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MINI REVIEW

Oxygen metabolism and a potential role for cytochrome c oxidase in the Warburg effect

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Abstract By manipulating the physical properties of oxygen, cells are able to harvest the large thermodynamic potential of oxidation to provide a substantial fraction of the energy necessary for cellular processes. The enzyme largely responsible for this oxygen manipulation is cytochrome c oxidase, which resides at the inner mitochondrial membrane. For unknown reasons, cancer cells do not maximally utilize this process, but instead rely more on an anaerobic-like metabolism demonstrating the so-called Warburg effect. As the enzyme at the crossroads of oxidative metabolism, cytochrome c oxidase might be expected to play a role in this so-called Warburg effect. Through protein assay methods and metabolic studies with radiolabeled glucose, alterations associated with cancer and cytochrome c oxidase subunit levels are explored. The implications of these findings for cancer research are discussed briefly.

Keywords Cancer · Cytochrome c oxidase · Metabolism · Oxygen · Warburg

Review

Few facets of nature are as elegant as respiration. While poets have long spoken of the “spark of life,” it was Lavoisier who initially described chemical similarities between oxidative respiration and combustion of carbon compounds (Holmes 1985). Although aerobes and fire both consume organic molecules and oxygen yielding carbon dioxide and water, the two processes are fundamentally different.

Examination of how the two processes handle the quantum mechanical properties of oxygen is illustrative of these differences. Ground state oxygen molecules contain two unpaired electrons yielding a quantum mechanically described triplet state. In contradistinction, most organic molecules have no unpaired electrons and consequently have a singlet ground state. Triplet and singlet state molecules resist chemical interaction with each other, just as a spinning top resists tipping because angular momentum must be conserved. In aerobic respiration as well as in combustion, this “spin forbidden” interaction between singlet and triplet state molecules must be circumvented. Combustion occurs when the energy levels of both the organic molecule and oxygen have been raised sufficiently to yield compatible spin states. The respiratory process, by contrast, alters the ground state of oxygen through binding with transition metal-containing enzyme active sites. This metal bound oxygen then accepts protons and electrons that have been stripped from organic molecules. The process is taxed to produce ATP, the cellular energy currency (Herrmann 1996). At the heart of oxidative respiration lies cytochrome c oxidase. In addition to a startling ability to harvest the large thermodynamic potential of oxygen reduction, catalysis by cytochrome c oxidase is even more surprising for not releasing any but trace quantities of toxic intermediates such as peroxide and superoxide. Only recently have the central mechanics of this enzyme from the inner mitochondrial membrane begun to be elucidated through a variety of spectroscopic experiments and biomimetic models (Collman et al. 1994, 1997, 1998, 2004, 2007; Tsukihara et al. 1996).

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The biosynthesis of cytochrome c oxidase is as marvelous as its catalytic function. Some of the cytochrome c oxidase subunits are expressed from nuclear genes while others are encoded within the mitochondrial genome (Hey et al. 1997; Hofmann et al. 1998; Lee et al. 1998). Within all cytochrome c oxidase-containing organisms, the core subunits responsible for the catalysis of oxygen reduction to water are expressions of the mitochondrial genome while the peripheral subunits, hypothesized by some to control the rate of the reaction, are expressed from genes of the nuclear genome (Yanamara et al. 1988; Vijayasarathy et al. 1998; Kadenbach et al. 2000). (Fig. 1a)

Even in the twenty-first century, Otto Warburg still looms large over the field of metabolism. In addition to first proposing an iron containing compound at the heart of oxidative metabolism (Warburg 1925), he recognized that tumor cells are physiologically different from non-tumor cells, a phenomenon known as the "Warburg Effect" (Warburg et al. 1924; Warburg and Kubowitz 1927; Warburg and Negelein 1928; Warburg 1929, 1930, 1956). While many mammalian cells can reversibly switch from anaerobic lactic acid production in a low oxygen environment, to aerobic metabolism when immersed in high oxygen environments, cancer cells differ by not utilizing oxygen when given the opportunity. Even in the presence of abundant oxygen, cancer cells continue a high anaerobic-like metabolism (Warburg 1956; Pedersen 1978; Nakashima et al. 1984). Why cancer cells seemingly avoid maximally utilizing their own cytochrome c oxidase remains one of nature's great enigmas.

Although Warburg long ago had demonstrated that cancer cells are metabolically different from normal cells (Warburg et al. 1924; Warburg and Kubowitz 1927; Warburg and Negelein 1928; Warburg 1929), and control steps for the altered metabolism of cancer cells have been demonstrated to lie within the glycolytic pathway (Mathupala et al. 1997; Chesney et al. 1999; Perrin et al. 2002; Mathupala et al. 2006), very little work has been performed to evaluate differences within the protein components of the oxidative metabolic machinery of cancer cells. Due to its central role in aerobic metabolism, cytochrome c oxidase could be expected to be altered or variably controlled in cancerous relative to normal cells (Krieg et al. 2004a).

