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Bioenergetics Analysis of Glucose Metabolism in Normal and Cancer Cells: Implications for Breast Thermography

***Abstract*— *Objective:* This study elucidates the observed "hot spots" in breast thermography (BT) by considering the sources of heat generation during glucose metabolism in normal and carcinoma breast cells and recommends two biosignatures for improving BT protocols. The bioenergetic differences during glucose metabolism in normal and carcinoma cells are examined, focusing on the negative enthalpy during lactate fermentation and respiration. *Methods:* To understand the preference of cancer cells for lactate fermentation over respiration to produce adenosine triphosphate (ATP) molecules and other metabolites, two aspects are analyzed. First, cancer cells switch to lactate fermentation (aerobic glycolysis) as it produces the maximum ATP required by the cells. Second, the increased demand for nicotinamide adenine dinucleotide molecules during cell proliferation increases the glucose uptake by cancer cells. Using two previously established methods, the heat generation during lactate fermentation and respiration metabolism is analyzed. *Results*: The lactate fermentation pathway lost 24–28% more heat than the respiration pathway when producing the same 36 molecules. Moreover, for cancer cells, the ATP produced via the fermentation pathway exceeds thrice that produced by normal cells via the respiration pathway. *Conclusion:* Rapidly proliferating cancer cells with adequate glucose supply exhibit approximately thrice the negative enthalpy of normal cells. Furthermore, the metabolic rate of glucose for lactate fermentation is 100 times faster than that for aerobic respiration. *Significance:* The biosignatures determined in this study could provide opportunities to significantly improve the sensitivity and specificity of BT, enabling it to become the preferred mass screening protocol for early breast cancer detection.**

***Index Terms*—adenosine triphosphate, bioenergetics, biosignatures, breast thermography, carcinoma, glycolysis, glucose metabolism, lactate fermentation, mammography, mitochondria, oxidative phosphorylation, pyruvate, respiration, tumor microenvironment, Warburg effect**

I. Introduction

IN 1927, Warburg *et al.* [1] published the seminal paper, *The Metabolism of Tumors in the Body*. They reported that carcinoma cells rapidly consume glucose and convert it to lactate even in the presence of oxygen, contrary to normal cells that favor the respiration pathway. This effect is recognized as a hallmark of carcinoma cells [2] and is known as the Warburg Effect [3].

The FDA approved breast thermography (BT) as an adjunctive diagnostic breast cancer screening procedure for the early detection of breast cancer in 1982 [4]. The BT technology was pioneered by Ray Lawson in 1956 using an early form of infrared scanning [5]. The technology, which was initially limited to detecting temperature differences lower than 0.1 °C [5], was subsequently improved to detect temperature differences as small as 0.025 °C. [4].

This study examines the bioenergetic differences in glucose metabolism between normal and carcinoma cells. In particular, it employs two previously established methods to investigate the negative enthalpy (heat loss) that occurs in two primary metabolic pathways, thereby aiming to elucidate the observed "hot spots" in BT.

II. Glucose Metabolic Pathways

A. Glycolysis

This study focuses on investigating the sources of the heat generated during the metabolism of glucose in normal and carcinoma breast cells. This analysis addresses the related bioenergetics [6] of the metabolism processes rather than the thermodynamics of the cellular chemistry involved. A distinguishing feature of the bioenergetics analysis is that it involves evaluating an open system in which different stages of the glucose metabolism occur in distinct compartments [7].

The primary metabolic pathways considered in this study have a common initial pathway, glycolysis. While glycolysis involves a series of reactions along the pathway, the significance of the process is that each glucose molecule entering the cell is broken down within the liquid cytosol to produce two pyruvate molecules, two molecules of nicotinamide adenine dinucleotide plus hydrogen, and a net gain of two adenosine triphosphate (ATP) molecules [8].

