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Gene Expression of Cellular Respiration Enzymes in Breast Cancer

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GENE EXPRESSION OF CELLULAR RESPIRATION ENZYMES IN BREAST CANCER

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GENE EXPRESSION OF CELLULAR RESPIRATION ENZYMES IN BREAST CANCER

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ABSTRACT

In the United States, approximately 2.0 million people will be diagnosed with cancer in 2024 (Siegel et al., 2024). 15% of those diagnoses will be breast cancer with 2% resulting in death (Siegel et al., 2024). Using normal breast tissue, metastatic breast cancer, and relapsed breast cancer to compare the gene expression of enzymes involved in glycolysis and the citric acid cycle could help with diagnosis and targeted treatment of altered metabolism. Cellular respiration is a set of metabolic processes; glycolysis, citric acid cycle, and oxidative phosphorylation. Normal cells use cellular respiration to convert nutrients into ATP (Chandel, 2021). Cancer cells undergo a phenomenon known as the Warburg effect (Chen et al., 2007). This effect causes cancer cells to favor glycolysis over oxidative phosphorylations in aerobic conditions (Chen et al., 2007). When metastatic and relapsed breast cancer is compared to normal breast tissue an increase in the expression of glycolytic enzymes is not observed but a decrease of enzymes associated with the citric acid cycle is. The obtained p-values indicate that the difference in gene expression is statistically significant but not if the metastatic or relapsed expression has increased or decreased compared to normal expression. If gene expression is altered enough to differentiate them from normal breast cells, drugs and other complementary health interventions (e.g. nutrition and exercise) can be developed to target cancerous breast cells while not affecting normal breast cells.

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INTRODUCTION

In the United States, approximately 2.0 million people will be diagnosed with cancer in 2024 (Siegel et al., 2024). 15% of those diagnoses will be breast cancer with 2% resulting in death (Siegel et al., 2024). Cancer occurs when sporadic or familial mutations accumulate in the genome. This can lead to cells dividing uncontrollably and resisting normal death signals (American Cancer Society, 2022). Breast cancer is cancer that originates in the breast tissue (American Cancer Society, 2022). Tumors can be benign or malignant (American Cancer Society, 2022). Benign tumors don't cross the basement membrane and invade the stroma (American Cancer Society, 2022). Malignant tumors can spread through the blood or lymph systems and grow in other body parts (American Cancer Society, 2022). When this occurs it is known as metastasis (American Cancer Society, 2022). Cancer can also relapse which is when it returns to the same primary site or a different area in the body after a period of remission (Sinn et al., 2019). Cancer cells still have the same needs as normal cells such as a blood supply, oxygen, and adenosine triphosphate (ATP) (Chandel, 2021). ATP stores and carries energy within the cell and can be hydrolyzed to release energy (Chandel, 2021). The energy release is used to power many cellular activities such as metabolic reactions, transport, cell division, and communication (Chandel, 2021). Cellular respiration is a set of metabolic processes; glycolysis, citric acid cycle, and oxidative phosphorylation. Normal cells use the products produced from cellular respiration in other cellular processes and to convert nutrients into ATP (Chandel, 2021). Cancer cells undergo a phenomenon known as the Warburg effect (Chen et al., 2007). This effect causes cancer cells to favor glycolysis over oxidative phosphorylations in aerobic conditions (Chen et al., 2007). This leads to

an increase in glucose uptake, the partial breakdown of glucose in the cytoplasm, and the production of lactate (Chen et al., 2007). Due to cancer cells favoring glycolysis, an increase in enzymes involved in glycolysis and a decrease in enzymes involved in the citric acid cycle may be observed when compared to normal cells. Using normal breast tissue, metastatic breast cancer, and relapsed breast cancer to compare the gene expression of enzymes involved in glycolysis and the citric acid cycle could help with diagnosis and targeted treatment of the altered metabolism.

