

**The Warburg Effect and Its Role in Cancer Detection  
and Therapy**

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## **ABSTRACT**

### **The Warburg Effect and Its Role in Cancer Discovery and Therapy**

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The Warburg Effect is a cellular phenomenon in cancer cells discovered by Otto Warburg in 1924. His findings showed that in normoxic conditions tumor cells primarily use glycolysis for energy production instead of mitochondrial oxidative phosphorylation like normal cells. This breakthrough has been the basis for much research. It has resulted in a successful and widely-used cancer detection method, the positron emission tomography (PET) scan. The PET scan uses radioactive isotopes and the fact that cancer cells exhibit higher rates of glycolysis to pinpoint tumors with advanced imaging tools. Furthermore, Warburg's work helped to show the potential for beneficial pharmaceuticals that could be developed by inhibiting certain chemical mechanisms of glycolysis to specifically target and kill cancer cells. This review covers research that has used the Warburg effect as a premise and the heretofore indications and applications of the Warburg effect.

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## **Introduction**

In the first half of the 20<sup>th</sup> century, the cell biologist and Nobel laureate, Otto Warburg, discovered that cancer cells have much higher rates of glycolysis than normal, healthy cells, even in the presence of oxygen. Warburg further postulated that cancerous growth is stimulated by cells mainly using the fermentation pathway to produce necessary energy [1]. Although a remarkable finding, Warburg's research has not been a focus of much cancer research until recently [2]. Starting with the premise that cancerous cells display higher rates of glycolysis than healthy ones, researchers are looking into detection techniques and potential treatments that are selective for this increased rate.

## **The Warburg Effect**

In the 1920's, Otto Warburg conducted a series of experiments where he compared the rate of oxygen consumption and the production of lactic acid, an end-product of the fermentation pathway, between cancerous and healthy cells. Warburg found that while both cultures of cells produce similar amounts of adenosine triphosphate (ATP), oxygen consumption was higher in healthy cells and the amount of lactic acid produced was higher in cancerous cells [1] (See Figure 1.) These findings led Warburg to his discovery that even under aerobic conditions cancerous cells selectively use the fermentation pathway to produce energy. In a 1966 lecture by Warburg, he put forth that, "the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar [3]." In a summary of his findings a decade earlier in 1956, Warburg postulated that damage to the respiration pathway and the consequent reliance on fermentation even in aerobic conditions was a step in cellular carcinogenesis, not a symptom [1]. Formally stated, the Warburg effect is the metabolic

change observed in cancer cells from oxidative phosphorylation to glycolysis as the primary source of cellular energy.

There has been strong evidence suggesting that the Warburg effect is a necessary change in a cell before it has full-blown cancer, as Warburg himself posited. For example, the hypoxic and thus anaerobic conditions found at solid tumor mass centers would require cells present there to rely on glycolysis. Furthermore, the metabolic change also works to elicit multiple cellular response mechanisms that stem from the concentrations of intracellular compounds that are altered by the increased rate of glycolysis. These cellular responses have been shown to cause distinct transformations like the upregulation of proteins such as hypoxia-inducible factor 1- $\alpha$  that help the tumor survive adverse conditions in which normal cells cannot persist [4-6] .

In a 2004 study, it was calculated that 62% of all known cancers show an increased expression of the genes involved in the glycolysis pathway [7]. The increase of glycolytic gene expression corroborates Warburg's finding. Furthermore, this finding is very significant because it verifies researchers' efforts of studying glycolysis in such great depth to better understand cancer.



## Cancer Detection

### Positron Emission Tomography

Warburg believed that aerobic fermentation should not be used as a test condition for the detection of cancerous cells [1]. However, in direct contrast to his statement, positron emission tomography does just that. The first positron emission tomography (PET) apparatus was created in 1953 and first used clinically in the 1960's [8]. Since then, the technology has advanced to become a common cancer discovery device that tracks the 3D origin of emitted positrons and can be used to pinpoint anatomical locations with high rates of glycolysis [9] (See Figure 2).

A PET scan begins with the intravenous injection of a radioactive tracer compound. In the detection of cancer, a substitute for glucose is used which is molecularly similar enough to be absorbed by cells. The molecule used is fluorodeoxyglucose (FDG) where the fluorine molecule is the radioactive isotope  $^{18}\text{F}$  (2- $^{18}\text{F}$ -fluoro-2-deoxy-D-glucose).  $^{18}\text{F}$  undergoes positive beta decay where the Coulomb repulsion forces in the nucleus provide the energy to convert a proton into a neutron, a neutrino and a positron [9]. The emitted positron will collide and annihilate with a nearby electron releasing two gamma rays at an approximately 180 degree angle [8]. The PET scanning machinery detects these two gamma rays and using computational software calculates the three-dimensional origin inside the body [9].

