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Review

Out of Warburg effect: An effective cancer treatment targeting the tumor specific metabolism and dysregulated pH

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ABSTRACT

As stated by Otto Warburg nearly a century ago, cancer is a metabolic disease, a fermentation caused by malfunctioning mitochondria, resulting in increased anabolism and decreased catabolism. Treatment should, therefore, aim at restoring the energy yield. To decrease anabolism, glucose uptake should be reduced (ketogenic diet). To increase catabolism, the oxidative phosphorylation should be restored. Treatment with a combination of α-lipoic acid and hydroxycitrate has been shown to be effective in multiple animal models. This treatment, in combination with conventional chemotherapy, has yielded extremely encouraging results in glioblastoma, brain metastasis and lung cancer. Randomized trials are necessary to confirm these preliminary data. The major limitation is the fact that the combination of α-lipoic acid and hydroxycitrate can only be effective if the mitochondria are still present and/or functional. That may not be the case in the most aggressive tumors. The increased intracellular alkalosis is a strong mitogenic signal, which bypasses most inhibitory signals. Concomitant correction of this alkalosis may be a very effective treatment in case of mitochondrial failure.

Contents

1. Introduction

In the early 1920's Otto Warburg demonstrated a unique feature of cancer cells, namely an increased uptake of glucose and secretion of lactic acid by cancer cells, even in the presence of oxygen (e.g. the aerobic glycolytic phenotype) [1,2]. This aerobic fermentation is the signature of cancer [3]. Warburg also noticed a concomitant decreased number of mitochondria (grana) [4]. In normal, differentiated cells, the yield of a molecule of glucose is 34 ATP. ATP is derived mostly from oxidative phosphorylation which takes place in the mitochondria [5,6]. In the absence of mitochondria the energy yield drops to two molecules of ATP per molecule of glucose [5,6]. As stated by Warburg in the 1920's, in cancer cells there is a decreased efficacy of the mitochondria resulting in lesser yield. Despite increased glucose uptake, there is a 50% drop in ATP level in human colon cancer cells compared to adjacent benign cells

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[7]. This decrease in ATP is a consequence of impairment of the oxidative phosphorylation [6–9].

To compensate for the decreased energy yield, the cell increases its glucose uptake [7,10]. The decreased activity of the mitochondria has many consequences, one of which is an increased secretion of lactic acid and another one is the activation of the pentose phosphate pathway (PPP). Another consequence is the activation of the glutaminolysis which is necessary for nucleic acid synthesis [6–9].

The activation of the Pentose Phosphate Pathway results from an increase in glucose uptake with a concomitant obstacle downstream of the pentose phosphate shunt, most probably at the level of pyruvate dehydrogenase and/or of pyruvate kinase [2,6,11]. The increased flux in the pentose phosphate pathway results in:

- A shift toward anabolism due to increased synthesis of NADPH that plays a crucial role in NDPH/$NADP^+$ ratio that determines the redox state of the cell via removal of reactive oxygen species (ROS) and so prevents cellular death and controls cellular fate [7,11].
- The shift toward the pentose pathway also results in the production of ribose-5-phosphate, required for the synthesis of nucleic acids [5].

One other crucial consequences of the mitochondrial defect is intracellular alkalosis [7]. Tumors show a 'reversed' pH gradient with a constitutively increased intracellular pH that is higher than the extracellular pH. This gradient enables cancer progression by promoting proliferation, the evasion of apoptosis, metabolic adaptation, migration, and invasion [12–15].

There is evidence that an acidic extracellular pH promotes invasiveness and metastatic behavior in several tumor models [14,16], proteolytic enzyme activation and matrix destruction [17–19].

In normal cells, the intracellular pH (pHi) oscillates during the cell cycle between 6.8 and 7.3 [7]. The oscillation of the pH during the cell cycle matches the value of the decompaction of the histones, RNA polymerase activation, DNA polymerase activation and DNA compaction before mitosis [7,11].

The intracellular pH of the cancer cells has been less studied. During the cell cycle, it oscillates between 7.2 and 7.5. Intracellular alkalosis is probably a consequence of the decreased oxidative phosphorylation resulting in decreased secretion of carbon dioxide ($CO_{2)}$ and the CO_2 reacts with water to create carbonic acid. Cell transformation or enhanced cancer cell division and resistance to chemotherapy are all associated with a more alkaline pHi [20–23].

The very reason of the dysfunction of the mitochondria is still open for debate. No tumor cell has yet been found with a normal content or composition of cardiolipin, the signature lipid of the inner mitochondrial membrane. This lipid regulates the efficiency of OxPhos [9,10].