Hypotheses

1) **Metastatic and relapsed breast cancer tissue will have an increase in enzymes associated with glycolysis when compared to normal breast tissue.**
2) **Metastatic and relapsed breast cancer tissue will have a decrease in enzymes associated with the citric acid cycle when compared to normal breast tissue.**

LITERATURE REVIEW

Cellular respiration occurs in all of our cells (Chandel, 2021). When a normal cell becomes a cancerous cell metabolic processes are altered (Chandel, 2021). 12% of women in the United States are diagnosed with breast cancer (Waks & Winer, 2019). 90% of the diagnoses are not metastatic at the time (Waks & Winer, 2019). There are three main types of breast cancer; hormone receptor-positive/*ERBB2* negative (HR+/*ERBB2*−), *ERBB2* positive (*ERBB2*+), and triple-negative (Waks & Winer, 2019). Comparing the gene expression of enzymes involved in cellular respiration between normal breast tissue, metastatic breast cancer, and relapsed breast cancer can help support the theory of Warburg metabolism.

Cellular respiration

Cellular respiration occurs in three stages; Glycolysis, Citric Acid Cycle, and Oxidative Phosphorylation (Electron Transport Chain) (Chandel, 2021). This metabolic process generates ATP/energy for required cellular functions (Chandel, 2021). Cellular respiration can vary based on the organism, oxygen availability, and metabolic demands (Chandel, 2021).

Glycolysis

Glycolysis is an important metabolic pathway to generate energy. It breaks down glucose (a six-carbon sugar) using ten enzymes to produce pyruvate, ATP, and other intermediates (Chandel, 2021).

The reaction of glycolysis is:

$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{Pi} \rightarrow 2\ \text{Pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O}$$

Glycolysis occurs in the cytosol and can proceed in aerobic and anaerobic conditions (Chandel, 2021). The 10 steps can be divided into two phases. Steps one through five are ATP investment and steps six through ten are ATP payoff (Chandel, 2021).

Step 1: Glucose Phosphorylation

Enzyme: Hexokinase

Reaction: $\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-phosphate} + \text{ADP}$

Step 2: Isomerization of Glucose-6-phosphate

Enzyme: Phosphoglucose isomerase (PGI)

Reaction: Glucose-6-phosphate → Fructose-6-phosphate

Step 3: Phosphorylation of Fructose-6-phosphate

Enzyme: Phosphofructokinase-1 (PFK-1)

Reaction: Fructose-6-phosphate + ATP → Fructose-1,6-bisphosphate + ADP

Step 4: Cleavage of Fructose-1,6-bisphosphate

Enzyme: Aldolase

Reaction: Fructose-1,6-bisphosphate → Dihydroxyacetone phosphate (DHAP) + Glyceraldehyde-3-phosphate (G3P)

Step 5: Isomerization of Dihydroxyacetone Phosphate (DHAP)

Enzyme: Triosephosphate isomerase (TPI)

Reaction: DHAP→ Glyceraldehyde-3-phosphate (G3P)

Step 6: Oxidation of Glyceraldehyde-3-phosphate (G3P)

Enzyme: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Reactions: G3P + NAD+ + Pi → 1,3-bisphosphoglycerate (1,3-BPG) + NADH + H+

Step 7: Phosphorylation of Glyceraldehyde-3-phosphate (G3P)

Enzyme: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Reaction: 1,3-BPG + ADP → 3-phosphoglycerate (3-PG) + ATP

Step 8: Isomerization of 3-Phosphoglycerate (3-PG)

Enzyme: Phosphoglycerate mutase

Reaction: 3-PG → 2-phosphoglycerate (2-PG)

Step 9: Dehydration of 2-Phosphoglycerate (2-PG)

Enzyme: Enolase

Reaction: 2-PG → Phosphoenolpyruvate (PEP) + H2O

Step 10: Phosphorylation of Phosphoenolpyruvate (PEP)

Enzyme: Pyruvate kinase

Reaction: PEP + ADP→ Pyruvate + ATP

The products from glycolysis are reactants in other cellular processes that occur. Pyruvate can enter the citric acid cycle in aerobic conditions or ferment in anaerobic conditions (Chandel, 2021).

Citric Acid Cycle

The citric acid cycle (CAC) which is also known as the Krebs cycle or tricarboxylic acid cycle produces energy in aerobic conditions. It is the final step in the

breakdown of carbohydrates, amino acids, and fatty acids (Ochoa, 1954). It occurs in the mitochondria, at a slightly more acidic pH, where each pyruvate is converted to acetyl CoA (Ochoa, 1954). For one molecule of glucose, the cycle turns twice (Ochoa, 1954).

