

The Mitochondrial Warburg Effect: A Cancer Enigma

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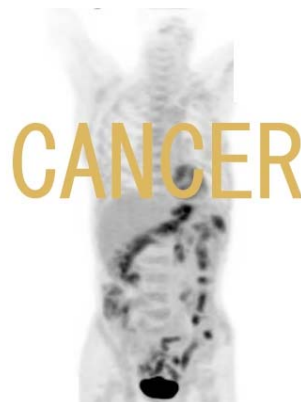
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SYNOPSIS

“To be, or not to be?” This question is not only Hamlet’s agony but also the dilemma of mitochondria in a cancer cell. Cancer cells have a high glycolysis rate even in the presence of oxygen. This feature of cancer cells is known as the Warburg effect, named for the first scientist to observe it, Otto Warburg, who assumed that because of mitochondrial malfunction, cancer cells had to depend on anaerobic glycolysis to generate ATP. It was demonstrated, however, that cancer cells with intact mitochondria also showed evidence of the Warburg effect. Thus, an alternative explanation was proposed: the Warburg effect helps cancer cells harness additional ATP to meet the high energy demand required for their extraordinary growth while providing a basic building block of metabolites for their proliferation. A third view suggests that the Warburg effect is a defense mechanism, protecting cancer cells from the higher than usual oxidative environment in which they survive. Interestingly, the latter view does not conflict with the high-energy production view, as increased glucose metabolism enables cancer cells to produce larger amounts of both antioxidants to fight oxidative stress and ATP and metabolites for growth. The combination of these two different hypotheses may explain the Warburg effect, but critical questions at the mechanistic level remain to be explored. Cancer shows complex and multi-faceted behaviors. Previously, there has been no overall plan or systematic approach to integrate and interpret the complex signaling in cancer cells. A new paradigm of collaboration and a well-designed systemic approach will supply answers to fill the gaps in current cancer knowledge and will accelerate the discovery of the connections behind the Warburg mystery. An integrated understanding of cancer complexity and tumorigenesis is necessary to expand the frontiers of cancer cell biology.



Keywords: Warburg, cancer, tumor, metabolism, glycolysis, mitochondria, dichloroacetate, pyruvate, lactate, pentose phosphate pathway, signaling protein, ATP, oxidative stress, apoptosis, programmed cell death, ROS, mtDNA mutation, mitochondrial proteome, systems biology, database mining