The Warburg effect may be a direct consequence of the activation of oncogenes [6]. Infection by an oncogenic virus or exposure to a carcinogen inhibits the mitochondrial function and causes the Warburg's effect [24–28].

As Warburg wrote in 1956 [4,29], "The chicken Rous sarcoma virus, which is labeled today as a virus tumor, ferments glucose, and lives as a partial anaerobe like all tumors." Infection by an oncogenic virus or exposure to a carcinogen inhibits the mitochondrial function and causes the Warburg's effect [24–28].

Alternatively, the Warburg effect results in oncogene activation and mutation. Oncogene up-regulation is needed to drive fermentation after OxPhos dysfunction. Mitochondria replacement will activate OxPhos and turn off oncogenes [9,10].

This Warburg's effect is responsible for the activation of the Pentose phosphate pathway, glutaminolysis and subsequent anabolism [9] and all thirty different oncogenes target and stimulate the anabolic pathways [9].

The introduction of normal mitochondria into cancer cells restores mitochondrial function, inhibits cancer cell growth and reverses chemoresistance [30,31]. Also the fusion of cancer cells with normal mitochondria results in increased ATP synthesis, oxygen consumption and respiratory chain activities together with marked decreases in cancer growth, resistance to anti-cancer drugs, invasion, colony formation in soft agar, and « in vivo » tumor growth in nude mice [31].

1.1. Reversing the Warburg effect

Cytotoxic chemotherapy has had tremendous benefits for pediatric or Hodgkin's patients. However, for most solid tumors, while there is a sizable response rate (complete and partial regression), there has been a limited gain in survival.

The mechanism of action of cytotoxic chemotherapy is debated. Response to cytotoxic drugs is assessed by PET scan. Effective treatment results in decreased radiolabelled glucose. Most cytotoxic drugs target (indirectly) the mitochondria and the Warburg effect [32–35].

As the Warburg aerobic glycolytic phenotype and its effects on metabolism are key to cancer, the obvious question is whether drugs can be designed to target it. To alleviate the Warburg effect, pyruvate should be converted into Acetyl-CoA, which would decrease the bottleneck that results in the activation of both the Pentose Phosphate Pathway and the glutaminolysis. The mitochondrial yield should be increased to stimulate the synthesis of CO_2 and the **i**ncreased secretion of CO_2 would result in a decreased intracellular alkalosis.

The combination of α-lipoïc acid and hydroxicitrate [36–39] has been reported to slow cancer growth, in murine xenografts. This inhibition appears to be independent of the primary tumor site and has been reproduced in different laboratories [40,41].

The most likely mechanism of action for α-lipoic acid in its reduction of tumor growth is the inhibition of pyruvate dehydrogenase kinase (the same target of Dichloroacetic acid (DCA)). This enzyme inhibits the activity of pyruvate dehydrogenase and is known to be up-regulated in cancer cells expressing the Warburg aerobic glycolytic phenotype. Pyruvate dehydrogenase catalyses the conversion of pyruvate to acetyl-CoA, the initial step of the final conversion of glucose to carbon dioxide and water in the TCA cycle, with the concomitant production of ATP. Therefore, it is reasonable to suggest that blocking the activity of pyruvate dehydrogenase kinase will at least partially restore the activity of pyruvate dehydrogenase, thereby increasing the flux of pyruvate through the TCA cycle in the mitochondria, while simultaneously reducing the production of lactic acid and most importantly decreasing the flux in the pentose pathway shunt [9].

There are several reports of metabolic treatment utilizing a combination of α-lipoic acid and hydroxycitrate together with conventional cancer therapy. Starting in January 2013, metabolic treatment (α-lipoic acid/hydroxycitrate with low doses of the chemotherapic plus Naltrexone) was offered to patients sent home after the failure of conventional cytotoxic chemotherapy for metastatic cancer (irrespective of the primary site) but with a Karnovsky performance status above 70 (quantification cancer patients' general well-being and activities of daily life) [42–46]. Of the first randomly selected eleven patients, five were alive and reasonably well 30 months after the start of treatment [43]. From our experience, the combination of α-lipoic acid/hydroxycitrate with low dose Naltrexone was able to prevent tumor recurrence in only two patients. The patients who survived for more than one year were treated with a combination of standard care with metabolic treatment.

