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### Cancer as a Metabolic Disease

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# Cancer as a Metabolic Disease

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## Abstract

Despite decades of intensive scientific and medical efforts to develop efficient and effective treatments for cancer, it remains one of the prime causes of death today. For example, in 2016, there will be an estimated 1,685,210 new cases of cancer and 595,690 deaths due to cancer in the United States alone (National Cancer Institute). Worldwide in 2012, there were an estimated 14 million new cases of cancer and 8.2 million deaths due to cancer. In order to come up with better methods of detection and more successful modes of treatment, it is crucial that scientists understand the depth of not only what causes cancer but also what sustains it. This literature review examines cancer as a metabolic disease. More specifically, it summarizes carbohydrate metabolism and compares and contrasts the roles of the glucose transporter, the metabolic enzymes hexokinase, pyruvate kinase, citrate synthase, succinate dehydrogenase, cytochrome c oxidase, ATP synthase, and the tumor suppressor protein p53 in normal versus cancer cells. The review focuses on the altered cellular function of these molecules and the significance of their dysfunctionality in the transformation of normal to cancer cells.

## Introduction

Research has established that cancer is not a single disease but multiple diseases and epigenetic change contributes to its formation (Hirschey et.al, 2015). Cancer is characterized by unrestrained cellular proliferation, which may arise from nuclear genetic mutations or from mitochondrial DNA damage which alters the metabolic pathways – a network of chemical reactions either building (anabolism) or breaking (catabolism) molecules in an organism – of a cell.

The hallmarks of cancer comprise six biological functions acquired during the development of human tumors, which include: 1) cell proliferative signals; 2) evading growth suppressors (also known as tumor suppressors); 3) inhibition of

apoptosis (cell death); 4) enabling replicative immortality; 5) inducing angiogenesis and 6) activating invasion and metastasis. Conceptual progress in the last decade has added two emerging hallmarks to this list: evading immune destruction and reprogramming of energy metabolism (Weinberg & Hanahan, 2011)

This review focuses on several differences in carbohydrate energy metabolism between normal and cancer cells and their significance in the cause and/or maintenance of the malignant phenotype. Carbohydrate metabolism comprises the different biochemical processes responsible for anabolism and catabolism of carbohydrates in an organism. Glycolysis and the citric acid cycle are two of the many biochemical processes used by cells for the

catabolism of glucose molecules, which are ultimately converted into useable energy in the form of ATP. Normal cells produce up to 32 ATP molecules per glucose molecule oxidized through glycolysis, citric acid cycle, and oxidative phosphorylation. 2 ATP molecules are produced from glycolysis in the cytosol and the other 30 in the oxidative phosphorylation using NADH and FADH<sub>2</sub> generated from glycolysis and the citric acid cycle.

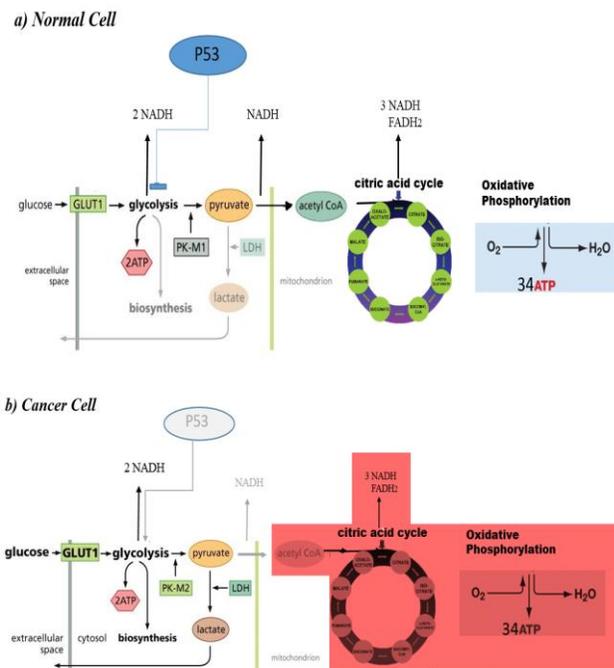
In the 1920s, Dr. Otto Warburg first observed the glucose metabolism in normal vs. cancer cells. He observed that the amount of glucose uptake in cancer cells was significantly greater than that in normal cells. He also observed that while normal mammalian cells usually convert all of their glucose into pyruvate via glycolysis, cancer cells converted more than half of their glucose into lactate (M.D. Hirschey et.al, 2015) in the presence or absence of oxygen (Bensinger & Christofk, 2012). This effect is known as the Warburg effect (Bensinger & Christofk, 2012). However, the mechanism of how cancers do this is yet to be determined.