ATP molecules serve as the energy currency for most cells. As such, they are necessary for various cellular functions, including cell division and the conversion of biochemical energy into mechanical energy in muscles. As will be discussed in Section III, the rate of ATP molecule generation is one of the two driving factors for increased glucose uptake in breast cancer cells. The other is the rate of nicotinamide adenine dinucleotide (NAD^+) molecule generation. The two primary metabolic pathways considered here are respiration and lactate fermentation. A discussion on these pathways is presented subsequently.

B. Respiration Pathway

Respiration proceeds from glycolysis. Here, pyruvate enters the mitochondria organelles, where several intermediate steps occur. The last two steps are the Krebs cycle, also known as the citric acid cycle, and oxidative phosphorylation (OXPHOS) [8]. The Krebs cycle and OXPHOS result in the production of an additional 34 ATP molecules, plus H_2O and

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CO_2.

Respiration (also termed aerobic metabolism) requires sufficient oxygen. The overall metabolism from glucose to OXPHOS produces 36 ATP molecules and is an efficient pathway in an aerobic cellular environment. When an oxygen deficiency occurs, such as in muscles during high-intensity exercise, lactate fermentation—the alternate subsequent pathway to respiration after glycolysis—occurs [9].

C. Lactate Fermentation

The alternate glucose metabolic pathway to respiration after glycolysis is lactate fermentation. This pathway does not require oxygen (anaerobic metabolism); it converts pyruvate to lactate, with 95% conversion occurring with minimal energy loss [10]. Although lactate is also considered a waste metabolite, it is generally available as a subsequent energy substrate. This may be within the fermentation tissue—as in the case when oxygen levels are restored in recovering muscles via the reverse of the pyruvate to lactate reaction in the mitochondria—or the lactate is exported to the bloodstream and carried to the liver, where it can be converted back to glucose [9][11][12][13].

An additional by-product of lactate fermentation is (NAD^+) molecules. For each glucose molecule that completes the overall lactate fermentation pathway, two NAD^+ molecules are produced. NAD^+ is essential in a myriad of metabolic processes, particularly for cell proliferation [14].

The importance of lactate fermentation in this relates to the Warburg effect [2], in which cancer cells were found to generate excessive amounts of lactate even in an aerobic environment. Warburg hypothesized that in cancer cells, the impairment of OXPHOS was caused by mitochondrial damage [15][16]. The hypothesis maintained that tumor cells cannot oxidize glucose via OXPHOS. However, through his experiments, Weinhouse [17] showed that the mitochondria were not necessarily damaged. In addition, it has been shown that OXPHOS continues to produce ATP in cancers as in normal cells [18].

Therefore, the overall glucose-to-lactate fermentation pathway produces two ATP molecules per glucose molecule. As such, it is an inefficient pathway for ATP production in the anaerobic cellular environment. However, this pathway proceeds up to 100 times faster than the respiration pathway, enabling a more rapid production of ATP molecules [3][19].

III. Cancer Cell Biosignatures

This section considers the differences between the cellular chemistry of cancer cells and that of normal cells. In particular, it examines the known preference of cancer cells to produce ATP molecules and other metabolites using lactate fermentation rather than respiration. In this study, these preferences are termed biosignatures, which could potentially be leveraged to improve BT protocols.

The tumor microenvironment (TME) is generally a heterogeneous environment in which specific cancer cells within the tumor have different levels of glucose, oxygen, and other metabolites, depending on their proximity to capillaries or veins. Even when the environment is oxygen-rich, cancer cells can induce hypoxia-independent mechanisms that cause pseudo-hypoxia. This, in turn, significantly increases glucose uptake within the cells through various cellular chemistry mechanisms, as detailed in [20].

As previously stated, normal cells completely metabolize glucose in a normoxic environment through respiration, switching to lactate fermentation only within a hypoxic environment, such as in skeletal muscles. Once oxygen returns to normal levels, the lactate is converted back to pyruvate and subsequently oxidized in the mitochondria [7][12][13].