The Warburg effect comes into the PET scanning process because of the tracer molecule that is used. Cancerous cells, due to their increased rate of glycolysis, as shown by the Warburg effect, take in greater amounts of glucose than healthy cells. This difference in uptake is exploited by FDG since it is a molecular analog to glucose. Tumor cells thus accumulate a larger amount of FDG and as the radioactive fluorine

decays, the positron annihilation events can be localized to the precise loci of cancer cells [9].

## **Protein Expression**

The Warburg effect and the distinct differences it states exist between cancerous and healthy cells directly led researchers to look more deeply into the metabolic pathway. These investigations studied multiple proteins involved in cellular fermentation and respiration, as well as regulators of those processes and other glycolytic metabolites and enzymes.

### **Hypoxia-Inducible Factor-1 $\alpha$**

One protein connected to the Warburg effect is hypoxia-inducible factor 1 (HIF-1) and specifically the oxygen-regulated  $\alpha$  subunit (HIF-1 $\alpha$ ) [4]. HIF-1 $\alpha$  is normally expressed in cells under hypoxic conditions [5] and it serves to upregulate the production of proteins that help the cell survive and adapt to a reduced supply of oxygen [6] (See Figure 3). It is important to note that in hypoxic conditions the rate of transcription of HIF-1 $\alpha$  is substantially lower than the rate found in tumor cells in normoxic conditions. HIF-1 $\alpha$  is post-transcriptionally activated in hypoxic cells to initiate the cell's response to the lack of oxygen [10]. Some of the cellular adaptations that HIF-1 $\alpha$  stimulates include the upregulation of red blood cell production [11, 12], increased transcription of vascular endothelial growth factor (VEGF) [12-14], and increased production and membranal localization of a glucose transporter, GLUT1 [14, 15]. While it still gives tumor cells a competitive advantage in normoxic conditions, the upregulation of HIF-1 $\alpha$  in solid tumor masses provides an even bigger edge. Many tumor microenvironments become so tightly

packed that oxygen is scarce and HIF-1 $\alpha$ 's hyperactivity helps keep tumor cells alive [14].

Tumor cells in normoxic conditions show an overexpression of HIF-1 $\alpha$  when compared to normal cells in the same environment [4]. The greater rate of aerobic glycolysis and lower rate of oxidative respiration in tumor cells leads to increased intracellular levels of glycolytic metabolites, specifically pyruvate [1]. Pyruvate promotes the production and stability of HIF-1 $\alpha$  thus creating a positive feedback loop that bolsters the proliferation of cancer cells [16].

### **Pyruvate Kinase**

Another potential marker for cancer based upon the findings of Warburg is pyruvate kinase (PK). In glycolysis, PK catalyzes the reaction of phosphoenolpyruvate into pyruvate by removing a phosphate group which PK uses to generate ATP. A specific form of PK, embryonic M2, has been found exclusively in cancer cells without the presence of the normal M1 isoform [17]. Christofk et al. showed that suppressing PKM2 while artificially adding PKM1 effectively reversed the Warburg effect and promoted aerobic respiration [18]. Tumorigenesis is greatly reduced or inhibited all together in cells with PKM2 knockdown [18]. PKM2 has also been shown to be a response marker to hypoxia as it becomes actively transcribed under low-oxygen conditions [10]. HIF-1 $\alpha$  is a known transcriptional activator of PKM2 [10, 19]. The regulatory relationship between PKM2 and HIF-1 $\alpha$  makes them both good candidates for tumor inhibition.

### **Transketolase**

In normal human cells, transketolase (TKT) is part of the metabolic pentose phosphate pathway. The pentose phosphate pathway contains both an oxidative and a nonoxidative part making it similar to glycolysis and oxidative phosphorylation and thus another candidate for cancer research. Certain carcinomas, specifically epithelial varieties, were shown to express a high level of transketolase-like-1 (TKTL1) [20]. Furthermore, the cellular concentration of TKTL1 directly correlated to the patient prognosis and survival [20]. TKTL1 has also been linked to cancer proliferation and the general regulation of other TKT enzymes [21]. Specifically in ovarian cancer, TKTL1 has been shown to be present at a higher rate and has also been linked to a poorer patient prognosis [22].

The importance of TKTL1 has not yet been fully elucidated, but progress has been made. An experiment that constructed a plasmid containing a coding region for siRNA against TKTL1 showed a substantial decrease in cancerous proliferation when the plasmid was activated [21]. This link demonstrates that TKTL1 plays a role in cancer.

## **Cancer Treatments**

Multiple avenues of treatment for cancer are being examined based upon Warburg's findings. These include inhibiting targets like upstream regulators and promoting the inhibitors of glycolysis. Other methods selectively target and kill cells based upon the concentration of select downstream products.