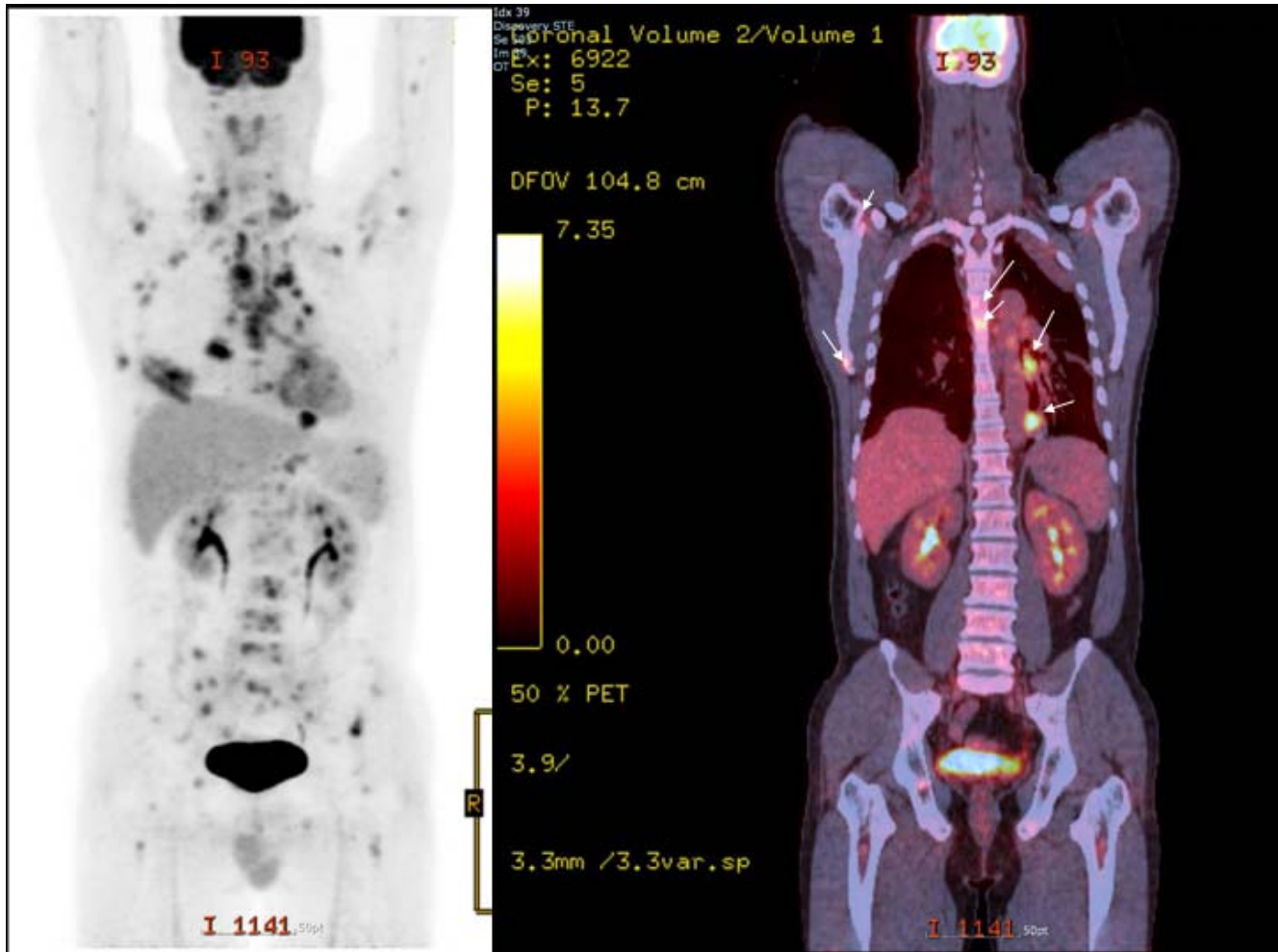


Figure 1. Functional metabolic flux imaging technology may help to detect cancer cells. 2-fluoro-2-deoxy-D-glucose (FDG)-PET/computed tomography in a 45-year-old male diagnosed with lung cancer. This demonstrates a hypermetabolic focuses (small arrows) in coronal PET-computerized tomography images. There are FDG-avid hypermetabolic nodules in the left lower lobe of lung and multiple FDG-avid bone lesions in thoracic, lumbar spines, right scapula, sacrum, and both femurs. Yellow and white colors in the positron emission tomogram indicate a typical high glycolysis rate of the cancer cells.

Answering Warburg's Invitation

More than 70 years ago, Otto Warburg reported that cancer cells exhibited a high glycolysis rate even in the presence of oxygen (Warburg and Negelein, 1924). This feature, known as the Warburg effect, is one of the most important characteristics of cancer cells, along with metastasis, angiogenesis, and endless replication (Kondoh et al., 2007). Thus, the Warburg effect provides a marker for detecting tumor cells. With positron emission tomography using a glucose radioisotope (^{18}F fluorodeoxyglucose), cancer cells can be visualized owing to their significantly higher than normal glucose uptake (Gatenby and Gillies, 2004). As shown in Figure 1, imaging based on a high glycolysis rate clearly demonstrates the importance of understanding the Warburg effect. Currently, there are two different hypotheses to explain the Warburg effect. The first states that the high glycolysis rate is the result of cancer cells' producing more energy and building blocks for proliferation and survival (DeBerardinis et al., 2008). This hypothesis provides an explanation for how cancer cells manage to supply additional energy and metabolites for their intense proliferation. However, no clear evidence has been provided to demonstrate why cancer cells consume more glucose and produce lactic acid via anaerobic glycolysis rather than using the more efficient Krebs cycle. Furthermore, this hypothesis overlooks high oxidative stress as a major metabolic burden in cancer cells. The second hypothesis suggests that a high glycolysis rate helps reduce oxidative stress,

because the product of anaerobic glycolysis, pyruvate, is a scavenger of superoxide. Glucose is also used in the pentose phosphate pathway, resulting in the production of NADPH, a cofactor that reduces free radicals (Spitz et al., 2000). Proponents of this view believe that cancer cells use more glucose to alleviate the high oxidative stress consequent to their aggressive growth.