A mathematical study of the Warburg Effect demonstrated that cancer cells switch to lactate fermentation (aerobic glycolysis, AG) because this pathway provides the maximum ATP production needed to meet cellular ATP demand [19]. This study determined that for mammalian cancer cells, the ATP production during the fermentation pathway was 3.08 times that of the respiration pathway. Coupled with the fact that AG requires 18 times the number of glucose molecules to produce the same number of ATP molecules, a rapidly prolific cancer cell with sufficient available glucose can metabolize over 50 times the number of glucose molecules compared with normal breast cells. This finding represents another potential biosignature that could be leveraged to improve BT protocols.

A second driving factor for increased glucose uptake in cancer cells is the increased demand for NAD^+ for cell proliferation [21][22]. The cited studies provide several results that are relevant to this study:

- Limiting NAD^+ production results in reduced cell proliferation.
- AG increases when the need for NAD^+ exceeds the need for ATP production.
- If ATP consumption is forced, AG will be increased.
- Increased cellular pyruvate increases cell proliferation in mammalian cancer cells.
- ATP is only regenerated as needed within a cell because it cannot be stored.
- Demand for NAD^+ regeneration can supersede the need for ATP in proliferating cells.
- Demand for NAD^+ is what drives AG in rapidly proliferating mammalian cells.
- The ratio of AG to OXPHOS is influenced by the need for NAD^+ in a proliferating cell. However, both pathways continue even in a cancer cell.
- A similar metabolism has been observed in both malignant and non-transformed cells; however, to a lesser degree in the latter.

IV. Bioenergetics Considerations

This study compares the exothermic energy expenditure (negative enthalpy) of the two metabolic pathways—respiration and lactate fermentation—using two previously established methods. First, the study by Scott and Djuristic is considered [23]. In this study, the authors separated the anaerobic and aerobic components of the total energy expenditure for glucose metabolism. The two components

occur in different compartments within the cell, with the anaerobic component occurring in the liquid cell cytosol and the aerobic component occurring within the walls of the mitochondria. They established a specific equation for calculating energy exchange during respiration, which is given by

$$19.6 \text{ kJ aerobic energy exchange} + 1.5 \text{ kJ anaerobic energy exchange} = 21.1 \text{ kJ (total energy exchange per L } O_2) \quad (1)$$

This provides the anaerobic negative enthalpy (1.5 kJ) for the two components. Notably, the anaerobic component, which occurs without oxygen, is often referred to as an oxygen deficit, and is an equivalent measure of the heat loss for the anaerobic component [7][11].

The negative enthalpy (heat loss) for only the anaerobic component, which corresponds to the lactate fermentation pathway, is approximately 7% of the total heat loss for a molecule of glucose in the respiration pathway. The ratio of heat loss in the two pathways is 1.5/21.1, or .0711.

Now, consider the enthalpy per ATP molecule. Lactate fermentation produces two ATP molecules per molecule of glucose, whereas respiration produces 36 ATP molecules. On a per ATP molecule basis, the lactate fermentation pathway requires 18 times the number of glucose molecules to produce the same 36 molecules of ATP as the respiration pathway. Using the above ratio, the lactate fermentation pathway produces 1.28, or approximately 28%, more heat loss than the respiration pathway to produce the same 36 molecules.

Using an alternative method of comparison, Forrest *et al*. [24] determined that the enthalpy change when glucose is fermented is -29.1 kcal/mole of glucose. One mole of glucose has a total of 686 kcal/mole [25].

For the respiration pathway, which produces 36 ATP molecules, the heat loss for one molecule of glucose was computed. Each ATP molecule has an energy of 7.3 kcal/mol [25], with a total of 262.80 kcal for the 36 molecules. The result is the heat loss corresponding to the difference of 686.0-262.8 kcal/mol, or 423.2 kcal/mol of glucose. This difference represents the heat loss or negative enthalpy. The ratio of the heat loss in the two pathways is 29.1/423.0, or .0688, which agrees with the Scott and Djuristic analysis [23].

