**Cancer Cell Metabolism: Change of Gene Expression to induce Proliferation in Some Cancer Cells**

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**Key Words:**

GLUT, Glutaminolysis, glutamine, proliferation, ROS, mitochondria, Warburg Effect

As one of the ten hallmarks of cancer, the deregulation of the metabolic processes in cancer cells is crucial for growth and proliferation. First demonstrated by Otto Warburg (1924), cancer cells alter their metabolism to an energetically inefficient catabolic processing of glucose. This process, now known as the Warburg effect, can only generate ~2 ATP per glucose molecule in comparison to the normal generation of ~32 ATP through normal catabolic processing. However, differential gene expression in cancer cells account for the lacking energy generation with changes in stringent response, non-essential amino acid uptake, and increased glucose uptake among many factors. Lactate, the alternative product of glycolysis (in comparison to pyruvate) can be used as an alternative indirect carbon source in the tricarboxylic acid (TCA) cycle with an increased efficiency over the glucose counterpart. Glutaminolysis, a prominent secondary metabolic pathway of cancer cells, uptakes glutamine from the environment to be used for processing of secondary metabolites as well as cell signaling. Recent research progress has shown the need for glutamine in cancerous cells as well as targeted treatment to disallow the use of glutamine by tumor cells. As exemplified, there are many pathways of cancer cells that are differentially changed for the sole purpose of growth and proliferation. In this review, the regulation of glucose uptake as well as the non-essential amino acid Glutamine will be discussed. With understanding of these pathways, inhibitors and other treatments can be hypothesized to assist in inhibiting proliferation of malignant cells.

**Introduction:**

With trillions of cells in the human body, nutrients are needed to keep each of them in a healthy condition to allow survival, growth and division. These nutrients, in the form of carbon sources, amino acids, and other non-essential components are taken up from the foods that we consume. Glucose (sugar) is the most common and abundant carbon source present to cells . Glucose will be sequentially broken down through glycolysis to generate a small amount of Adenosine Triphosphate (ATP) as well as other cofactors such as NADH to be used in later parts of the metabolic pathways. The primary product of this pathways though is pyruvate, a molecule with 3 carbon linkages that will be sequentially broken down in the tricarboxylic acid cycle (TCA). This breakage will generate more NADH as well as carbon dioxide that will be released as well exhale. Finally, a proton gradient will be generated by pumping electrons out of the cell, allowing the generation of a large amount of ATP for the cell to use on the many processes it needs to survive. Overall, this process generates ~28-32 ATP for use by the cell, a process we know now as respiration and is the primary sourcing of energy for the cell.

 As first hypothesized by Otto Warburg in 1942, cancerous cells perform a different type of metabolic processing to generate the energy needed for their cellular processes. Termed “aerobic glycolysis” by Warburg, cancerous cells use glycolysis in an anaerobic way to generate a small fraction of ATP (~2) as well as the byproduct lactate in contrast to pyruvate that will be generated by normal human cells. (Warburg, O. 1956) As expected, this hypoxic generation of lactate cannot traffic to mitochondria for use in the TCA cycle and cannot be further broken down to generate the rest of the ATP that can be used for cellular processes. This aerobic respiration, also known as the Warburg Effect is still a topic of debate today. Regardless, we have seen extensive research into how cancerous cells compete with normal human cells for resources such as glucose, amino acids, and other non-essential elements.

 One may then ask the major question, “If cancer cells cannot generate as much energy in comparison to normal cells, how do cancerous cells compete with normal cells so well?” This can be determined due to the changes of gene expression in cancerous cells. While all cells of the body have the same genotype, the phenotype is different due to the difference in gene expression of each cell type in the body. Additionally, cancer cells have secondary phenotype expression due to the mutations present in the genotype of the cell. These secondary phenotype expressions lead to an increased metabolism due to overexpression of a certain protein in the cell membrane.

**Glycolysis:**

Glucose uptake is the first regulation of cell growth in the human body. Without glucose, cells cannot grow and proliferate to continue the normal processes that are required of them. The uptake of glucose, mediated by the facilitative glucose transporter (GLUT) proteins are the main transporter protein to uptake glucose into the cell for use in glycolysis. (Macheda, M. et al. 2004). With thirteen members of this transporter identified and 12 hydrophobic a-helical domains, they are responsible for the uptake of glucose, hexose and all other sugars as well as some amino acids into the cell.

While present in normal cells, GLUT is tightly regulated as they are not the all-encompassing factor of energy production. Secondary processes as stated before generate large amounts of energy with a normal flux of glucose into the cell. By comparison, most cancer cells do not generate pyruvate from glycolysis, instead opting for the generation of lactate in hypoxic conditions. Limited by substrate-level oxidation, cancer cells adapt higher energy generation rates through the overexpression of the GLUT protein on the cell membrane. With increased expression of GLUT, tumor cells can uptake large amounts of glucose to be used to generate metabolites, as well as the main product lactate. This will be explored more in the recent progress.