One metabolic aberration in cancer cells includes a broken link between glycolysis and the oxidative phosphorylation (OP). OP is oxygen dependent whereas glycolysis is oxygen independent. Cancer cells take advantage of glycolysis being independent of oxygen and thus excel at it, breaking the homeostatic link between glycolysis and OP. The rate of ATP production in cancer cells is 100 times faster than that of normal cells. Thus, even by taking an insufficient pathway for ATP production, cancer cells are able to proliferate at high rates. Where normal cells are strategically programmed to work

together to build various types of tissues needed for organismic survival, cancer cells have a different agenda. All they want to do is make more copies of themselves and the Warburg effect helps them with that (Weinberg, 2014).

### 1. Glucose transport

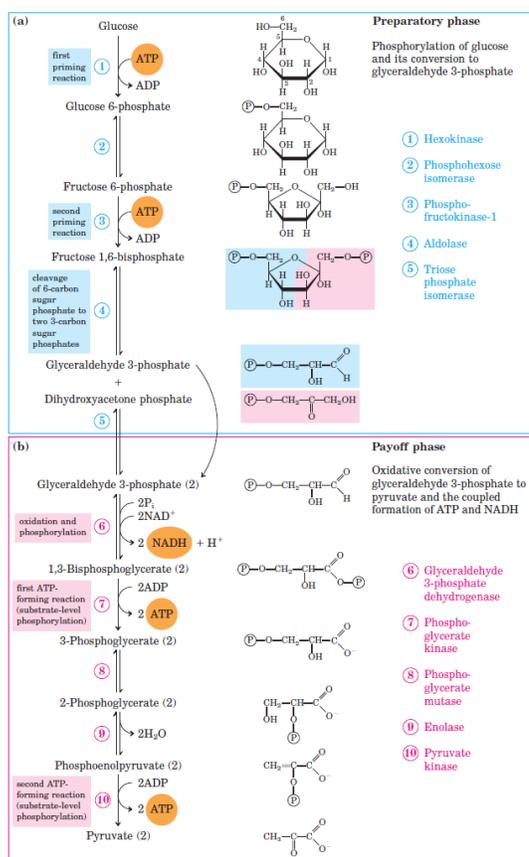
Normal and cancer cells alike need glucose for survival. Our cells acquire glucose from the blood stream via transporters located on the cell's membrane. The glucose transporters (GLUT) are a group of 14 different uniporter membrane proteins that transport glucose through the plasma membrane into the cell. GLUT-1, which is encoded by the SLC2A1 gene in humans, is of primary importance to this review (Serra et.al, 2014). In normal cells, the level of GLUT-1 on the membrane fluctuates depending on how much glucose is available in the blood stream, such that the GLUT-1 level is inversely proportional to glucose level. However, it has been observed that GLUT-1 is expressed in high levels in many tumors, regardless of the level of glucose present in the blood stream (Serra et.al, 2014). This allows for an increase in glucose uptake to serve the high energy demands of cancer cells (i.e, rapid, uncontrolled cell division). High levels of GLUT-1 are also found in fetal tissues, adult erythrocytes, and endothelial cells. Interestingly, cancer cells are known to mimic fetal and undifferentiated cells in many ways.



**Fig. 1: Difference in Metabolism between Normal vs. Cancer cells.** The figure shows the glucose metabolic pathway in a) a normal cell and b) in cancer cell. In a) a normal cell, glucose is transported into the cell via GLUT-1 where catabolism takes place through glycolysis, where glucose is committed to catabolism by HK (not shown) and broken down in to pyruvate by PKM-1. 2 ATP molecules are generated in this process. Pyruvate is then transported in to the mitochondria where it is converted to acetyl-coA which goes through the citric acid cycle. The NADH and FADH<sub>2</sub> generated from glycolysis and citric acid cycle goes through oxidative phosphorylation (OP) through which the proton gradient (not shown) is established, O<sub>2</sub> is reduced to form water, and 32 ATP molecules are synthesized. Increase levels of P53 (*refer to 5. P53*) inhibits glycolysis when necessary blocking glucose metabolism with no net ATP production. In b) cancer cell, glucose is transported in the cell via GLUT-1 where catabolism takes place through glycolysis, where glucose is committed to catabolism by HK2 (not shown) and broken down in to pyruvate by PKM-2. Pyruvate is then converted to lactate by LDH and leaves the cell creating an acidic environment (not shown). The chain between glycolysis and OP is broken (marked as red) and P53 is either silenced or completely lost from the cell.

## 2. Glycolysis

Once glucose enters the cytosol, it can undergo oxidation by glycolysis, a series of 10 enzyme catalyzed chemical reactions in which glucose is broken down to two pyruvate molecules (as shown in **Fig.2**). Energy is required in the first half of the process and then generated in the second half. For each glucose molecule, 2 molecules of pyruvate, 2 NADH, and 2 ATP molecules are generated (Pratt & Cornely, 2014).



