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Combination therapy targeting cancer metabolism

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Abstract

Cancer cells undergo significant metabolic adaptation. Cellular transformation enhances both glycolysis and mitochondrial respiration efficiency through the induction of HIF-1 α and HIF-2 α . In this process, energy production and synthesis of macromolecules are maximized with minimal ROS accumulation. Furthermore, a series of antioxidant enzymes are induced to mitigate the damaging effects of ROS. Examination of these metabolic changes provides rationale for a synergistic approach to combination anti-cancer therapy –targeted inhibition of HIF and inhibition of cellular defenses against oxidative stress.

Keywords

HIF-1 α ; HIF-2 α ; glycolysis; mitochondrial metabolism; oxidative stress

Metabolic adaptation: A balance between aerobic glycolysis and mitochondrial respiration in tumor cells

Cellular transformation relies on molecular signals that promote cell proliferation, obstruct cell death, inhibit cellular differentiation, and induce angiogenesis. Underlying these cellular processes, transformed cells also undergo significant metabolic adaptation. This has led to the suggestion that altered metabolism should be considered the “seventh” acquired function of the cancer phenotype; with the other six being self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, sustained angiogenesis, tissue invasion and metastasis, and limitless replicative potential (1, 2).

Before proceeding with a discussion of metabolic shifts in cancer metabolism, we will briefly review the biochemical components of glycolysis and respiration (Figure 1A) (3, 4). Glucose is transported into the cytoplasm by glucose transporters (GLUT); and, while in the cytosol, glucose is metabolized to pyruvate by a series of glycolytic enzymes. Glycolysis

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serves three major functions. First, glycolysis generates 2 ATP via substrate-level phosphorylation; and, provides 2 NADH as reductant for subsequent redox reactions. Second, glycolysis produces a variety of carbon intermediates, which may be removed at various steps to serve as substrates for anabolic pathways. Third, glycolysis produces 2 pyruvate molecules, which follow either one of two pathways, dependent on the presence or absence of oxygen: anaerobic respiration, or aerobic respiration through the coupling of the tricarboxylic acid (TCA) cycle and oxidative phosphorylation.

In anaerobic respiration, pyruvate is metabolized to lactate with the regeneration of NAD^+ for the continuation of glycolysis. In aerobic respiration, pyruvate enters the mitochondrial matrix, where it undergoes oxidative decarboxylation to acetyl-CoA. Acetyl-CoA then enters the TCA cycle, where it is dehydrogenated by a series of enzymes to generate NADH and succinate, which in turn, provide reductants that drive the electron transport chain and oxidative phosphorylation. During oxidative phosphorylation, electrons are transferred from electron donors to acceptors within the inner mitochondrial membrane. The energy from electron transfer is tightly coupled to the transfer of protons from the mitochondrial matrix into the intermembrane space to generate a proton gradient. Oxidative phosphorylation is powered by the electron transport chain, which consists of 4 complexes, I-IV. Redox reactions in complexes I and III contribute to the proton-motive force through the transfer of protons from the matrix to the intermembrane space. In complex IV (cytochrome c oxidase), electrons are transferred from reduced cytochrome c to the final electron acceptor oxygen to form water, while additional protons are pumped across the inner mitochondrial membrane. The final stage of oxidative phosphorylation involves the F_1F_0 ATP synthase (complex V). The F_1F_0 ATP synthase complex allows protons to flow down the proton-gradient, from the mitochondrial intermembrane space into the matrix; thus, providing the driving force for the generation of ATP from ADP. Oxidative phosphorylation is efficient in energy production, netting 38 ATP per molecule of glucose metabolized.

Although aerobic respiration is efficient in ATP production, disturbances in redox balance within the electron transport chain may lead to the generation of reactive oxygen species (ROS) that promote cellular damage (5, 6). Mitochondrial ROS generation is thought to occur at complexes I and III, where electrons may be transferred directly to oxygen, forming superoxide (Figure 1A). Superoxide is then converted by superoxide dismutases into hydrogen peroxide. These ROS species can cause cell death or growth arrest, as they oxidize proteins, lipids, and DNA. Complex IV is usually highly efficient at reducing oxygen to water. Inhibition of complex IV results in a shift in redox balance within the electron transport chain. The subsequent inefficiency of mitochondrial electron transport leads to the production of ROS through increased reduction of oxygen to superoxide at complexes I and III.