**Glutaminolysis:**

As expressed earlier, glycolysis primarily is used to generate lactate, as pyruvate transporters to mitochondria are not present. While lactate is generated and moved to the cytosol, glutaminolysis is also taking place to drive ATP and GTP production in the TCA cycle. Glutamine, the most abundant amino acid in the world, is a non-essential amino acid that many people obtain through foods. In cancer however, it is an important amino acid used to sequentially generate ATP for the cell.

Glutamine, upon consumption, will be acted upon by an enzyme called Glutaminase will convert glutamine into glutamate, which in turn is converted to alpha-ketoglutarate by an enzyme named glutamine dehydrogenase. Later in the cycle, this is sequentially broken down to succinate, fumarate, malate, and oxaloacetate by their respective enzymes. This products energy carriers such as NADH as well as ATP and GTP for energy in the cell. (Weinberg, Robert 2014). Additionally, we see an upregulation of many importing proteins for glutamine, as well as the enzymes used for conversion of this amino acid into other forms to generate ATP or GTP in the TCA cycle. Similarly, fatty acids that are consumed can be broken down in a similar pathway, often resulting in production of ATP.

**Recent Progress:**

**Lactate indirectly feeding the TCA cycle:**

As state earlier, lactate is the primary byproduct of glycolysis in cancerous cell types. While glutaminolysis has the highest turnover of ATP and GTP for cancerous cells, we must also turn to lactate as a potential carbon source for the TCA cycle. While lactate cannot be uptaken directly through mitochondria to be broken down in the cycle, our body instead uses gluconeogenesis in the liver to recover pyruvate from lactate generated in the first step of glycolysis (Hui, Sheng et. al 2017). With an enzyme named lactate dehydrogenase (LDH), the backwards conversion back to pyruvate can drive precursors to be used in the TCA cycle indirectly. This was also proved further as the turnover rate for the catabolism of lactate was about 1.1 times the normal catabolism rate for glucose breakdown.

 There are also complements present that drive cancer proliferation to happen at a faster rate. P53 as well as other genes such as c-myc when mutated cause an upregulation of enzyme production in both the conversion of lactate to pyruvate as well as many enzymes that are present in the TCA cycle to convert substrates. We also now know that there is some type of metabolic switch present in certain types of cancer, between hypoxic and normal aerobic conditions, enabling a normal, coupled TCA cycle and ETC.

**Reactive Oxygen Species (ROS):**

 While energy production is one of the main byproducts of oxidative phosphorylation in the TCA cycle, the other major byproducts are energy carriers as well as reactive oxygen species. With the TCA cycle happening in the mitochondria from both lactate being converted back to pyruvate as well as glutaminolysis yielding other precursors for the TCA, NADH+ are shuttled to be harnessed as a proton gradient for large amounts of ATP to be generated.

 Reactive oxygen species are radicals with single unpaired electrons that are often used for the killing of bacteria and other pathogens in the lysosome. In cancer though, it is an unlikely byproduct due to the abusive production of ATP in the mitochondria. With cyclic production of H+ protons throughout the TCA cycle, some cancer cells are able to generate the proton gradient for mass production of ATP as well as the generation of these reactive oxygen species.

 ROS elevated rates are detected in almost all cancers, which can lead to additional mutations, DNA breakages, and damaging of the mitochondria. However, cancer cells also possess an overproduction of antioxidants, often leading to the neutralization of these superoxide radicals, protecting themselves from the overproduction of the ROS themselves. (Geou-Yarh. 2014). This neutralization of ROS into that of hydrogen peroxide can also be used for cell signaling for cancer cells, leading to increase activation for genes such as protein synthesis.

 This also designates a secondary problem for the human body when it comes to cancerous cells. The immune system itself (such as macrophages) use superoxide stress for killing of cells that could be marked for death. If macrophages were to attempt to kill a tumor cell with increase neutralization activity, it can lead to chronic immune damage as well as an increase in tumor proliferation.

**ASCT2/SLC1A5 and Glutaminolysis:**

 Current research into glutaminolysis is targeted to the many amounts of cancers that allow for the process to be abused. Among these heterogenous cancers, breast cancer is becoming the largest of the researched relationships as it is one of the highest risks for tumor addiction to glutamine. Upon the metabolism of glutamine in breast cancer, it will activate pathways used to regulate cell growth and protein translation. This activator (known as mTORC1) is a checkpoint in the sensing pathway for nutrients which is also a prevalent target for possible therapeutic drugs and inhibitors (Van Geldemalsen, M. et al 2016). However, the main focus of research is rather the transporters that are responsible for the uptake of glutamine in the first place.