**Fig.2: Glycolysis.** A series of 10 enzyme catalyzed chemical reactions in which glucose is broken down to two pyruvate molecules. There is a preparatory phase where two ATP molecules are consumed and a payoff phase where 4 ATP and 2 NADH molecules are generated, producing a total of 2 ATP, 2 NADH, and 2 Pyruvate molecules in the end (Giri, 2016).

### *Hexokinase*

Hexokinase (HK) catalyzes the first step of glycolysis in the cytosol (in most glycolytic pathways) converting glucose to glucose-6-phosphate (G6P). This is a rate-limiting step for glycolytic pathway, which commits glucose molecules to undergo complete oxidation (Bioinformatics, 2016). The role of HK in committing glucose molecules to energy metabolism may play a crucial role in supporting cancer, since these cells require an abundance of glucose energy for survival. HK is an enzyme located on the outer membrane of mitochondria, which is expressed in four isoforms, HK1, HK2, HK3, and HK4 all of which are found in humans (Patra, et.al 2013). HK1 is common in various types of cells in adults, HK4 is common in liver and pancreatic cells, little is known about HK3, and high levels of HK2 have been observed in many types of cancer including epithelial ovarian, breast, and colon cancer.

HK2 has two catalytic domains, the N- and C-terminals (Ahn et.al, 2009). Both domains have enzyme activity with N-terminal having a higher activity than the C-terminal. Even though there are two binding sites for glucose on HK2, only one molecule binds at a time. Studies suggest that when one glucose molecule binds on one of the two sites, it causes the enzyme to go through a conformational change, which then prevents another glucose molecule from binding to the enzyme during catalysis (Cardenas et.al, 1998). It is not completely known why HK2 has two glucose binding sites when it only catalyzes one molecule at a time.

High levels of HK2 are of great significance to cancer cells as observed in

many different studies. In one of the studies, tissues of 48 colorectal cancer patients were studied for mRNA expression of proteins including but not limited to HK2, GLUT-1, PKM2, and VDAC-1 (Graziano et.al, 2016). Significantly high levels of these proteins were observed in primary tumor and liver metastasis with respect to the levels in normal mucosa. Other experiments have shown enhanced glycolytic rate when solubilized HK2 was added in a liver tumor cytosol (Hirschey et.al, 2015). In mouse models of kRas-driven lung cancer and Erb-driven breast cancer, HK2 was shown to be necessary to initiate and maintain tumor cells (Patra et.al, 2013).

Studies have shown a correlation between low levels of HK2 and down-regulation of the Warburg effect (a decrease in the rate of glycolysis). In one study of epithelial ovarian cancer (EOC), low levels of Glucose transporter 1 (GLUT1), HK2, and down-regulation of the Warburg effect and cell proliferation were observed when a transcription factor known as FOXM1 gene expression was suppressed (Wang et.al, 2016). FOXM1 bind directly to the promoter region of GLUT1 and HK2 gene and promotes synthesis at the transcriptional level, so when FOXM1 was knocked down, the levels of GLUT1 and HK2 were observed to decrease (Wang et.al, 2016). In another study, the inhibition of HK2 was shown to improve the effects of anticancer drugs (Peng, et.al, 2009). Thus high levels of HK2 may very well be involved in the alteration of cancer cell's metabolism and the transformation of normal cells into cancer cells. The mechanism of how HK2 may be involved in tumorigenesis, however, is still unknown.

### *Pyruvate Kinase*

Pyruvate Kinase catalyzes the last step in glycolysis where it converts phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP. This is another rate limiting step in the glycolytic path after HK2 (Bensinger & Christofk, 2012). The PKM gene encodes for isoenzymes PKM1 and PKM2. They are located in the cytosol within the glycolytic enzyme “complex”. The two isoforms only differ in 23 amino acids within a 56 amino acid band on the C-terminus. PKM1 is commonly found in normal cells and assures the import of pyruvate in to the mitochondria, whereas PKM2 is active in embryos and more common in tumor cells where it diverts pyruvate to lactate dehydrogenase-A (LDH-A), which reduces pyruvate to lactate. Our focus is on PKM2 which is expressed in organs with high energy demands such as muscle, brain, and all cells that have high rate of nucleic acid synthesis, such as normal proliferating cells, embryonic cells, and tumor cells (Gupta & Bamezai, 2010).