As an experimental starting point, the relative amounts of intracellular cytochrome c oxidase subunits produced from the nuclear and mitochondrial genomes were measured. Evaluation of the ratio of nuclear-encoded to mitochondrial-encoded cytochrome c oxidase subunit quantities provides a glimpse of the holoenzyme's synthesis and consequently the potential for isoform alteration. Immunohistochemical studies of whole mounted prostatic tissue quickly revealed a difference in the staining intensity ratio of nuclear-encoded to mitochondrial-encoded cytochrome c oxidase subunits in cancerous tissue relative to normal prostate glandular epithelium. To quantitate these observed differences in cytochrome c oxidase subunit levels, micro-dissection of normal and cancerous glandular epithelium was followed by Western blotting and reverse phase protein micro-array analysis. The results demonstrated an increased ratio of nuclear-encoded to mitochondrial-encoded cytochrome c oxidase subunits, corroborating the immunohistochemical observations. In addition, the quantitation (when normalized to a variety of intracellular benchmarks) further demonstrated that the effect was predominantly due to an increase in nuclear encoded subunits in the cancer cells with relatively constant levels of mitochondrial encoded subunit quantities observed in normal and cancerous cells. The same trends in altered cytochrome c oxidase subunit levels were observed in certain nonprostatic carcinomas including those of breast, esophagus, gastrointestinal tract, and uterus (Herrmann et al. 2003).

Although the subunit levels were different in normal and cancerous prostatic tissue, it still was not clear that these findings were associated with alterations in metabolic function. Consequently, studies were undertaken to evaluate for a possible association between subunit level variation and anaerobic cancer-associated metabolism. Since such studies require live respiring cells, appropriate established cell lines were chosen. Three urothelial derived cell lines were compared. One of these cell lines had been derived from normal epithelium through immortalization, another from low-grade urothelial carcinoma, and an additional one from high-grade urothelial carcinoma. In addition, a unique set of prostate derived cell lines, both of which originated within the same surgically resected prostate gland, were obtained. One of these prostate derived cell lines had been generated from the organ's cancerous cells and the other from the surrounding normal glandular epithelium (Krieg et al. 2004a).

Carbon dioxide (CO_2) produced by these respiring cells during incubation experiments in the presence of strategically radiolabeled glucose was trapped with the use of a specially designed portable gas capture device (Krieg et al. 2004b). The captured radiolabeled CO_2 was then quantitated. Analysis of the ratio of the $^{14}CO_2$ produced from 1-[^{14}C]-glucose to the $^{14}CO_2$ produced from 6-[^{14}C]-glucose permitted an estimation of the ratio of cellular utilization of the glycolytic pathway relative to that of the hexose monophosphate (HMP) shunt in the respiring cell cultures. The relative utilization of these two pathways has been correlated with the level of anaerobic relative to aerobic metabolism (Krieg et al. 2004a). (Fig. 1b–d)

The same cytochrome c oxidase subunit ratio trends observed with dissected cancer tissue were observed between the normal derived and tumor derived cell lines with increasing ratios of nuclear to mitochondrial encoded

subunit levels correlating with decreased levels of cancer differentiation. In addition, the tumor derived cell lines demonstrated increased use of the HMP shunt relative to the normal derived cell lines with the more poorly differentiated cell lines showing greater relative flux through the HMP shunt. These findings demonstrate correlated trends of increased anaerobic metabolism and increased nuclear to mitochondrial encoded cytochrome c oxidase subunit ratios with decreasing degrees of cancer differentiation (Krieg et al. 2004a; Herrmann et al. 2003). (Fig. 1)

