tumor growth (see below). Additional studies have found roles for phosphofructo-2-kinase (PFK-2) [39–41] (Fig. 1G), hypoxia [42–44] (Fig. 1H), and VHL protein deficient in von Hippel-Lindau disease [45,46,47**] in metabolic control of cancer growth.

Altered activity of mitochondrial shuttles and transporters

Since most ions and metabolites are unable to cross the mitochondrial membranes passively, significant attention has been directed towards possible alterations of active transport processes through the mitochondrial membranes. Increased uptake of Ca^{2+} , but impaired release of the signaling ion, have been observed in numerous tumor cells [48,49] (Fig. 1I). Transport capacities for inorganic phosphate, succinate, and citrate were mainly found to be unchanged, while transport capacities for malate and glutamate were found to be decreased [50,51] (Fig. 1K). Import of ADP was found to be increased [52], while ATP export from mitochondria decreased [53,54].

Lastly, aberrant pathways to convert reduced nicotinamide derivatives from glycolysis into a mitochondrially available form, specifically by oxidation of malate into pyruvate employing malic enzyme, have been described to be upregulated in cancer cells [55,56] (Fig. 1K).

Altered mitochondrial metabolism in cancer

Since tumors exhibit an increased rate of glycolysis, researchers have addressed the question of whether tumor mitochondria may have lost the ability to metabolize substrates other than carbohydrate derivatives, that is pyruvate. Weinhouse and colleagues [57–59] observed a decreased rate of fatty acid utilization especially in highly malignant tumors, while well differentiated tumors retained the ability to metabolize fatty acids (Fig. 1L). Specifically, they showed that activity of the key activating enzyme of β -oxidation, acyl thiokinase, is reduced by up to 85% in malignant tumors [59]. Accordingly, amino acid metabolism has been studied in malignant cells. Early reports suggest that the branched-chain amino acids valine and isoleucine are preferentially metabolized by tumor cells [60] (Fig. 1M). Since valine and isoleucine generate succinate before entering the Krebs cycle, these findings suggest that tumor cells exhibit alterations of upstream citrate metabolism resembling a truncated Krebs cycle (see below). Metabolism of glutamine and glutamate appears to be intact in tumor mitochondria [61]

(Fig. 1N). Intriguingly, recent findings suggest that the mitochondrial activity of tumor cells can be restored by offering glutamine as the only fuel source [62].

For many years tumor cells have been found to carry mitochondria containing increased amounts of membrane cholesterol [17,63] (Fig. 1O). Subsequently it was found that tumor mitochondria export comparably large amounts of citrate [64,65], an essential precursor of cholesterol biosynthesis. The reasons for this observation remained unclear; nevertheless, impaired activity of mitochondrial aconitase, an enzyme carrying Fe/S clusters and converting citrate into isocitrate, would be sufficient to cause such truncation of Krebs cycle activity (Fig. 1P). Aconitase is the enzyme most severely affected by aging [66*] and has been found to be reduced in tumor cells [67,68] (Fig. 1P). Of interest, citrate is also required for de-novo synthesis of fatty acids found to be increased in subsets of cancers [69–71] (Fig. 1F). Recent findings suggest that suppression of this synthesis pathway may inhibit cancer growth [72**]. Whether the anticarcinogenic effects of some nuclear receptors and coactivators may be linked to induction of mitochondrial biogenesis remains to be evaluated [73–76].

As stated above, Fe/S clusters are essential components of redox-active enzymes within the Krebs cycle (aconitase) (Fig. 1P) and within the respiratory chain (complexes I, II and III) (Fig. 1Q) [5**]. Mitochondrial synthesis of Fe/S is controlled by a mitochondrial matrix protein called frataxin [77] (Fig. 1R). Humans with decreased expression of this protein develop a neurodegenerative disease named Friedreich's ataxia [78], accompanied by fatal cardiomyopathy, diabetes mellitus, and occasionally cancers atypical for their young age [79]. Frataxin controls efficiency of OXPHOS [80,81]. Targeted disruption of frataxin causes impaired Fe/S enzyme activity and decreased OXPHOS, leading to tumor formation in mice [82*]. Whether disruption of frataxin causes a truncated Krebs cycle as found in cancers (see above) remains to be determined.