PKM2 is present in two forms, tetrameric and dimeric. The tetrameric form has high affinity to PEP and is highly active at physiological PEP concentration, whereas the dimeric form has low affinity to PEP and is almost inactive at physiological PEP concentration. The dimeric form of PKM2, termed as Tumor M2-PK, is observed in many tumor cells (Mazurek, et.al, 2005). An increase in glycolysis helps to meet energy demands of high synthesis in cancer cells and PKM-2 plays an important role in generating synthetic intermediates. The low tetramer to high dimer ratio of PKM2 effects the conversion of glucose. High levels of dimeric form of PKM2 favors the conversion of glucose to lactate, whereas the

tetrameric form favors the conversion of glucose to pyruvate.

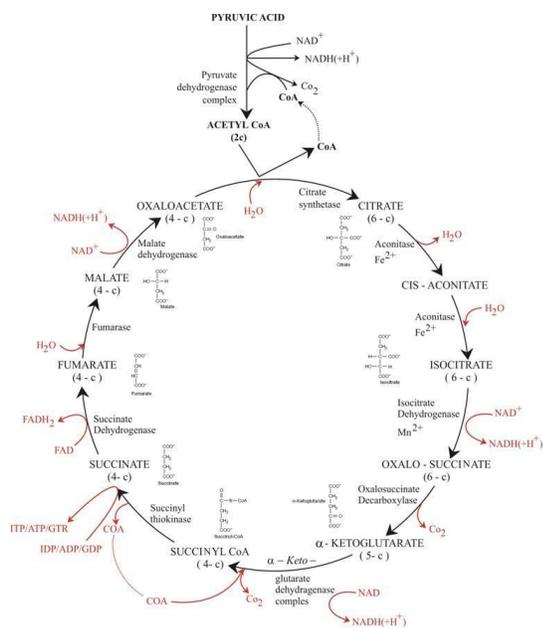
The alteration of normal enzymes within the glycolytic complex hinders how the intermediates are processed. The shift from oxidative phosphorylation to glycolysis for ATP production with feedback inhibition accelerates glycolysis, which leads to enhanced lactate production. Glycolysis becomes the fastest way for energy production as well as the best pathway to synthesize nutrients (that are the intermediates) to help with cell proliferation. (Icard, 2011).

In one study that investigated the effects of PKM2 expression in osteosarcoma (Liu, et.al, 2016); PKM2 expression was observed to be elevated in cancerous tissues with respect to the adjacent normal tissues. The same study also showed an overall decrease in patient survival rate in the presence of high PKM2 levels than low PKM2 levels. In another study, silencing of PKM2 was shown to increase docetaxel (a chemotherapy drug) sensitivity in cells leading to strong suppression of cell viability (Yuan, et.al, 2016). High levels of PKM2 can be detrimental for patients and inhibition may increase the survival rate. This suggests the great importance of PKM2 levels present in cancer cells.

Other experiments have also shown that the growth of tumors depend on PKM2 expression, high levels of GLUT-1, and LDH-A. Inhibition of either of these proteins have been observed to noticeably slow down tumor growth. These observations show that the altered glucose metabolism of cancer cells creates a physiological system favorable for cancer growth and proliferation (Weinberg, 2014).

### 3. Citric Acid Cycle

Citric acid cycle, also known as the Tricarboxylic acid (TCA) or krebs cycle, is a pathway located in the inner matrix of mitochondria. When pyruvate enters the mitochondria it is converted into acetyl-co-A, which then proceeds in the TCA cycle, producing 2 CO<sub>2</sub>, 3 NADH, 2 FADH<sub>2</sub>, and 1 ATP molecule. Note that amino acids, fatty acids, and carbohydrates can enter the cycle and go through the same process as well. The cycle is a series of enzyme catalyzed chemical reactions (as shown in **Fig. 3**), where the intermediates can serve as precursors of many reactions and products of different biological molecules (Pratt and Cornely, 2014).



**Fig.3: Citric Acid Cycle.** The cycle is a series of enzyme catalyzed chemical reactions that take place in the mitochondria to catabolize pyruvate. The intermediates can serve as precursors of many reactions and products of different biological molecules (not shown). 2 CO<sub>2</sub>, 1 ATP, 3 NADH, and 2 FADH<sub>2</sub> molecules are generated (<http://www.chemistrylearning.com/krebs-cycle/>).

### Citrate Synthase

Citrate synthase (CS) is an enzyme in the first step in the citric acid cycle (commonly assumed to be the rate limiting step) in which it catalyzes the condensation reaction of acetyl-coA and oxaloacetate to citrate. It is located in the mitochondrial matrix, transcribed by the nuclear DNA, and serves as a central enzyme in the process of glucose oxidation. It is comprised of 437 amino acids, which are organized into two subunits with 20 alpha helices each. Three quarters of the helices have tertiary structure while the remaining have irregular structure and a beta sheet composed of 13 amino acid residues. The active site is roughly located between the two subunits where each subunit has a binding site, one for acetyl-coA and the other for oxaloacetate (<http://pdb101.rcsb.org/motm/93>). High levels of ATP:ADP, acetyl-coA:CoA, and NADH:NAD ratio inhibit CS; these signal that the cell has enough energy and does not need to generate more energy until the ATP, acetyl-coA, and NADH levels decline.