### **3-Bromopyruvate**

The compound 3-bromopyruvate (3-BrPA) was studied for its effects on glycolysis [23]. It functions by inhibiting the activity of hexokinase II, the enzyme that first phosphorylates glucose when it enters a cell to prevent glucose from freely diffusing out of the cell due to the natural diffusion potential created by the active cellular import of glucose [24]. Initial results with 3-BrPA show a significantly lower number of viable tumor cells than healthy cells when treated with 3-BrPA, as well as an appreciably decreased concentration of ATP in tumor cells versus healthy ones [23]. The decrease in ATP concentration leads to cell death by the activation via dephosphorylation of a proapoptotic protein BAD, a member of the Bcl-2 family [25]. The increase in cellular concentration of BAD occurs along with the movement of BAX from the cytosol into mitochondria, an increase of cytochrome c and other known precursors of apoptosis [23]. Cell death occurs via this pathway at a much faster rate in tumor cells than in healthy cells due to the greater dependence of tumor cells on glycolysis than healthy ones for generation of ATP [23].

3-BrPA is also a natural toxin to normal cells which presents a natural problem for its use as a pharmaceutical, but the understanding of its inhibition of hexokinase II

could lead to chemical derivatives that are less toxic but equally or more effective at targeting tumors. The mechanism of toxicity may be due to 3-BrPA's ability to alkylate a variety of compounds that could also produce a cytotoxic effect in cells outside of hexokinase II inhibition [26]. Given by localized injection *in vivo*, 3-BrPA has a higher success rate and reduced cytotoxic affect [27].

### **Koningic Acid**

Koningic acid (KA) blocks the enzymatic activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by covalently altering a cysteine residue that is essential for GAPDH's metabolic function [28, 29]. GAPDH serves as a catalyst with inorganic phosphate and NAD for the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate, a crucial step in glycolysis. The inhibition of GAPDH by KA causes cells that are dependent upon glycolysis to undergo apoptosis [30]. GAPDH's function in glycolysis causes an increase of phosphofructokinase (PFK) activity in the presence of KA [30]. PFK converts fructose-6-phosphate to fructose-1,6-bisphosphate, a precursor to GAPDH's substrate, while consuming one molecule of ATP. The increased activity of PFK in high-glycolytic cells only serves to further deplete ATP stores and, in the presence of glucose, prompts ATP-depletion related apoptosis by caspase 3 [30]. KA is only effective in cells with a supply of glucose otherwise alternate starvation-induced metabolic pathways may be initiated to prolong cell life and reestablish the tumor's dominance [30].

KA is not a viable option for human cancer therapy because of toxic effects on certain human cells that rely heavily upon glycolysis for energy, such as erythrocytes [30].

### **Inhibition of Hypoxia-Inducible Factor-1 $\alpha$**

Due to the overexpression of HIF-1 $\alpha$  in cancer cells [4], it is a natural target for therapeutic agents (See Figure 3). One potential therapeutic agent is nitric oxide (NO). NO acts as an inhibitor of HIF-1 $\alpha$  by enhancing the activity of HIF-1 $\alpha$ -prolyl hydroxylase (HIF-PH) [31]. HIF-PH selectively ubiquitinates HIF-1 $\alpha$  for degradation by a proteasome. This ability of NO does require at least 1% oxygen concentration [31] so it may not be an ideal treatment for solid tumor masses, but could be used in addition to another drug.

FK228 is another candidate for HIF-1 $\alpha$  suppression. FK228 is a histone deacetylase (HDAC) inhibitor that has shown promise for decreasing cellular HIF-1 $\alpha$  levels as well as lowering HIF-1 $\alpha$ 's binding activity with DNA [32]. HDACs condense chromatin by removing acetyl groups that prevent DNA from binding with histones. FK228 specifically tightens the DNA that is used in the transcription of HIF-1 as well as the DNA regions to which HIF-1 $\alpha$  acts as a transcription factor [32].

FK228 is currently undergoing clinical trials for use as a cancer therapeutic agent.

Direct downregulation of HIF-1 $\alpha$  may also prove as a potential treatment. Short interfering RNA, or RNAi, has been used to knockdown HIF-1 $\alpha$  and has shown an effect on early stage tumor cells [33] prompting the need for further research.

### **2-Deoxyglucose**

An analog of glucose, 2-Deoxyglucose (2-DG), is known to compete with glucose for hexokinase II, an enzyme in glycolysis. Hexokinase II phosphorylates 2-DG to 2-DG-P but cannot progress further down the glycolytic pathway thus inhibiting

hexokinase II from phosphorylating glucose-6-phosphate. Since 2-DG acts as an inhibitor to glycolysis it has the potential to select for cells using anaerobic respiration. The buildup of 2-DG in cells relying upon glycolysis for energy eventually becomes cytotoxic. Cells that are able to use oxidative phosphorylation can rely on other sources such as fats and proteins to produce ATP. This was tested using osteosarcoma cells and healthy bone tissue. Results showed that healthy cells had a 1300-fold increased resistance to the susceptibility of 2-DG's cytotoxic effects versus the osteosarcoma cells [34].