Although less energy efficient, cancer cells activate glycolysis and suppress mitochondrial respiration even in the presence of oxygen; hence, referred to as aerobic glycolysis, or the Warburg effect, as first described by Dr. Otto Warburg in the 1920's (7). It should be emphasized that the Warburg effect does not mean complete shutdown of mitochondrial respiration. In fact, in a majority of cancer cells, energy derived from aerobic glycolysis does not exceed 50% of total cellular production; and, the remaining is derived from mitochondrial respiration (8).

There are several advantages for cancer cells to utilize both aerobic glycolysis and mitochondrial respiration (1, 9-12). First, cancer cells would maximize energy production. Glycolysis allows rapid breakdown of glucose for ATP production about ~100 times faster than the metabolic flux through the mitochondria. For dividing cells with virtually unlimited supply of glucose, energy production can actually be increased through the induction of

glycolysis. Any residual pyruvate, not converted to lactate, can be channeled to the mitochondria for more efficient energy production. Second, some ROS production from mitochondrial metabolism may stimulate cancer cell growth. To prevent excessive ROS accumulation, cancer cells induce the expression of antioxidant enzymes. Third, both glycolytic and TCA cycle intermediates are required for anabolic pathways for the synthesis of macromolecules. For example, the glycolytic intermediate glucose-6-phosphate is shuttled to the pentose phosphate shunt to provide ribose sugars for the synthesis of nucleotides (13). The TCA cycle intermediate citrate is transported from the mitochondria to the cytosol for lipid synthesis. In addition, oxaloacetate and α -ketoglutarate are converted to non-essential amino acids for the synthesis of proteins and nucleic acids (12).

HIF-1 α and HIF-2 α coordinately regulate glucose metabolism in cancer cells

The hypoxia-inducible factors-1 α and -2 α (HIF-1 α and HIF-2 α) are transcription factors that are overexpressed in cancer and linked to cancer progression and poor prognosis (14, 15). Structurally, HIF-1 α and HIF-2 α are partially related, sharing 48% amino acid homology and two identical proline residues in their oxygen-dependent degradation domains (16, 17). HIF-1 α and HIF-2 α dimerize with HIF-1 β to form HIF-1 and HIF-2, respectively.

HIF-1 α and HIF-2 α overexpression are driven by intratumoral hypoxia and genetic mutations in oncogenes and tumor suppressor genes (18-20). Under normoxia, HIF-1 α and HIF-2 α are ubiquitinated through an oxygen-dependent interaction with the von Hippel-Lindau protein (pVHL) and degraded by the 26S proteasome (21-23). However, hypoxia decreases the activity of prolyl hydroxylases (PHDs) that hydroxylate specific proline residues in the oxygen-dependent degradation domain (ODD) of HIF-1 α and HIF-2 α leading to protein stability (24). HIF-1 α and HIF-2 α then accumulate, translocate to the nucleus, dimerize with HIF-1 β , and transactivate target genes. In cancer, growth factor signaling and genetic alterations in tumor suppressor genes and oncogenes also induce HIF-1 α and HIF-2 α overexpression under normoxic conditions, and lead to the transactivation of target genes.

Both HIF-1 α and HIF-2 α induce the expression of target genes important in tumor angiogenesis, cell growth/survival, and metastasis. To date, regulation of cancer glucose metabolism has been predominantly linked to HIF-1 α rather than HIF-2 α (25). HIF-1 α induces the expression of glucose transporters, glycolytic enzymes, and LDHA that promote glucose uptake, glycolysis, and lactate formation (11, 26). This has been well demonstrated under hypoxic conditions, and more recently under normoxic conditions (27, 28). HIF-1 α was also recently shown to induce the expression of PDK1 (pyruvate dehydrogenase kinase 1) under hypoxic conditions (29, 30). PDK1 phosphorylates and inhibits pyruvate dehydrogenase (PDH), an enzyme that catalyzes the conversion of pyruvate to acetyl-CoA. This leads to the suppression of pyruvate entry into the TCA cycle, with consequent suppression of mitochondrial oxygen consumption (29, 30). More recently, both HIF-1 α and HIF-2 α were shown to regulate the exchange of COX IV (cytochrome *c* oxidase 4) subunits under hypoxic conditions, thereby increasing mitochondrial respiratory efficiency and decreasing ROS production (31). These findings implicate HIF-1 α and HIF-2 α in balancing glycolysis and aerobic respiration to maintain ATP production and prevent toxic ROS generation. It remains to be determined if the exchange of COX IV subunits plays a role in cancer.