The catalysis of the condensation reaction of acetyl-coA and oxaloacetate to citrate by CS means that CS activity is proportional to citrate concentration. Increase in CS activity will yield high levels of citrate, which has shown anti-cancer properties that sensitize cancer cells to chemotherapy (Icard, et.al, 2012). In normal cells, the Pasteur Effect – the process of fermentation inhibited in the presence of oxygen (opposite of the Warburg effect) – is regulated by ATP and citrate. Under hypoxic conditions, the cell undergoes oxidative stress that results in low level of ATP and citrate in the mitochondria. This in turn increases the activity of phosphofructokinase-1 (PFK1) – the enzyme

catalyst in the third step of glycolysis – which in turn increases the rate of glycolysis to generate sufficient energy to keep the cell alive (Icard, et.al, 2012).

Alterations in CS activity have been observed in many types of cancers (Chen et.al, 2014). In a study of ovarian carcinoma, the cell line of benign tumor was compared with normal human ovarian surface epithelium, and ovarian tumor cell line was compared with ovarian cancer cell line (Chen et.al, 2014). High levels of CS were observed in benign versus normal and tumors versus cancer cell line. These observations show the proportionality between cell transformation and CS levels and suggest that CS expression is correlated with tumor progression. It has also been observed that the knock down of CS in ovarian cancer cell lines slowed down the process of cell proliferation and inhibited invasion and migration of cancer cells in vitro. The knock down also helped sensitize cancer cells to chemotherapeutic drugs like cisplatin, which subsequently showed enhanced apoptotic results (Chen et.al, 2014). In another study, however, the effects of knocked down CS were analyzed in human cervical cancer cell lines in which the silencing of CS showed an increase in cell proliferation, invasion, and migration in vitro (Lin, et.al, 2012). The metabolic shift of normal cells to cervical tumor cells was observed and results showed that low levels of CS corresponded to the switch from normal aerobic respiration to glycolysis (Lin, et.al, 2012). The contradiction regarding the effects of CS activity in different types of cancer is one of the many examples demonstrating that cancer is not a single disease but multiple diseases with distinct alteration in metabolism that thus require different treatments.

It is interesting to note how CS activity regulated in normal cells under hypoxic conditions resembles the activity in cancer cells. The majority of tumor cells are found to be hypoxic and exhibit the same kind of behavior, which is catabolizing glucose in to higher yields of lactate than pyruvate.

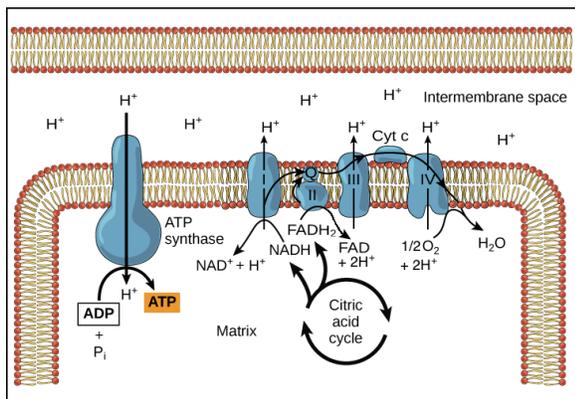
#### *Succinate Dehydrogenase*

Succinate Dehydrogenase (SD) is one of the many tumor suppressor protein (encoded by a tumor suppressor gene, which regulates cell cycle by slowing down cell division, repairing DNA, and inducing apoptosis when necessary) located on the inner membrane of the mitochondria (King et al, 2006). It is involved in both, the citric acid cycle and the oxidative phosphorylation (OP) also known as the electron transport chain (ETC), which will be discussed later in this review. SD catalyzes step 7 of the citric acid cycle and is also known as complex II in OP where it couples the oxidation of succinate to fumarate in the citric acid cycle with the reduction of ubiquinone to ubiquinol in OP. Mutations in SD are found to promote cancer transformation through the production of carcinogens (Ciriolo & Cardaci, 2012). The mechanism and detailed effects of SD in cancer metabolism is still under investigation.