As a cancer treatment, 2-deoxyglucose is promising, but its use is limited because of the cellular reaction that it induces. 2-DG makes ATP production via glycolysis increasingly difficult and thus limits the quantity of ATP present. This activates increased production of HIF that increases transcription of hexokinase and the glucose transport protein, GLUT1 as well as translocation of GLUT1 to the cellular membrane. These two actions by HIF serve to make the cell less susceptible to 2-DG since glucose has more opportunities to enter the cell via GLUT1 and be phosphorylated by hexokinase [34]. Cancerous cells may be more susceptible to treatment with 2-DG in the presence of HIF inhibitors.

2-DG has been shown to be a candidate for dual-drug treatment [35]. By understanding the glycolysis pathway and the precise effect 2-DG has on the metabolic process, it was studied in conjunction with 3-BrPA, a hexokinase inhibitor. 2-DG competes against glucose for phosphorylation by hexokinase II, 3-BrPA reduces the total number of active hexokinase II molecules in the cytosol. Since both drugs work to inhibit glycolysis at the same point in the pathway they were good candidates to study for



a cooperative result. Testing against rabbit tumor cells has shown that the synergistic effect of both drugs results in a strong inhibition of glycolysis and even cell death [24]. However, this strong focus on one target in glycolysis pathway may prove harmful for certain healthy cells too. Healthy cells will be less susceptible to treatment by 2-DG with 3-BrPA because they can rely on other energy sources, like lipids and proteins, and oxidative phosphorylation, but human cells that use glucose as a primary energy source may be adversely affected.

### **Inhibition of AMP-Activated Protein Kinase**

AMP-activated protein kinase (AMPK) is a cellular energy monitor that is activated by high levels of adenosine monophosphate (AMP), which can be induced by hypoxia. While the extent of AMPK's role in carcinogenesis is not fully elucidated, links have been made between AMPK activity and tumor survivability [36]. In addition, inhibition of AMPK has been shown to work synergistically with a chemotherapy agent, cisplatin, by readily inducing p53 mediated apoptosis [37]. This effect is believed to occur because cisplatin-induced cellular stress promotes the activity of AMPK which serves to protect the tumor by increasing ATP concentration and halting replication [37].

### **Glucose Transport Proteins**

Increased transcription of the membranal glucose transport protein GLUT1 has been linked to the presence of a malignancy [38]. This discovery ties into the effect HIF has on GLUT1 transcription [15], GLUT1's role in cellular reproduction, and the results of PET scans because of the increased localization inside cancer cells of the glucose

analog FDG [9]. GLUT1 is a strong candidate for treatment because of cancer cells' dependency on it.

GLUT1 inhibition has been studied in combination with an existing chemotherapy drug, daunorubicin. Phloretin is a known inhibitor of glucose uptake [39]. Using phloretin to prevent glucose uptake and hinder glycolysis made tumor cells more vulnerable to daunorubicin and led to apoptosis [40].

The potential for GLUT1 as a target was further confirmed by transfecting cancer cells with antisense GLUT1 cDNA that successfully suppressed tumor growth. In addition, monoclonal antibody treatment specific for GLUT1 has been shown to be efficacious in slowing tumor proliferation [41]. This treatment has also worked to improve the ability of other chemotherapy agents [41]. While antibody therapy looks promising results have only been gathered *in vitro*. *In vivo* studies may show lethal effects on cell lines that exhibit high levels of GLUT1 such as erythrocytes and blood-brain barrier epithelial cells.

### **Oxythiamine**

Because of its high levels of expression in certain cancer lines, transketolase is a possible target for anticancer therapies. Oxythiamine has shown potential as an inhibitory agent of TKT, a metabolic enzyme in the pentose phosphate pathway. *In vivo* studies in mice resulted in a decrease in tumor growth without toxic effects [42]. While transketolase-like 1 (TKTL-1) siRNA has shown to be effective in restricting cancer proliferation [21], oxythiamine has not been linked to decreased TKTL-1 expression [43]. As well as inhibiting TKT activity, oxythiamine is known to block pyruvate dehydrogenase [44] which could potentially lead to blocking aerobic respiration in

normal cells. Oxythiamine has also been shown to work well in conjunction with dehydroepiandrosterone (DHEA) to arrest cancer cell growth and proliferation.

### **Lonidamine**

Lonidamine was an early candidate for cancer therapy because it was shown to inhibit glycolysis in tumor cells [45]. It is believed to act on mitochondrially-bound hexokinase but the exact mechanism is unknown. Furthermore, lonidamine improves the success of the chemotherapy drug cisplatin [46]. Research with lonidamine and other chemotherapy and alkylating agents is still on-going.