To summarize, activation of HIF-1 α and HIF-2 α in cancer cells induces glucose uptake, glycolytic metabolism, and enhances the efficiency of aerobic respiration. The advantages for cancer cells would be to utilize glycolysis and mitochondrial respiration for maximal

energy production, stimulation of cell growth, and generation of intermediates for the synthesis of macromolecules.

Oncogenic induction of both glycolysis and mitochondrial respiration in cancer

Oncogenes, such as c-myc and ras, have been shown to play prominent roles in the activation of glycolytic enzymes. C-myc is a key contributor to normal structure/function of mitochondria, in addition to its stimulatory effect on nuclear-encoded mitochondrial genes and mitochondrial mass. Zhang et al demonstrated the impact of c-myc overexpression on mitochondrial metabolism; c-myc stimulated an increase in mitochondrial DNA content, mitochondrial biogenesis, and oxygen consumption (32). Amplification of the proto-oncogenic c-myc is quite common in lymphomas and may serve as the driving force for energy acquisition through its stimulatory impact on mitochondrial content and glycolysis (33). The function of c-myc on energy production appears to reside in the bulk quantity of mitochondria through increased mitochondrial mass.

HRAS and KRAS have been shown to upregulate glucose transport and activation of glycolysis. Yun et al demonstrated that GLUT1 was substantially overexpressed in colorectal cancer cell lines harboring the oncogenic KRAS mutation; and glycolytic activation coincided with the overexpression of GLUT1 (34). Ramanathan et al further demonstrated that tumor cells with oncogenic HRAS developed a strong dependency on glycolysis for energy acquisition (35).

Targeted inhibition of HIF-1 α and HIF-2 α and inhibition of cellular defenses against oxidative stress would likely be synergistic

Due to their integral roles in regulating metabolism, it is likely that targeted inhibition of HIF-1 α and HIF-2 α would lead to a combination of insults to the mitochondria: decreased glycolysis, excessive metabolic flux through the TCA cycle, and inefficient aerobic respiration (Figure 1B). This would lead to increased ROS generation and decreased ATP production. Given that ROS are highly reactive and short-lived molecules, the maximal effect of ROS is likely in the immediate vicinity of their site of generation. Cardiolipin is a vulnerable target of ROS due to its unsaturated acyl chains and its close proximity to ROS generation sites (36). ROS causes peroxidation of cardiolipin, neutralizing its insulator effect on complexes I-IV and F₁F₀ ATP synthase, which leads to further ROS generation (36). In addition to the deleterious effects of ROS on cardiolipin levels, mitochondrial cytochrome c also plays a prominent role in apoptosis through lipid peroxidation. Kagan et al elucidated the role of cytochrome c in the peroxidase reaction resulting in the oxidation of mitochondria-associated cardiolipin (37). It has been extensively shown that the heme moiety of cytochrome c functions normally as an electron carrier, located within the intermembrane space of the mitochondria. In apoptotic mitochondria, there is a substantial shift of cardiolipin from the inner to the outer leaflet of the inner mitochondrial membrane; therefore, allowing physical interaction between cytochrome c and the negatively charged phospholipid, cardiolipin. It is this direct physical contact between cytochrome c and cardiolipin that converts cytochrome c from an electron carrier to a peroxidase enzyme with a strong affinity for cardiolipin (37). In cancer cells with excessive ROS production, the threshold in reaching irreversible mitochondrial injury and apoptosis due to cardiolipin peroxidation is likely significantly lower (37, 38). Beyond the mitochondria, excessive ROS leakage oxidizes membrane lipids, nucleic acids, proteins, and carbohydrates; causing necrotic cell death (Figure 1B).