Energy Supply and Building Blocks for Cancer Growth

Otto Warburg initially hypothesized that cancer cells were more dependent on glycolysis to generate ATP because of defective mitochondrial function, but this Warburg hypothesis was later disproved (Vander Heiden et al., 2009; Gatenby and Gillies, 2004). The question remains, why do cancer cells convert glucose to lactate in the presence of oxygen, even though anaerobic glycolysis produces ATP less efficiently than aerobic respiration via mitochondrial oxidative phosphorylation? It may be related to the surprising fact that although aerobic respiration produces 18 times the ATP per mole of glucose compared with anaerobic glycolysis, the rate of anaerobic glycolysis is 100 times that of aerobic respiration (Bartrons and Caro, 2007). According to a population biology model developed at the Max Delbrück Center for Molecular Medicine in Germany, ATP production at a higher rate but lower yield may confer a selective advantage in competing for shared energy resources (Pfeiffer et al., 2001). Lactate, also a product of

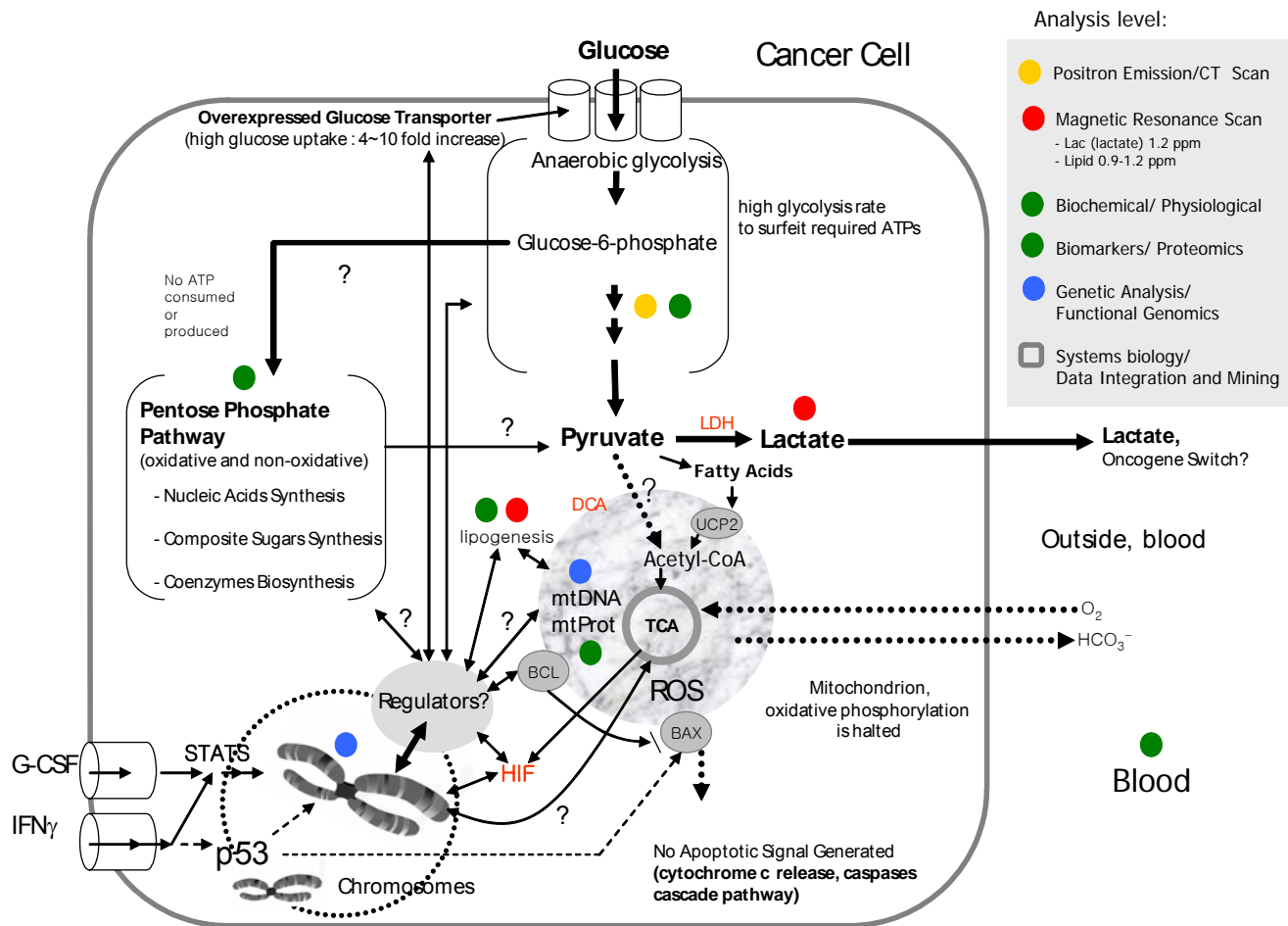


Figure 2. Typical cancer cell metabolism and a rendering of important signaling pathways. The Warburg effect may simply be a consequence of damage to the mitochondria in cancer? The answer is absolutely not. To sustain the rapid and prolonged cell proliferation, cancer cell uses high glycolysis metabolism instead of impaired mitochondrial oxidative phosphorylation. The main role of pentose phosphate pathway (PPP) is to provide a source of NADPH for biosynthetic reactions and to offer pentose phosphate for the synthesis of nucleotides. The production rates of nucleic acids and NADPH are very high and the imbalance on PPP was demonstrated in most cancer cells. Lipogenic enzymes are upregulated or activated in cancer cells, representing increased lipogenesis (Robey et al., 2008). Survival factors such as G-CSF and IFN γ may stimulate signal transducers and activators of transcriptions (STATs), and they finally influence the mitochondrial BCL and BAX proteins which regulate the opening of voltage dependent anion channel (VDAC). Due to cancer cells switch mitochondrial apoptosis program off, the release of cytochrome c from VDAC was stopped. Pyruvate dehydrogenase complex (PDC) is down-regulated by pyruvate dehydrogenase kinase (PDK). Dichloroacetate (DCA) inhibits mitochondrial pyruvate dehydrogenase kinase (PDK) and facilitates transport of pyruvate into mitochondria, turning impaired mitochondria circuits back on (detailed functions are not known).

glycolysis, induces several oncogenes. In addition, lactate surrounds cancer cells, providing an acidic environment that protects cancer cells from the immune system (Gatenby and Gillies, 2004). Many terminal cancer patients experience intense pain, which is often relieved with narcotics. It is suspected that lactic acid produced by cancer cells causes the pain, possibly by attacking nerve cells.

Both *in vitro* and *in vivo* studies have shown that cancer growth can be stopped by drugs that inhibit the pentose phosphate pathway (Boros et al., 1997; Rais et al., 1999). The pentose phosphate pathway uses glucose to produce ribose, which is used to synthesize DNA and RNA. Owing to their rapid growth, cancer cells must continually produce these building blocks for proliferation. The percentage of glucose that is normally used in the pentose phosphate pathway versus the glycolytic pathway is not known, but recent studies provide evidence that the pentose phosphate pathway is closely linked to the abnormal glucose metabolism in cancer cells. A key enzyme of the pentose phosphate pathway, transketolase, was shown to play an important role in cancer proliferation and malignancy (Boros et al., 1997). Among colon and

uroepithelial cancer patients, the expression level of transketolase-like gene 1 (one of the transketolase genes) was strongly related to the patients' survival rate. Autopsy results confirmed the correlation between increased expression of transketolase-like gene 1 and a higher mortality rate (Langbein et al., 2006). Furthermore, an *in vitro* study demonstrated that knocking out transketolase-like gene 1 via interference RNA successfully halted tumor proliferation (Zhang et al., 2007).