### **Resveratrol**

Resveratrol is a polyphenol and phytoalexin that has been shown to promote tumor apoptosis [47, 48]. It works by both interfering with phosphatidylinositol-3-kinase (PI3K) signaling pathways as well as inhibiting glucose metabolism [48]. PI3Ks have a wide range of effects including the regulation of metabolism and cellular replication. Specifically, resveratrol decreased the activation of PI3K by reducing the phosphorylation of Akt, a PI3K upstream regulator [48]. In addition, Resveratrol was shown to decrease glycolysis through additional inhibitory effects on the PI3K pathway [48]. It has also been found to inhibit glucose intake in ovarian cancer cells [47].

Resveratrol is a naturally occurring compound in red grapes among other foods and can also be chemically synthesized making it easily available and affordable.

Table 1. Summary of prospective therapeutic targets and the effect of selected drugs.

<b>Target</b>	<b>Drug(s)</b>	<b>Effect</b>
Hexokinase II	2-DG	Glycolysis inhibition leading to cell death
	3-BrPA	Decrease in intracellular ATP; apoptosis
	Lonidamine	Glycolysis inhibition
GAPDH	KA	Apoptosis by caspase 3
HIF-1 $\alpha$	FK228	Histone condensation; HIF-1 $\alpha$ downregulation
	NO	HIF-1 $\alpha$ ubiquitination and degradation
GLUT1	mAb	Reduced tumor proliferation
Transketolase	Oxythiamine	TKT inhibition
	siRNA	TKTL1 knockdown
PI3K	Resveratrol	Inhibition of Akt\PI3K signaling pathway

## **Future Research**

The Warburg effect, known for over 80 years, has provided the premise for numerous advances in cancer research. There are still yet many areas that need to be elucidated.

### **Pyruvate Kinase M2**

Even though PKM2 has been directly linked to the Warburg effect [18], the mechanism by which this occurs is unknown. One possible explanation is that in tumor cells without PKM1, excess growth factor produced by other carcinogenetic processes may trigger the suppression of fructose-1,6-bisphosphate which is an allosteric activator of PKM2. It has been suggested that without the activity of both PKM1 and PKM2 cells switch to aerobic glycolysis for energy production [18]. Another suggested explanation is that PKM1 and 2 play a role in intracellular metabolite motility and that PKM1 upregulates the movement of pyruvate to the mitochondria, while PKM2 sequesters pyruvate to lactate dehydrogenase [18].

While certainly plausible, the precise pathway by which cellular metabolism switches from aerobic respiration to aerobic glycolysis and what function PKM2 has are not clear. The interactions of PKM1 and 2 with pyruvate dehydrogenase and lactate dehydrogenase should be studied further to determine if there is preferential movement between either of the PKs and the subsequent enzyme in the glycolysis pathway.

### **GLUT1**

A plethora of research has been done connecting the upregulation of GLUT1 transcription and increased localization of GLUT1 in the cellular membranes of tumor cells [15, 38]. The selective increased utilization of GLUT1 for glucose intake by cancer cells makes it a potent target for the development of therapeutic agents. As was already mentioned, treatment by GLUT1 inhibition may adversely affect normal human cells that also rely heavily on GLUT1. This problem could potentially be avoided by increasing the selectivity of the treatment or by using a lower dosage in combination with another drug.

The monoclonal antibody inhibition of GLUT1 demonstrated by Rastogi et al. tested *in vitro* [41] should be repeated in an animal model to test for wider physiological toxicity. Direct injection of the antibody treatment into a solid tumor mass may prove to limit any potential toxic effects. Furthermore, studying different antibody doses in conjunction with chemotherapy agents in an animal model could increase the potency of other drugs while limiting adverse physiological effects.

### **Dual-Drug Treatments**

While many of the aforementioned treatments only provide partially successful results, some may show further efficacy when used in combination with other treatments. Some studies have already been done that show the potential for synergistic effects.

### **2-Deoxyglucose and Lonidamine**

Following in the footsteps of the study conducted utilizing both 3-BrPA and 2-DG [24], another study should be done looking at the effectiveness of 2-DG with lonidamine. 2-DG and lonidamine both work to inhibit glycolysis at the conversion of glucose to

glucose-6-phosphate by hexokinase. 2-DG actively competes with the normal substrate of hexokinase II, glucose [34] and lonidamine has been shown to reduce the activity of hexokinase II altogether [45]. Using these two drugs together for treatment could prove to effectively decrease the rate of glycolysis in tumor cells more than either one individually and should be the subject of future research.

Due to 2-DG's strong inhibition of glycolysis it may prove to be harmful to certain human cell types that rely heavily on glucose for energy such as erythrocytes, epithelial cells in the brain, and male germ cells [26]. However, a clinical trial testing the efficacy and safety of 2-DG administration in coordination with radiation therapy showed that it could be given safely to patients at 250mg/kg [35].