To date, high-throughput small compound screens and mechanistic studies have identified several classes of anticancer agents that disrupt HIF-1 function, including inhibition of its transcriptional activity and HIF-1 α protein synthesis or stability (39, 40). Based on partial structural and functional similarities between HIF-1 α and HIF-2 α , some of the already identified HIF-1 inhibitors could also inhibit HIF-2. Many of these agents are currently in clinical trials or clinical use. It would also be of interest to determine whether these agents inhibit glycolysis and induce inefficient mitochondrial respiration in cancer cell lines and cancer in patients.

To mitigate the damaging effects of ROS, a series of antioxidant enzymes are induced in cancer cells including superoxide dismutase, catalase, glutathione peroxidase, and glutathione transferases. The mechanism of induction may result from ROS-mediated dissociation of Keap1-NRF2 complex (41, 42). NRF2 then binds to antioxidant response elements and induces the expression of antioxidant enzymes. Another potential method by which mitochondria combat increased oxidative stress is via expression of PGC-1 α that induces the expression of oxidative stress protective genes, increases the intracellular levels of mitochondrial antioxidant proteins, and reduces the cellular concentrations of reactive oxygen species (43). Superoxide dismutase converts the highly reactive superoxide radical (O₂⁻) to less reactive hydrogen peroxide (H₂O₂) that can ultimately be inactivated by catalase or glutathione peroxidase. In addition, the glutathione transferases also participate in this integrated cellular defense strategy against oxidative stress, by catalyzing the inactivation of peroxidation products through linkage to glutathione (GSH). In cancer cells in which the glutathione transferase -- GSTP1 -- is inhibited, there is increased lipid peroxidation, increased apoptosis, and decreased tumorigenic potential (44, 45). Peroxiredoxins consist of a family of ubiquitously expressed peroxidases that catalyze the peroxide reduction of hydrogen peroxide, organic hydroperoxides, and peroxynitrate (46). Currently, a number of agents inhibiting these antioxidant enzymes are in clinical development (47).

We anticipate that combined with inhibition of HIF-1 α and HIF-2 α , inhibition of cellular defenses against oxidative stress would lead to catastrophic effects on the structure/function of the mitochondria; and, ultimately lead to cellular death. Figure 1C demonstrates the widespread mitochondrial insults induced by increased ROS production and decreased ROS clearance. Increased oxidative stress will denature proteins and lipids resulting in the disruption of the electron transport chain protein complexes, and the matrix-associated enzymes of the TCA cycle. Besides the insults to the organelle's metabolic machinery, ROS will damage the structural integrity of the mitochondria through extensive lipid peroxidation. These oxidative efforts will result in not only the destruction of the mitochondria, but will ultimately lead to cellular death through the release of apoptotic proteins and ROS into the cytosolic compartment. Mitochondrial escape of these degradative factors will result in the peroxidation of cellular macromolecules, leading to increased cell death, and inhibition of tumorigenesis.

The mechanism of efficacy is through a vicious cycle of mitochondria stress/dysfunction→enhanced ROS production→decreased clearance of peroxidation products of oxidative stress→further mitochondria stress/dysfunction→cell death (Figure 1C). We anticipate that this approach would also increase the effectiveness of radiation therapy and chemotherapy, as the anti-tumor effects of these modalities depend partly on the generation of ROS

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Figure 1.

Diagrams showing: **A**, metabolism of glucose in cancer cells with the induction of both glycolysis and efficient mitochondrial respiration. Glucose is transported into the cell and is metabolized to pyruvate. In aerobic respiration, pyruvate enters the mitochondria, where it is converted to acetyl-CoA. Acetyl-CoA combines with oxaloacetate to form citrate and is then catabolized through the TCA cycle. Electrons are donated, through NADH and FADH₂, to the electron transport chain. Electron transfer is coupled to the translocation of protons (H⁺) from the mitochondrial matrix to the intermembrane space to generate a proton gradient. Oxygen (O₂) is the final electron acceptor at complex IV, and in this process is reduced to water (H₂O). The final step of oxidative phosphorylation involves F₁F₀ATP synthase whereby protons flow down the electrochemical gradient to generate ATP from ADP. Early transfer of electrons to oxygen at complexes I and III results in the generation of ROS. **B**, effect of inhibiting HIF-1α and HIF-2α. **C**, effect of inhibiting HIF-1α and HIF-2α and inhibiting cellular antioxidant defenses.