#### **4. Oxidative Phosphorylation**

Oxidative Phosphorylation is the main source of ATP production in normal cells. It is the final stage of catabolism of metabolic fuels, a process whereby free energy from the transfer of electrons is conserved in a transmembrane gradient of protons that is then used to power ATP synthesis (Pratt & Cornely, 2014). The OP pathway is comprised of 5 complexes as shown in

**Fig.4**, two of which are of particular significance in cancer cell metabolism.



**Fig.4: Oxidative Phosphorylation.** There are five complexes involved in oxidative phosphorylation located in the inner mitochondrial membrane space. Complexes I-IV are in sequence on the right and complex V is shown on the left. Complexes I and II take the electrons from NADH and FADH<sub>2</sub> generated from the citric acid cycle and pass it on to complex III and IV through ubiquinone and cytochrome c. During this process, protons are pumped from the matrix into the intermembrane space creating a proton gradient. Complex V then utilizes the proton gradient to synthesize ATP from ADP and P<sub>i</sub> (<https://www.khanacademy.org/science/biology/cellular-respiration-and-fermentation/oxidative-phosphorylation/a/oxidative-phosphorylation-etc>).

#### *Cytochrome c oxidase (Complex IV)*

Cytochrome c oxidase (COX), also known as complex IV, is a large transmembrane protein located on the inner membrane of mitochondria. It is the central part of the oxidative phosphorylation metabolic pathway where oxygen is reduced. In mammals, it is composed of 13 subunits, 10 of which have a nuclear origin and the other three have a mitochondrial origin (Krieg, et.al, 2004). Each complex has multiple metal prosthetic groups that are integral to the electron transfer process. There are two hemes present on the complex, one of which forms a binuclear center (a<sub>3</sub>-Cu<sub>B</sub>) that carries out oxygen

reduction. Four cytochrome c molecules transfer 4 electrons, one at a time, to Cu<sub>A</sub>, which passes the electrons to cytochrome, and then on to the binuclear center where oxygen is reduced and 2 water molecules are formed. During this process, COX attracts 8 protons, 4 of which are used in the formation of water molecules and the other 4 are pumped out in the intermembrane space enhancing the proton gradient (Pratt & Cornely, 2014).

COX is present in three configurations: fully oxidized (also known as pulsed), partially reduced, and fully reduced. Different types of inhibitors have affinity towards different conformational states. Cyanide, for example, is a competitive inhibitor and has a high affinity to the partially reduced state of COX. It binds slowly but efficiently to binuclear site when the complex is in that state. On the other hand, high levels of ATP can allosterically inhibit cytochrome c oxidase (Arnold & Kadenbach, 1997).

Since COX is a central part of the oxidative metabolism, an alteration in this enzyme may have proportional effects on alterations in metabolism seen in cancer cells (Krieg et.al, 2004). In cancer cells, the homeostasis between the glycolytic pathway and oxidative phosphorylation is disrupted. The high level of glycolysis is used to acquire immense level of ATP production and the cell is not dependent on oxidative phosphorylation (OP) to fulfill its energy needs, unlike normal cells. Studies have shown an increase in the level of nuclear encoded versus mitochondrial encoded COX subunits in tumor derived cell lines of prostate and urothelial epithelium (Krieg et.al, 2004). High activity of COX was also observed in these cell lines and was

correlated with an increase in glycolysis. High activity of COX is thought to assist tumor by depleting the citric acid cycle intermediates, which will lower the citrate concentration so it cannot inhibit PFK, therefore creating an environment that favors high rate of glycolysis (Krieg et.al, 2004). Further research needs to be done to understand the correlation between the COX proteomics and its link to metabolic alteration.

Another study has shown the correlation between altered COX function and the Warburg effect, a hallmark of metabolic reprogramming. In this study, two of the 10 nuclear encoded COX subunits, IVi1 and V<sub>b</sub> were knocked down to disrupt the COX normal function (Srinivasan et.al, 2016). These cytochrome c oxidase knock down (COXKD) cells showed a loss of COX activity, which was linked to the metabolic switch to glycolysis. The knock down of either subunit showed an increase in HK and phosphofructokinase (PFK) in cells. This led to an increase in glucose consumption and GLUT4 mRNA levels by greater than 2 folds compared to normal cells. However, the level of GLUT-1 was not affected, which is quite interesting as GLUT-1 is found in high levels in many cancer types. All these observations show a link between the disrupted COX and metabolic reprogramming in cancer cells. The COXKD cells were observed to have invasive phenotype, a decrease in transmembrane potential, and upregulation of genes that regulate cell growth, motility, invasiveness, and other hall marks of cancer cells. The induction of tumorigenesis may potentially be caused by defected COX, which may very well be used as a biomarker (Srinivasan et.al, 2016).