### **2-Deoxyglucose and HIF Inhibition**

The main efficacy problem with 2-DG as a cancer therapeutic is the intrinsic cellular response that follows its absorption and interaction with hexokinase. The scarcity of ATP caused by 2-DG competition with glucose stimulates a cell stress response mediated by HIF that amplifies the transcription of hexokinase, increasing the quantity of the molecule that 2-DG and glucose compete over, and also promotes the transcription and translocation of GLUT1 to the membrane, thereby increasing the uptake of both 2-DG and glucose into the cell from the extracellular environment. This natural cell response makes the effective dose of 2-DG very high and thus more likely toxic to healthy cells [34].

To circumvent this cellular response, a combined treatment with NO or FK228 may prove to be a viable option. By downregulating the transcription of HIF-1 $\alpha$ , the

cellular stress response to 2-DG and the induced ATP shortage may be restricted and thus help hinder the growth of cancer cells or kill them altogether.

### **Alternate Theories**

Although the Warburg effect has led to many promising advances in the study of cancer, alternate theories exist that present contradictory evidence. One such theory involves cancer cells using glutamine metabolism in the citric acid (TCA) cycle [49] (See Figure 4). This is particularly controversial because one of the main facets of Warburg's theory is that oxidative metabolism is damaged and glycolysis is the pathway used to meet the tumor's energy needs. In tumor cells, glutamine metabolism has been specifically linked to the production of oxaloacetate, which is a required molecule for the first step in the TCA cycle. In healthy cells undergoing rapid growth, the consumption of glutamine is exhibited at high rates. In cancer cells, this base utilization has additionally been tied to an increase of NADPH production by malic enzyme that suggests that glutamine may also play a role in the synthesis of fatty acids and nucleotides, potentially from precursors in the TCA cycle, that may be critical to tumor proliferation [49].

With the TCA cycle active in cancer cells, this knowledge goes beyond Warburg's theory because it opens up many potential biosynthetic pathways that could contribute to the maintenance and growth of cancer cells. Additionally, certain cancers have been shown to not exhibit the Warburg effect. This may be because they do not use glucose as



their primary source of energy and instead rely on another molecule. Glutamine is an interesting candidate for further research because it may provide another target for treatment that extends beyond the limitations of Warburg's original postulate [50].

## **Conclusion**

A report released by the American Cancer Society estimates 1,437,180 new cancer cases in the United States in 2008. This report also states that approximately one out of every four deaths in the US is cancer related [51]. These statistics clearly show the importance and need for cancer research. In the 84 years since Otto Warburg demonstrated aerobic glycolysis as a trait of tumor cells, scientific progress has been immense. The PET scan has been proven to be an effective, safe and non-invasive detection tool. Many drug candidates such as FK228, 2-DG and 3-BrPA have shown promising results [23, 32, 34].

Research on cancer is far from over. Efforts must continue to improve drug therapy and to find the most efficacious and safe treatments. Studies looking into other detection methods such as screening for genetic predispositions should still be carried on. To this end, research needs to be done to gain a better understanding of the exact cause for the hypoxic response and the unique metabolic profile exhibited by tumor cells that is already advantageously used by existing therapies. This information will be an invaluable asset in selecting the best diagnostic methods and therapeutic agents.

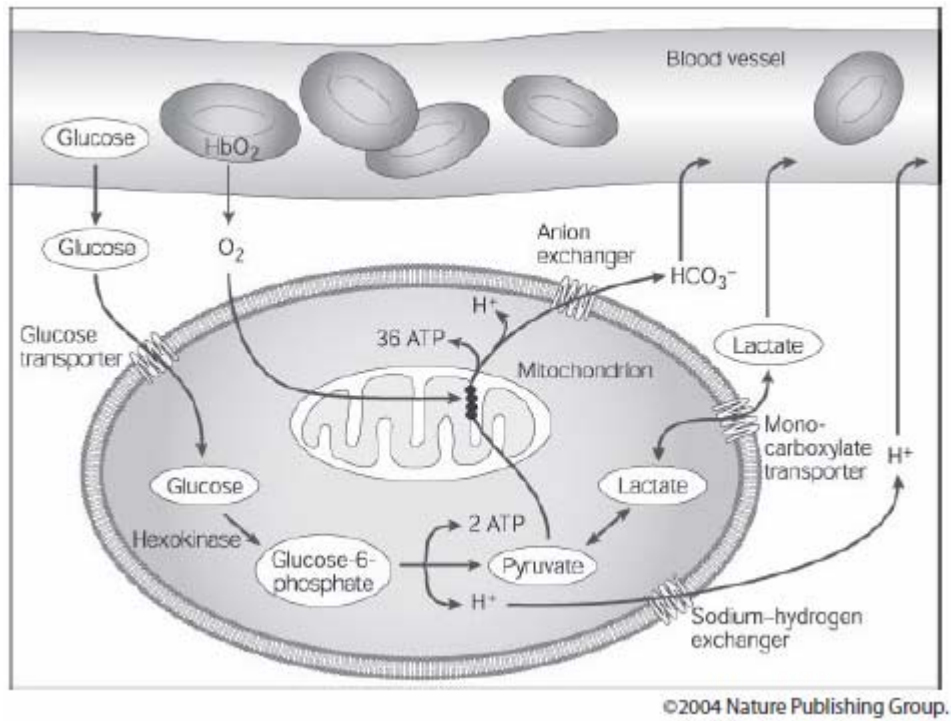


Figure 1. Summary of cellular metabolism. (Source: Garber, K., *Energy Boost: The Warburg Effect Returns in a New Theory of Cancer*. J. Natl. Cancer Inst., 2004. **96**(24): p. 1805-1806.)