Protection from Oxidative Stress

Several factors contribute to cellular oxidative stress, which occurs when the balance between oxidants and antioxidants is disrupted, resulting in an overall increase in reactive oxygen species (ROS). ROS are produced as a result of various metabolic events; for example, in the formation of water molecules during mitochondrial respiration. Molecular oxygen (O_2) is the terminal electron acceptor in the electron transport system of mitochondria and is converted to water (H_2O). In some cases, O_2 receives just one electron, becoming a superoxide anion. It is estimated that 4-5% of O_2 molecules are normally converted to superoxide anions

(Spitz et al., 2000). Superoxides are then converted to peroxides by an enzyme called superoxide dismutase. Subsequently, pyruvate scavenges the peroxides and converts them into water (Nath et al., 1995). Thus, an increased glycolysis rate that leads to increased pyruvate production may reduce oxidative stress.

There are two more ways in which the Warburg effect may reduce oxidative stress. Mitochondrial dysfunction may result in reduced oxidative stress, given that mitochondria are a main source of ROS generation (Orrenius, 2007). Alternatively, the antioxidant production associated with the Warburg effect may protect cancer cells from the negative effects of their explosive glycolysis. As previously mentioned, the balance between oxidants and antioxidants is vital to maintaining a healthy cell. As cancer cells grow rapidly and spread throughout the body, they require more energy and cellular building blocks, and a high metabolic rate is necessary to sustain their growth. Relatively high oxidative stress is a consequence of a highly active metabolism, and the Warburg effect results in increased production of antioxidants, as follows. After glucose is taken up into cells, it is used in two main metabolic pathways: glycolysis and the pentose phosphate pathway, which comprises oxidative and non-oxidative branches. The oxidative branch of the pentose phosphate pathway converts glucose into ribulose and generates the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH, from the oxidized form, NADP⁺. NADPH reduces oxidized agents, thereby decreasing oxidative stress. NADPH also supplies charged chemical energy for the synthesis of DNA, lipids, and proteins. More importantly, NADPH is a cofactor of glutathione reductase, which reduces glutathione disulfide to glutathione. Glutathione then reduces hyperoxide to water, maintaining a low level of oxidative stress in cells (Spitz et al., 2000). The production ratio of [NADPH]/[NADP⁺] in the pentose phosphate pathway is usually high in rapidly proliferating tumor cells, whereas the ratio of reduced to oxidized nicotinamide adenine dinucleotides, [NADH]/[NAD⁺], is low (Klein et al., 1990; Bernstein et al., 2002). The high production rate of NADPH indicates vigorous activity of the pentose phosphate pathway. Protection against oxidative stress is especially important for cancer cells, as the defective mitochondrial electron transport system in cancer cells makes them more susceptible than normal cells to oxidative stress. A study from the Free Radical and Radiation Biology Department at the University of Iowa demonstrated that the survival fraction of cancer cells was substantially reduced upon exposure to oxidative stress, whereas normal cells were not affected; furthermore, when cancer cells were deprived of glucose, they died due to oxidative stress and not because of the lack of ATP production (Ahmad et al., 2005). Although more questions remain to be answered, these data strongly suggest that the Warburg effect protects cancer cells from oxidative stress.

Can We Make a Silver Bullet to Kill Cancer?

Oncologists are still debating the cause of the Warburg effect, and intensive studies are being conducted on both glycolysis and the pentose phosphate pathway (Chen et al., 2007). It has been shown that increased glucose metabolism reduces oxidative stress in cancer cells, and the Warburg effect may be a defense mechanism protecting cancer cells from oxidative stress. Alternatively, many researchers believe that the Warburg effect is a symptom (i.e., result) of cancer metabolism, related to energy production and synthesis of basic building blocks for cancer cell growth. Nevertheless, blocking both glycolysis and the pentose phosphate pathway in cancer cells definitely increases oxidative stress and results in the production of less energy and fewer materials required for growth. The overall abnormal glucose metabolism in cancer cells is still poorly understood, and the links governing the systematic metabolic changes are still unknown. A study comparing changes in oxidative stress, energy metabolism,

and ribose synthesis may reveal which of these has the most significant role in explaining the Warburg effect. In addition, further elucidating the contribution of the pentose phosphate pathway in reducing oxidative stress would provide a better understanding of the Warburg effect. Moreover, it is still not clear whether the alteration of metabolic flux is caused solely by mitochondrial dysfunction.