### *ATP Synthase (Complex V)*

ATP Synthase (ATPs), also known as complex V, is a part of the OP metabolic pathway. It is involved in the catalysis of ATP synthesis by forming phosphoanhydride bond between ADP and P<sub>i</sub>, as well as in the decomposition of ATP to ADP and P<sub>i</sub>, and thus serves as the rate limiting step in OS (Whitford, 2005). ATPs is located on the inner membrane of mitochondria and has two domains, F<sub>o</sub> and F<sub>1</sub>, both of which are known to have different biological and chemical properties. F<sub>o</sub> is the hydrophobic domain embedded in the inner mitochondrial membrane where it functions as a proton translocator using the proton gradient created by the electron transport chain. F<sub>1</sub> is the hydrophilic domain located in the mitochondrial matrix and consists of 5 different subunits. F<sub>o</sub> and F<sub>1</sub> domains of ATPs work together by using the energy generated from the proton gradient to form or break the phosphoanhydride bonds for the synthesis or decomposition of ATP (Whitford, 2005).

ATPs plays an important role in human carcinoma, and its malfunction is known to be involved in mediation and progression of different types of human pathologies (Cenizo et.al, 2010). Studies have shown the importance of ATPs and how the malfunctioning could contribute in carcinogenesis. In normal cells, a protein known as inhibitory factor 1 (IF1) is known to prevent ATPs from shifting to ATP hydrolysis under hypoxic conditions. The expression of IF1 varies among different tissues, as it is highly expressed in heart, moderately in liver, and almost negligible in breast, colon, and lung. In human carcinoma, studies have shown that IF1 is found in abundance even in the cells that

once had negligible levels. This resulted in the malfunctioning of ATPs and promoted the switch to high rates of aerobic glycolysis. As expected, the silencing of IF1 was shown to have reverse effects as it decreased the rate of glycolysis (Arago et.al, 2012). What causes the upregulation of IF1 is still unknown. One may wonder what causes IF1 to not interfere with ATPs activity when it is highly expressed in normal tissues such as the heart. It is hypothesized that perhaps the IF1 protein goes through post-translational modifications which do not allow it to interfere with normal ATPs activity and perhaps that modification is subdued in carcinoma (Arago et.al, 2012).

In another study, elevated expression of IF1 was shown to be involved in mediating the switch to Warburg phenotypes by regulating tumor metabolism through ATPs activity. Monoclonal antibodies were used against IF1 to show high expression of IF1 in human carcinomas compared to normal cells and high levels were shown to increase the rate of aerobic glycolysis with a decrease in OP. The silencing of IF1 had the opposite effects as a decrease in the rate of aerobic glycolysis and an increase in the rate of OP was observed. These observations were correlated to the Warburg effect in this study (Cenizo et. al, 2010).

## 5. P53

P53, also known as TP53, is a well-known tumor suppressor encoded by a tumor suppressor gene. A tumor suppressor gene is an anti-oncogene that regulates cell cycle by slowing down cell division, repairing DNA, and inducing apoptosis when necessary. One of the hallmarks of

cancer cells is its ability to evade tumor suppression (Weinberg & Hanahan, 2011).

P53 gene is located on chromosome number 7 and it is a phosphoprotein with 393 amino acids that build its 4 domains (Bioinformatics). Each domain has a distinct function to activate transcription factors, recognize specific DNA sequences, tetramerize the protein, or recognize DNA damage.

P53 also regulates mouse double minute 2 (Mdm2), also known as E3 ubiquitin-protein ligase, encoded by the Mdm2 gene, and uses it to regulate its own levels. P53 proteins that are not phosphorylated binds to Mdm2, which then degrades it through ubiquitination (Bioinformatics). P53 helps maintain the cell's genome stability by preventing mutations through regulating DNA damage repair proteins (Jones & Thompson, 2009). Low levels of P53 are present in normal cells which are activated and increased if the cell has been insulted with DNA damage, hypoxia, and oxidative stress, shunting the cell to go under apoptosis (Jones & Thompson, 2009).

With the tumor suppressor functions, P53 also has an important role in terms of metabolism. It has the ability to reduce the glycolytic flux, thus oppose the Warburg effect (Hirschey, et.al, 2015). Approximately 50% of all tumors have mutations in p53 (Bioinformatics).

Energy homeostasis pathways are regulated by stress-induced transcriptional programs, which in turn are regulated by p53 (Jones & Thompson, 2009). When a cell goes under hypoxia induced oxidative stress, the level of (cyclic adenosine monophosphate) cAMP:ATP increases which activates 5' adenosine

monophosphate-activated protein kinase (AMPK), which then not only activates p53 but also inhibits lipid and protein synthesis, crucial for cell growth. The increase in p53 then triggers a protein called TIGAR (TP53-inducible glycolysis and apoptosis regulator), which inhibits the glycolytic pathway.

Studies have also shown that the activity of P53 favors the formation of ATP from OP, which is common in normal cells (Jones & Thompson, 2009). It does this by regulating fructose-2-6-biphosphate (in the glycolytic pathway), TIGAR, and the assembly of COX by cytochrome c oxidase assembly protein (SCO2). It is interesting to note that a lack of SCO2 assists the cell to shift from OS to glycolysis to generate ATP (Jones & Thompson, 2009).