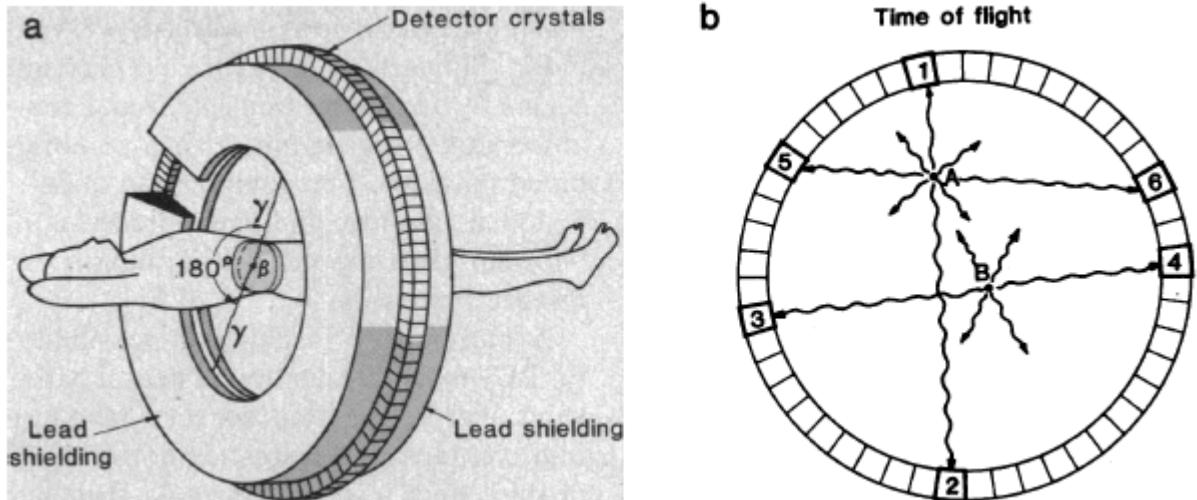


Figure 2. (a) Schematic drawing of a PET machine. (b) Concept drawing of positron decay. (Source: Brownell, G.L., et al., *Positron Tomography and Nuclear Magnetic Resonance Imaging*. Science, 1982. **215**(4533): p. 619-626.)