With respect to the therapeutic potential of targeting the Warburg effect, the key metabolic regulators implicated in metabolic flux changes and cell proliferation must be checked (shown briefly in Figure 2), but this is not to minimize the critical importance of other cancer-related signaling pathways. Recently, dichloroacetate (DCA) was studied as a potential metabolic tuning agent for cancer therapy (Bonnet et al., 2007). DCA blocks the inactivation of pyruvate dehydrogenase kinase and thereby eventually increases the flow of pyruvate into the defective mitochondria, which activates oxidative phosphorylation and enables the mitochondrial apoptotic process. The *in vivo* effects of DCA are probably associated with a resistance to cell proliferation. Currently, this controversial salt is being used in preliminary trials for the treatment of brain tumors (2009, <http://www.thedcasite.com>). By activating oxidative phosphorylation and the originally coded mitochondrial self-killing program, DCA may prove to be an effective agent in the treatment for cancer, because it attacks the fundamental energy metabolism of cancer cells. Given the cessation of mitochondrial oxidative phosphorylation and the unique reactive glycolysis in most tumor cells, this therapeutic approach could destroy cancer cells despite their irreversible nature. Cancer cells could be selectively killed without reversion to normal cells. Mitochondrial revitalization is a promising method for promoting naturally encoded programmed cell death and kill cancer cells, as demonstrated by Michelakis and colleagues at the University of Alberta, Canada.

Systems Approach for the Interpretation of Cancer Cell Language.

Combined computational and experimental approaches may help to elucidate the Warburg effect. Developing a model of cancer signaling networks that interconnect overall metabolic pathways is an important goal, as enhanced glycolytic metabolism may be more than just a symptom of cancer (Mandal and Davie, 2007). In an integrated approach, genetic alterations, including gene mutations, of both chromosomal and mitochondrial DNA (mtDNA) should be investigated together with biochemical analysis, to identify the Warburg inducer network. Chromosomal instabilities and mtDNA mutations have been frequently reported in cancer or tumorigenic cells (Zanssen and Schon, 2005). Hypervariable regions of mitochondrial D-loop mutations that control mtDNA replication and transcription are associated with various types of tumors (Coller et al., 2001). A high frequency of homoplasmic mutations in mtDNA and the subsequent altered expression of mtDNA-encoded COX proteins have been reported in tumors harboring p53 mutations (Zhou et al., 2003; Hsu and Sabatini, 2008; Fliss et al., 2000), although it is still unclear whether p53 is directly linked to the control of mitochondrial genome integrity and protein expression levels. Nuclear-encoded mitochondrial proteins are of particular interest, because most mitochondrial proteins are imported from the cytoplasm. For example, mutations of two metabolic enzymes, succinate dehydrogenase and fumarate hydratase, were shown to induce severe tumorigenesis (Selak et al., 2005). Both enzymes are imported TCA cycle enzymes and are direct regulators of hypoxia inducible factor-1 (HIF-1), which is regarded as a major controlling factor for a multiplicity of glycolysis enzymes related to reactive glycolysis, and also suppresses mitochondrial function (Zhong et al., 1999). However, it is difficult to determine whether the alteration of metabolic flux is caused by HIF-1 alone, because the upregulation of reactive glycolysis does not occur exclusively through HIF-1

Table 1. Mitochondrial proteome analysis indicates a tissue-dependent enrichment level for known and unknown proteins in mitochondria.