In the update of a subsequent study, patients with multiple brain metastasis (n = 4) or glioblastoma (n = 6) were treated with

a combination of conventional and metabolic treatments (α-lipoic acid/hydroxycitrate) as well as ketogenic diet. Five out of six patients with glioblastoma were alive and stable after two years, while two of the four patients with multiple brain metastases are alive and well three years later [46]. These glioblastoma patients had concomitant radiation therapy and chemotherapy (temozolamide).

In another study, four lung patients (primary adenocarcinoma) were treated with a combination of the small molecule EGFR inhibitor, Gefitinib, and metabolic treatment (α-lipoic acid/hydroxycitrate). They all responded to treatment and are stable one year after the start of therapy. These very encouraging results need to be confirmed by further randomized clinical trials.

1.2. Correcting the intracellular alkalosis

Correcting the intracellular pH may be an alternative or an adjunct to a metabolic treatment as there is extensive literature that many effective cancer treatments decrease the intracellular pH (pHi) [2,23,47]: literature on increased survival support for the combined use of antacids (which prevent proton extrusion from the tumour cells) with standard chemotherapy [15,48–51]. Hyperthermia [50] decreases the intracellular pH.

There are two possibilities to decrease the intracellular pH (pHi). The first is a calorie restricted ketogenic diet [8], which will reduce the availability of glucose, the principal metabolite for glycolysis and the PPP. This diet will result in increased levels of acidic ketone bodies that cannot be metabolized by the cancer cells (while normal cells are able to do so) and this probably results in a decrease of the intracellular pH. Similarly, it has been proposed that an acid diet in general might lead to a supply of lactate (exogenous lactate) that might lead to regression of the tumour growth [51].

The rapid turnover of aberrantly dividing cancer cells within the tumor mass, sugar fermentation and increased ATP hydrolysis, all lead to the production and release of large amounts of protons into the extracellular compartment [1,52]. The H^+ accumulation, in turn, leads to the progressive setting of a highly hostile microenvironment, mostly characterized by low pH. The acidic microenvironment produces a sort of "selective pressure," that, in fact, favors the cells that are best adapted to survive in these hostile conditions. The hostility of the microenvironment is increased by hypoxia and low nutrient supply, making it virtually impossible for "normal" or more differentiated cells to survive in these unsuitable conditions. To thrive in such an unfavorable microenvironment, tumor cells must develop systems to actively extrude excess protons [48,52–55]. These mechanisms include the V-ATPase, the Na^+/H^+ exchanger (NHE), monocarboxylate transporters (MCTs) and carbonic anhydrase 9 [52]. Of course, we must consider these proton exchangers as key "survival options" for cancer cells, and, therefore, depriving cancer cells of their function should inevitably lead to a rapid cell death due to internal acidification, as it has been shown for different cancer cell types in pre-clinical settings [56,57]. Unfortunately, it is very hard to convince clinical oncologists to use this approach in a first line treatment of cancer patients. However, it was possible to increase the proof of principle that at least proton pump inhibitors may be included in new anti-cancer protocols together with the standard treatments. Clinical evidence has been provided in human tumor patients with either osteosarcoma [58], breast cancer [59] or GI cancers [60]. Further evidence has been accumulated in clinical trials have been performed in domestic animals with spontaneous tumors of different histotypes, with very encouraging results [61,62]. Altogether, these pre-clinical and clinical results suggest that a way to counteract intracellular alkalinity might be to inhibit the above mechanisms designed to avoid intracellular acidification.

2. Conclusion

Today, cancer is thought to be a set of very complex diseases with thousands of different mutations. That apparent complexity has led to personalized medicine. However, modern biology has confirmed the universality of the Warburg aerobic glycolytic phenotype. Furthermore, the fact that the combination of α-lipoïc acid and hydroxycitrate slows down cancer growth in every tumor model studied to date suggests that at least some targets are the same in a large spectrum of tumors.

The Warburg effect can be caused by two intertwined phenomena. The first is a metabolic bottleneck, which can be corrected. The second is the destruction/disappearance of the mitochondria.

Cytotoxic drugs injure or even destroy the mitochondria [32–35]. At the time of failure of chemotherapy, there is a sharply increased glucose uptake such as seen on PET scan [63]. Resistance to chemotherapy has been correlated with mitochondrial functionality (perhaps oxidative phosphorylation) [64] and alkaline pHi [1,23]. The oxidative phosphorylation is further reduced; the pH is more alkaline, and the PPP is activated. Cancer grows relentlessly, and further chemotherapy is usually ineffective [9].

Further studies should assess the role of low dose chemotherapy together with a combination of metabolic treatment and treatments to lower the intracellular pH.

Conflict of interest statement

The authors declare that they have no competing interests.

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