On a per ATP molecule basis, the lactate fermentation pathway requires 18 times the number of glucose molecules to produce the same 36 ATP molecules as the respiration pathway. Using the above ratio, the lactate fermentation pathway produces 1.24 times more heat loss (approximately 24%) than the respiration pathway to produce the same 36 molecules.

This analysis can be considered a baseline for the expected heat generation difference between the two metabolic pathways. Cancer cells produce ATP molecules both anaerobically and aerobically. Therefore, depending on the available glucose substrate and the availability of oxygen, the accrual heat generation in the TME can be greater than the baseline heat generation by the cell. As previously stated, the TME is a heterogeneous environment.

For mammalian cancer cells, the ATP production during the fermentation pathway was 3.08 times that of the respiration pathway [19]. Therefore, for a rapidly proliferating tumor, the local heat generated could be over three times that of the adjacent non-transformed tissue.

Finally, the dynamics of the TME differ significantly from those of the non-transitioned tissue. The lactate fermentation pathway proceeds up to 100 times faster than the respiration pathway, enabling a more rapid production of ATP molecules [3][19].

A. Implications for BT

Mammography is the only FDA-approved mass screening for the early detection of breast cancer (specifically carcinomas) [26]. This protocol has several negative aspects, one of which is that it has a biosignature that is far removed from the bioenergetics of a breast tumor. In particular, it relies on a passive, non-dynamic sensing of abnormal localized cell density [27]. For dense breasts, the sensitivity of the protocol decreases significantly. Dense breasts are more prevalent among women under the age of 50 (50%). Despite mammography being the gold standard for breast cancer screening, it suffers from false positives, false negatives, and overdiagnosis [28]. Furthermore, the protocol requires exposure to low-intensity radiation, making it unsuitable for women below the age of 50. This is even more the case for periodic screening, which results in repeated radiation exposure.

Conversely, BT is discouraged by the FDA as a mass screening protocol for breast cancer screening. At best, it is offered as an adjunct screening protocol [4][26]. The reasoning behind the claim that BT is inferior to mammography is that BT produces false positives and, to a lesser extent, false negatives. In addition, it requires a higher degree of manual interpretation by the analyst. However, this last limitation can be overcome with machine learning and other AI technologies. Moreover, this technology has been found to be more sensitive to precancerous cells and in the early detection of breast cancers [4].

BT creates a thermal mapping of the breast, and the standard protocol seeks to detect abnormal localized hot spots. The hot spots in a carcinoma are usually attributed to several sources as follows [4]:

1) Angiogenesis
Similar to a non-transitioned tissue, the need for a greater blood supply leads to the formation of new capillaries, which in turn generate increased heat due to the increased blood supply. The formation of new capillaries is a complex, multi-step process that occurs much more slowly compared with the rapid changes in the available glucose within the supplied blood [29].

2) Dilation of existing blood vessels
The creation of nitric oxide in the cancer tissue increases blood supply, which in turn increases heat generation.

3) General increased metabolic activity
This cause is typically discussed without providing an

approximate bioenergetics evaluation.

CONCLUSION

This study provided a bioenergetic evaluation of the differences in the glucose metabolism of normal and breast cancer cells. The study revealed that, as a baseline, cancer cells can exhibit up to three times the negative enthalpy (exothermic heat production) compared with the corresponding non-transformed (normal) breast cells. Furthermore, the metabolic rate of glucose for lactate fermentation is 100 times faster than that for aerobic respiration. These biosignatures could provide opportunities to significantly improve the sensitivity and specificity of BT by leveraging measured dynamic changes in the glucose blood supply, enabling it to become the preferred mass screening protocol for early breast cancer detection.

Furthermore, this study has benefited from over 100 years of research outcomes from a vast effort of cellular biology researchers, starting with the original study by Warburg *et al.* This study is expected to contribute to that effort.

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