GO name	heart	T-leukemia	hair shaft	coexist in heart+T-leukemia	coexist in heart+hair shaft	coexist in T-leukemia+hair shaft	coexist in heart+hair shaft+T-leukemia
antioxidant activity	4	-	1	1	-	-	-
auxiliary transport protein activity	1	-	-	-	-	-	-
binding	110	185	82	57	2	13	5
catalytic activity	123	71	24	46	1	-	3
chaperone regulator activity	-	-	-	-	-	-	-
chemoattractant activity	-	-	-	-	-	-	-
chemorepellent activity	-	-	-	-	-	-	-
enzyme regulator activity	9	3	6	3	1	-	1
metallochaperone activity	-	-	-	-	-	-	-
molecular transducer activity	3	6	2	2	-	-	-
motor activity	5	3	-	1	-	-	-
nutrient reservoir activity	-	-	-	-	-	-	-
protein tag	-	-	-	-	-	-	-
structural molecule activity	28	27	9	12	-	-	-
template for synthesis of G-rich strand of telomere DNA activity	-	-	-	-	-	-	-
transcription regulator activity	3	10	2	2	-	1	-
translation regulator activity	-	-	-	-	-	-	-
transporter activity	26	11	-	13	-	1	-
unknown	395	261	102				
total	707	557	228	137	4	15	9

(Kondoh, 2008).

Mitochondrial dysfunction is clearly involved in carcinogenesis and the deregulation of cell apoptosis. Ongoing studies also indicate that mitochondrial proteins are involved in tumorigenesis and an altered metabolism. These findings initiated comparative proteomic studies of cancer-cell mitochondria (Verma et al., 2003; Kulawiec et al., 2006). Mitochondria contain about 1000 proteins, and almost all are imported from the cytoplasm via the translocase of the outer membrane (TOM) complex (Schatz, 1996). Using a proteomic approach and data mining for cancer biology, researchers at the Purkyne Military Medical Academy identified proteins whose expression was significantly altered in γ -irradiated human T-lymphocyte leukemia cells (Szkanderová et al., 2005). At the same time, a systematic characterization of T-leukemia mitochondria performed by another research group identified 227 known mitochondrial proteins, including membrane and soluble proteins, and 453 additional proteins thought to be associated with mitochondrial functions (Rezaul et al., 2005). Although only a few studies have been reported, they suggest the feasibility of a proteomic method for precisely monitoring mitochondrial responses in cells. In addition, the interrelationships among the cancer biomarkers discovered throughout the last decade must be established to better understand complex cellular networks.

A comparative analysis of the distribution of mitochondrial protein enrichment may provide a new approach to inferring different (or abruptly altered) cellular functions. Table 1 shows the disparate migrations of proteins from heart, hair shaft, and T-leukemia mitochondria. These mitochondria appear to have very few proteins in common. The mitochondria from T-leukemia cells show significantly high abundances of transcription regulators, molecular transducers, and many binding proteins, although their roles and mechanisms are unclear (Kim et al., 2009; Gabaldón and Huynen,

2004). A well-characterized mitochondrial proteome database and a systems approach will be necessary to identify the unknown mitochondrial proteins and their functions.

Conclusion and Future Directions

Network modeling of the interconnections among the crucial factors involved in metabolic flow and signaling pathways is a necessary future undertaking. In addition, the mitochondrial uncoupling effect should not be overlooked. Although cancer cell energy generation is mainly dependent on reactive anaerobic glycolysis, most malignant tumors still breathe, in part by an uncoupling protein-mediated mitochondrial pathway (Samudio et al., 2009). Uncoupling proteins help import fatty acids and are overexpressed in various types of chemo-resistant cancer cells. This may increase an apoptotic threshold level. A better understanding of metabolism in cancer cells may lead to the development of novel therapeutic strategies exploiting their uniqueness.

Current technologies may help accomplish this systematic work. In addition to PET and magnetic resonance scans and next-generation sequencing, the newly developed multiparametric live cell analysis system is needed to precisely study cancer cell biochemistry (Mayevsky, 2009). As evidenced by current proteomics and biomarker studies, detection limits should be less than femto- to ato-mole levels, considering that significant proteins or small peptides secreted from a tiny tumor cell may represent only 1% of the total protein and are extensively diluted throughout the human body (Chung, 2006; Simpson and Dorow, 2001)

An integrated analysis of an entire tumor genome with mtDNA variations, signaling networks, and accompanying metabolic flux

changes will unveil new insights into the Warburg effect, potential therapeutic targets, and mechanisms of drug resistance in cancer cells. Obtaining proteome profiles and building databases will enable a new approach to elucidating how cells function and communicate with each other. An integrated approach is essential to expand the frontiers of cancer cell biology and to better understand the complexity of cancer and the causes of tumorigenesis

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