Another study has shown an interesting relationship between PKM2 and p53. An increase in PKM2 resulted in a decrease in p53 protein level with a short half-life (Wu, et.al, 2016). However, when PKM2 was knocked down in the cell, the p53 level increased with a greater half-life. It was also observed that PKM2 is capable of directly binding to p53 and MDM2 complex and promote p53 ubiquitination (dimeric form of PKM2 has a greater significance) (Wu, et.al, 2016).

Defects or a loss in P53 results in abnormal cell proliferation, which is one of the hall marks of cancer. This provides a great growth opportunity for normal cells to transform in to cancer cells. When normal cells are insulted with multiple DNA damage, hypoxic oxidative stress, and other cell insults, the absence of p53 would mean the absence of repair mechanism and apoptosis induction, allowing the cell to

behave abnormally (Jones & Thompson, 2009). The silencing or complete loss of P53 allows the switch in the metabolism from OP to glycolysis without blocking the cell cycle and inducing apoptosis.

	<i>In Normal Cells</i>	<i>In Cancer Cells</i>
<b>Glucose transporter (GLUT-1)</b>	GLUT-1 level on the membrane is inversely proportional to glucose level in the blood stream.	High GLUT-1 level are present with no relation to glucose level in the blood stream.
<b>Hexokinase (HK)</b>	HK1 is more common in adults and HK2 is more common in embryos.	High level of HK2 is present and inhibition improves the effects of anti-cancer drugs.
<b>Pyruvate Kinase (PKM)</b>	PKM-1 is more common and assures the import of pyruvate in to the mitochondria.	PKM2 is active in embryos, more common in tumors, and diverts pyruvate to lactate dehydrogenase-A (LDH-A) to make lactate.
<b>Citrate Synthase (CS)</b>	Pasteur effect is regulated by ATP and citrate.	High CS activity will yield high levels of citrate which has shown anti-cancer properties sensitizing cancer cells to chemotherapy.
<b>Succinate Dehydrogenase (SD)</b>	Part of the citric acid cycle and OP.	Mutation promotes cancer transformation through the production of carcinogens.
<b>Cytochrome c Oxidase (COX)</b>	Part of OP where oxygen is reduced to water.	High activity is thought to deplete citric acid cycle intermediates which lowers the citrate concentration so it cannot inhibit PFK, therefore creates an environment which favors high rate of glycolysis.
<b>ATP Synthase (ATPs)</b>	IF1 (inhibitory factor of ATPs) highly expressed in heart tissues, moderately in liver, and almost negligible in breast, colon, and lung tissues.	Inhibition of hydrolase activity by high levels of IF1 present in tissues where it was once negligible shown in carcinomas.
<b>P53</b>	Cell insults increase P53 levels.	Silenced or completely lost.

**Table. 1:** Compares the significance of GLUT-1, HK, PKM, SD, CS, COX, ATPs, and P53 in cancer verses normal cells.

## Conclusion

All that is known about cancer and the advances in treatment seen today have been a direct result of a terrifying historical background. It was an era of trial and error in the scientific community where thousands of cancer patients had no choice but to go through treatments that were nothing more than an educated guess from doctors and scientists. The patients ended up having severe side effects, making the approach to death as horrific as it could be. The trials ranged from directly injecting toxins in patients with no knowledge of dosage and side effects, to performing radical mastectomy with no knowledge of what

cancer actually is and how it metastasizes. It is important to note, however, that all of this was done with an intention to save lives because the rate of patients coming in with cancer was increasing and scientists did not understand what cancer is, let alone know how to cure it. (Mukherjee & Burns, 2015).

Research in mitochondria has enabled us to discover the differences in the structure and function of this organelle between normal and cancer cells. These differences have been used to develop novel site-specific targets for chemotherapy. Mitochondria-targeted drugs or mitochondria-anticancer agents (mitocans) have shown both efficacy and selectivity to kill cancer cells in pre-clinical and early clinical trials. However, these drugs as single agents are not yet sufficient to go beyond phase III clinical trials, which requires high selectivity, efficacy, and low toxicity (Napolitano & Weissig, 2015). This challenge has led scientist to come up with new strategies to enhance the selectivity of mitocans to achieve the desired results in vitro and vivo.

Further research on mitochondria and targeting it for cancer therapies will undoubtedly lead to new drug discoveries and new methods that will enable us to create more efficient and selective treatments for a variety of cancers. Perhaps it may take another era to ultimately save all the lives suffering from cancer, but progress is what gives us hope and the battle against cancer will continue.

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