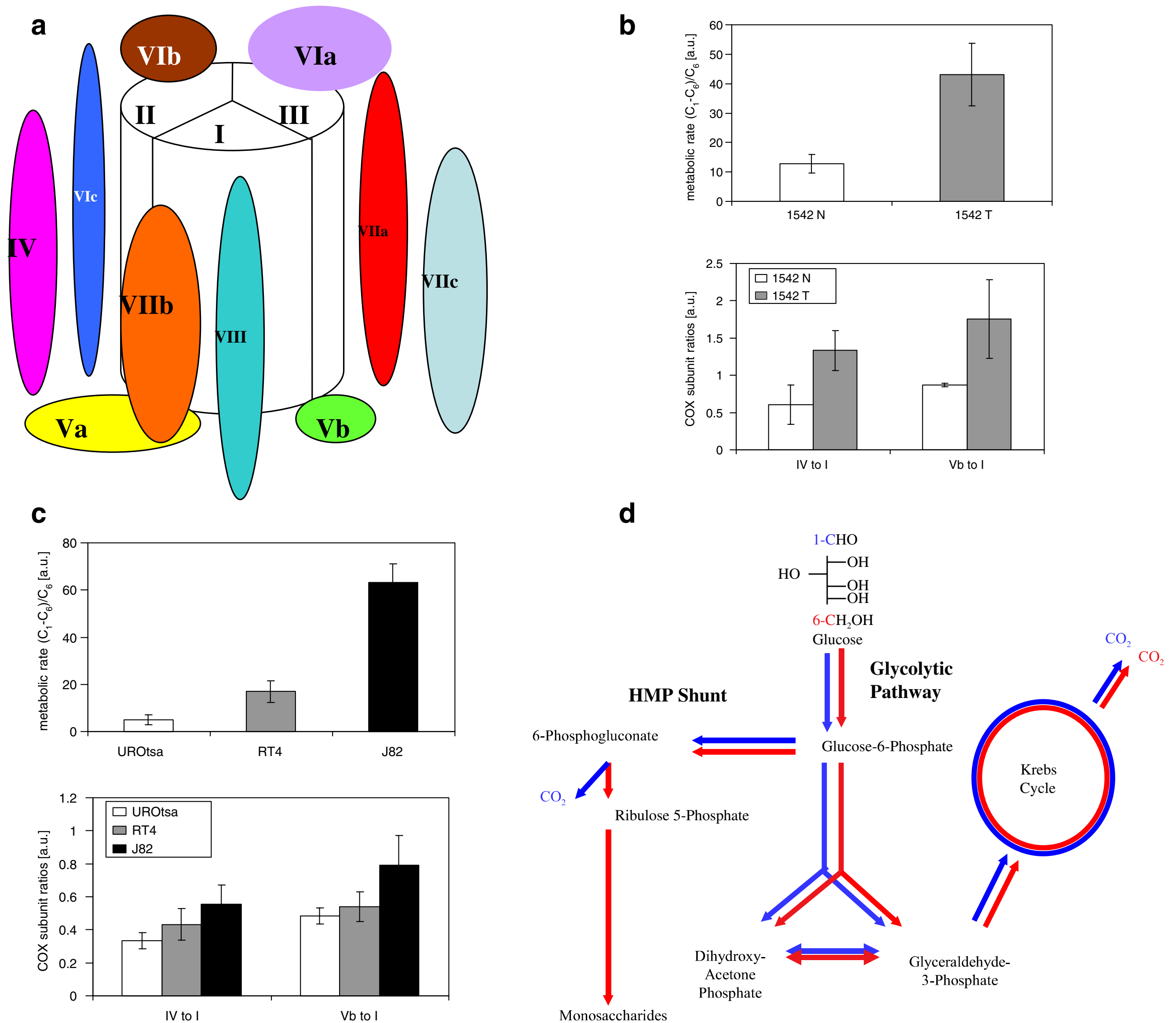

Fig. 1 Glucose metabolism and cytochrome c oxidase subunit ratios. **a** cartoon depicting the 13 individual subunits comprising human cytochrome c oxidase. The core subunits (I, II, III) represented in *white* are entirely encoded in the mitochondrial genome and compose the catalytic core of the enzyme. The remaining subunits (IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, VIII) represented by *various colors* are encoded in the nuclear genome. The upper graph in **b** shows the ratio of hexose monophosphate shunt derived metabolism to glycolysis passing through the Krebs cycle. The lower graph depicts cytochrome c oxidase subunit ratios for the cell lines evaluated. Data for the normal prostate derived cell line 1542N are represented by the *white bars*, while data for the prostatic tumor derived cell line 1542T are depicted by the *gray bars*. The upper graph in **c** shows the ratio of hexose monophosphate shunt derived metabolism to glycolysis passing through the Krebs cycle. The lower graph depicts cytochrome c oxidase subunit ratios for the cell lines evaluated. Data for the normal urothelium derived UROtsa cell line are represented by the *white bars*. Data for the well differentiated urothelial carcinoma derived cell line RT4 are depicted by the *gray bars* while data for the poorly differentiated urothelial carcinoma derived cell line J82 are represented by the *black bars*. All ratios are unitless. The basic metabolic pathways for glucose are broadly sketched in **d**. The metabolic pathway for carbon 1 of glucose is highlighted in blue while the metabolic pathway for carbon 6 of glucose is highlighted in *red*. The figure demonstrates that carbon dioxide released from carbon 1 of glucose is produced from the Krebs cycle as well as from the hexose monophosphate shunt, while carbon dioxide released from carbon 6 of glucose is only produced via the Krebs cycle. *HMP* denotes hexose monophosphate shunt (Herrmann et al. 2003; Krieg et al. 2004a)

From such studies, it is clear that cancer cell metabolism differs from normal cellular metabolism in association with differences in the concentrations of protein components of the aerobic metabolic machinery. Stepping back to take a more all-encompassing look at the organism reveals that cancer cells must exploit the organism's noncancer cells for survival (Tisdale 1997). By metabolizing glucose normally destined for noncancer cells to lactate, the cancer cell robs the organism's normal cells twice. First, cancer cells starve the normal cells by voraciously gobbling glucose at an ever-increasing rate and consequently decrease the total available glucose within the circulation. To add insult to injury, the cancer cells then force the noncancer cells, according to their characteristic enzyme composition, to either utilize the increased circulating lactic acid or regenerate it back to glucose through gluconeogenesis, an energetically costly process. Such parasite-like behavior has been hypothesized to cause the cachexia often observed in late stage cancer patients (Bongaerts et al. 2006). An altered metabolic process so tightly associated with both cancer cell function as well as the natural history of the disease should be exploitable therapeutically as well as diagnostically, a point not missed by Otto Warburg (Warburg 1956; Ko et al. 2004).

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