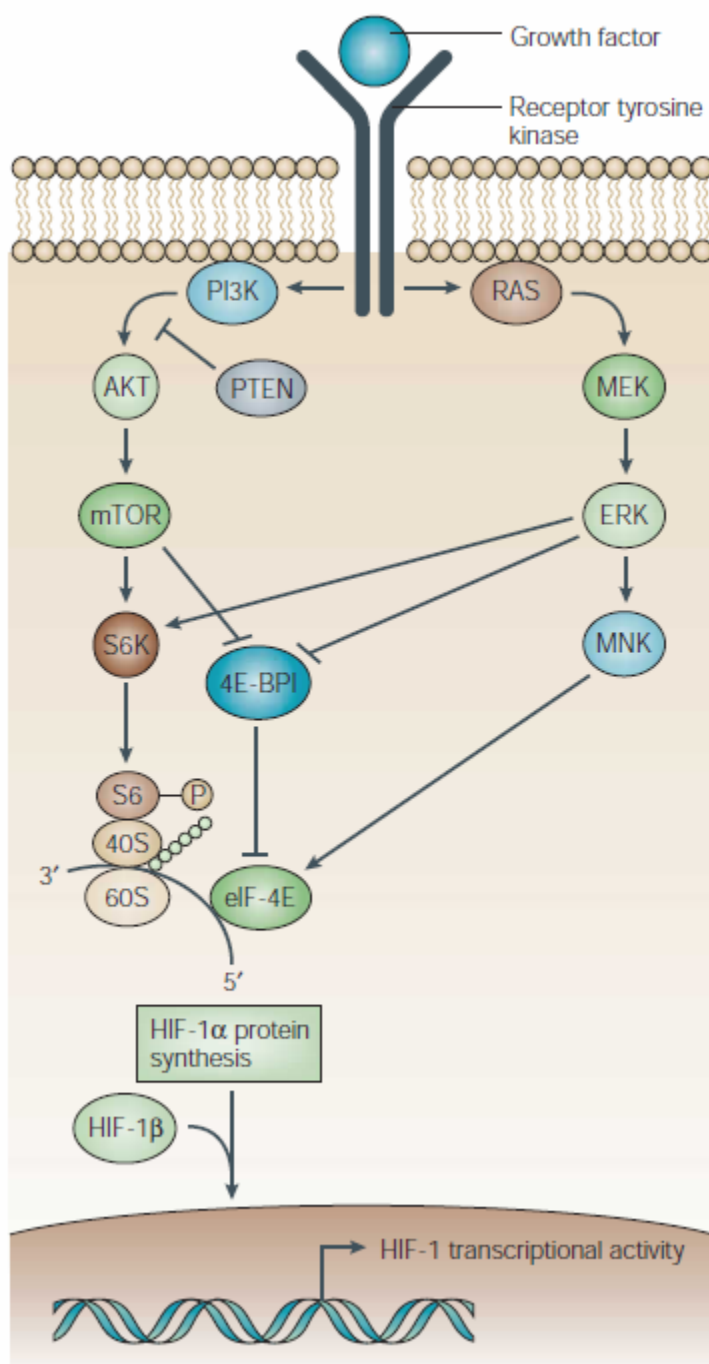


Figure 3. Hypoxia-Inducible Factor 1 $\alpha$  Regulatory Pathway. (Source: Semenza, G.L., *Targeting HIF-1 for cancer therapy*. Nat Rev Cancer, 2003. **3**(10): p. 721-732.) HIF-1 $\alpha$  is a transcription factor that activates the synthesis of numerous proteins that promote angiogenesis, cellular growth and metabolism, and survival pathways. Study of the inhibition of HIF-1 $\alpha$  is undergoing as a potential cancer treatment.

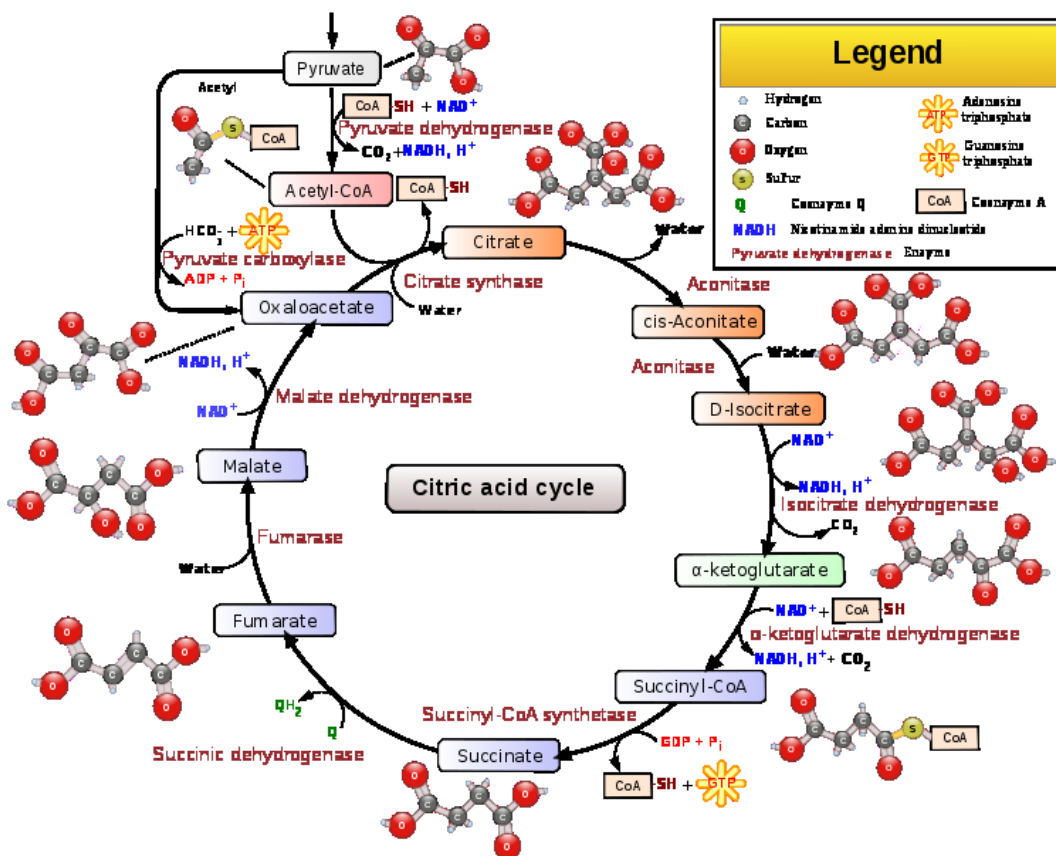


Figure 4. The TCA cycle

(Source: [http://en.wikipedia.org/wiki/File:Citric\\_acid\\_cycle\\_with\\_aconitate\\_2.svg](http://en.wikipedia.org/wiki/File:Citric_acid_cycle_with_aconitate_2.svg). Licensed under the GNU Free Documentation License. Accessed 19 January 2008.)

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