 Identified as the alanine, serine, cysteine-preferring transporter 2 (ASCT2; SLC1A5), this protein transporter is responsible for the initial uptake of glutamine in glutaminolysis. Without the glutamine present in the system of metabolism of the cell, conversion to other metabolites as well as the generation of ATP in these pathways can be inhibited. This will also play into the activator known as mTORC1 that was explained earlier. Not being able to make it to the critical amount of glutamine needed to activate mTORC1 could have positive effects on possibly limiting cell proliferation and protein synthesis in tumor cells.

 In both their in vitro as well as in vivo studies, their use of the inhibitor known as L-gamma-glutamyl-p-nitroanilide (also known as GPNA23) yielded a decrease in overall cell proliferation in some cells (those who are sensitized to glutamine). They added also that while it blocked glutamine, it also blocks other neutral amino acids that could present themselves as assistants to tumor cell growths.

**Discussion:**

**Lactate and the TCA Cycle:**

 In terms of metabolism, lactate is an important possibility for generation of ATP through LDH and the TCA cycle. It may seem strange to see the machinery of the human body used in such a primitive way but is not as primitive as many make it out to be. While lactate is a weak base often created by cancer cells in hypoxic conditions, lactic acid (its corresponding acid) is often generated in the body under strenuous activities. Progressive overload of the human muscle groups yields a forceful hypoxic response, generating lactic acid in the muscles used (also one factor that plays into soreness as a result).

 In terms of the turnover rate, we must ask what the limitations of the 1.1x turnover rate between glucose and lactose really means. In their paper, they explained simply as the turnover of lactate and glucose after generated as a product themselves, yet did not account for the amount of time needed to generate the substrate of lactate. It has been proven through many journal entries that the upregulation of many of the enzymes could possibly play into this also, such that if the concentration of enzymes as well as substrates are so high, it is absolutely dependent on the enzymes ability to turn-over the substrate to its products.

 However, while the turn-over time is trivial, it does show that while it may seem primitive, it is another way that tumor cells can hoard large amounts of glucose to not only fuel the anabolic reactions it must rely on, but also stripping other cells of the necessary components for them to survive and thrive.

**Reactive Oxygen Species (ROS):**

 While reactive oxygen species are highly researched, we still have yet to find many treatments to stop chronic inflammatory response by macrophages other than via radiological or drug therapies. These therapies, while important for treatment in urgent patients in later stages of metastasis, can cause severe problems of the immune system. This is due to the non-selectivity of the drugs used to treat patients, such that it will also “wipe” the immune system back to basic innate immunity. This has been exemplified as many cancer patients who have gotten these treatments are incredibly vulnerable to secondary infection and complications.

 As for reactive oxygen species themselves, this is an important matter as these ROSs can be used for signaling to other cells. The research itself is valid, yet does not hypothesize about potential treatments, only giving information unto the problems that arise with ROS in cancer cells.

**ACST2/SLC1A5 Glutaminolysis:**

 In their studies, it has been accepted that the primary transporters of glutamine could be possible ways to treat breast cancer. They also stated explicitly that while all cancers have minor similarities, they have major differences that must be handled in the case to case basis. However, there is possible problems to glutaminolysis such that the pathways that it holds could also be followed by fatty acids. While glutamine is brought in later in the TCA cycle (from glutamine to glutamate and eventually alpha-ketoglutarate), fatty acid biosynthesis could also be problematic as it generates acetyl-CoA earlier in the TCA cycle.

 Fatty acid biosynthesis, while an independent problem, ties into the ultimate goal of stoppage of the chronically activated TCA cycle. Further research into this would be of great interest to those who wish to understand cancer biosynthesis in the TCA cycle but is more geared towards other cancers rather than breast cancer.

**References:**

Hui, S., et al. "Glucose Feeds the TCA Cycle Via Circulating Lactate." Nature, vol. 551, no. 7678, 2017, pp. 115-118. SCOPUS, [www.scopus.com](http://www.scopus.com), doi:10.1038/nature24057.

Liou GY, Storz P. Reactive oxygen species in cancer. *Free Radic Res*. 2010;44(5):479-496. doi:10.3109/10715761003667554

Macheda, M.L., Rogers, S. and Best, J.D. (2005), Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J. Cell. Physiol., 202: 654-662. <https://doi-org.argo.library.okstate.edu/10.1002/jcp.20166>

Van Geldermalsen, M., et al. "ASCT2/SLC1A5 Controls Glutamine Uptake and Tumour Growth in Triple-Negative Basal-Like Breast Cancer." *Oncogene* 35.24(2016):3201-8. *ProQuest.*

Warburg O. On the origin of cancer cells. Science. 1956 Feb 24;123(3191):309-14. doi: 10.1126/science.123.3191.309. PMID: 13298683.

Weinberg, R. (2014), The Biology of Cancer – 2nd Edition pp. 53-56.

 ISBN:978-0-8153-4220-5.