Other OXPHOS-related enzymes have been implicated in induction and promotion of tumors. Reduced expression of succinate dehydrogenase (SDH), a multifunctional enzyme conglomerate employed in the Krebs cycle as well as complex II of the electron transfer chain (Fig. 1S), has been linked to paraganglioma and pheochromocytoma, both tumors of the nervous system [83–86]. More recently, accumulation of succinate due to SDH insufficiency has been mechanistically linked to hypoxia inducible factor-1 α (HIF1 α) translocation [87*–89*]. Other mitochondrial enzymes linked to tumor growth are complex I deficiencies [90,91] (Fig. 1T), as well as fumarate hydratase deficiencies [92,93] (Fig. 1U).

Dietary restriction and cancer

It has been known for many decades that dietary restriction, i.e. reduced uptake of metabolizable calories from macronutrients, may not only prolong life span [94], but also efficiently inhibit cancer growth in mammals [95], an effect which can potentially be mimicked by blocking glucose metabolism [96*]. Most researchers in this particular field believe that decreasing calorie uptake will decrease mitochondrial respiration, and concomitantly suppress generation of reactive oxygen species (ROS) [97,98]. The latter are believed to induce oxidative damage of proteins and DNA, including mtDNA, phenomena which have been linked to induction of cancer and acceleration of aging [99]. Contrary to this mainstream hypothesis, Lin and colleagues [100] recently showed that, at least in the eukaryote *S. cerevisiae*, caloric restriction induces respiration, that is mitochondrial activity. Published evidence as summarized above strongly suggests that cancer cells show a specific impairment of mitochondrial activity. Future research will have to show whether the anticarcinogenic effects of caloric restriction and the global impairment of mitochondrial function in cancer merely coincide.

Induction of mitochondrial metabolism inhibits cancer growth

The mitochondrial matrix protein frataxin (see above) has been shown to be a useful tool to manipulate mitochondrial metabolism [80,101] (Fig. 1R). Overexpression of this protein in nontransformed cells causes increased mitochondrial metabolism and OXPHOS, while lactate production is decreased, altogether suggesting a preferential activation of mitochondrial metabolism [80]. Based on these findings, frataxin was overexpressed in various colon carcinoma cell lines. Such cells have increased oxidative metabolism, shown by concurrent increases in aconitase activity, mitochondrial membrane potential, cellular respiration, and ATP content. Consistent with Warburg's hypothesis, frataxin-overexpressing cells also have decreased growth rates and population doubling times, show inhibited colony formation capacity in soft-agar assays, and exhibit a reduced capacity of tumor formation when injected into nude mice [102**]. Taken together, these results support the view that an increase in oxidative metabolism induced by mitochondrial frataxin may inhibit cancer growth in mammals. Additionally, recent findings suggest that re-instating normal OXPHOS in mitochondrially impaired cancer cells may not only inhibit cell growth and proliferation, but also impair metastatic capacity of malignant cells [103*].

Conclusion

For many decades, an observational association between cancer growth and oxidative metabolism has been described. More recently, several pathways and mech-

anisms linking these autistic entities of interests have been proposed. On the one hand, it appears that oncogenic transformation and mitochondrial perturbation of cancer cells are not mutually exclusive but rather synergistic or even functionally linked [104**]. On the other hand, forcing cancer cells back into mitochondrial metabolism efficiently inhibits cancer growth, and suppression of glycolysis may exert its tumor-inhibiting activity by this alternative and more efficient mechanism for ATP generation that seems to be readily functional in normal tissues but obviously impaired to a certain extent in cancer cells.

Acknowledgements

The author thanks Dr Marc Birringer for the electronic version of Fig. 1, and Dr Lutz Schomburg for essential corrections of the text.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 510).

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