The net reaction for one turn of the CAC is (Ochoa, 1954):

Acetyl-CoA + 3 NAD+ + FAD + GDP + Pi + 2 H2O → 2 CO2 + 3 NADH + 3 H+ + FADH2 + GTP + CoA

There are 8 steps involved in the CAC (Ochoa, 1954):

Step 1: Acetyl-CoA Formation

Enzyme: Citrate synthase

Reaction: Acetyl-CoA + Oxaloacetate → Citrate + CoA

Step 2: Isomerization

Enzyme: Aconitase

Reaction: Citrate → Isocitrate

Step 3: First Oxidation

Enzyme: Isocitrate dehydrogenase

Reaction: Isocitrate + NAD+ → Alpha-Ketoglutarate + NADH + CO2

Step 4: Second Oxidation

Enzyme: Alpha-ketoglutarate dehydrogenase complex

Reaction: Alpha-Ketoglutarate + NAD+ + CoA → Succinyl-CoA + NADH + CO2

Step 5: Substrate-Level Phosphorylation

Enzyme: Succinyl-CoA synthetase

Reaction: Succinyl-CoA + ADP + Pi → Succinate + ATP + CoA

Step 6: Oxidation of Succinate

Enzyme: Succinate dehydrogenase

Reaction: Succinate + FAD → Fumarate + FADH2

Step 7: Hydration of Fumarate

Enzyme: Fumarase

Reaction: Fumarate + H2O → Malate

Step 8: Final Oxidation

Enzyme: Malate Dehydrogenase

Reaction: Malate + NAD+ → Oxaloacetate + NADH

The CAC obtains energy from the breakdown of acetyl- CoA to produce NADH and FADH2 which are electron carriers and used in the electron transport chain (Ochoa, 1954).

Warburg Effect

In the presence of oxygen normal cells use oxidative phosphorylation to generate energy in their mitochondria (Chen et al., 2007). Cancer cells in aerobic conditions favor glycolysis for energy production, known as the Warburg effect (Chen et al., 2007). Cancer cells have an increased rate of glycolysis and convert pyruvate into lactate (Chen et al., 2007). They have reduced mitochondrial activity and continuously consume glucose to meet energy needs (Chen et al., 2007). Although the reason behind the Warburg effect is unknown, there are multiple theories as to why it occurs (Chen et al., 2007).

Some theories suggest that oxidative phosphorylation occurs in the mitochondria of the cell and an increase in mitochondrial DNA mutations could lead to malfunctions related to energy production and cellular functions (Chen et al., 2007). A decreased ability to generate ATP through oxidative phosphorylation could lead cancer cells to increase glycolysis for energy production (Chen et al., 2007). The Warburg effect could be an adaptive advantage to cancer cells. Mutations that occur in nuclear DNA and changes in enzyme levels could support growth and proliferation, and provide resistance to apoptosis, and therapies (Chen et al., 2007). Tumor microenvironments have limited oxygen, and nutrients, and are more acidic (Chen et al., 2007). Increased glycolysis leads to more lactic acid which could promote the spread of the tumor and invasion of the immune system (Chen et al., 2007). It also allows cancer cells to continue to make and produce energy in environments that lack oxygen and provides materials necessary to make new cells without the required catabolic processes in the mitochondria taking place

(Chen et al., 2007). The Warburg effect is not seen in all cancers but it is being continuously studied to understand it better and help improve treatment (Chen et al., 2007).

Breast cancer

Cancers arise from mutations that occur in the DNA (Waks & Winer, 2019). When those mutations originate in the cells of the breast it is known as breast cancer (Waks & Winer, 2019). Mutated cells will continue to grow and divide forming a lump or tumor (Waks & Winer, 2019). Those cells can continue to invade surrounding tissue and metastasize, spreading to other parts of the body (Waks & Winer, 2019). After a period of remission, cancer can return due to some cancer cells surviving the initial treatment. When this occurs, it is known as a relapse and cancer can return in the same or different part of the body (Sinn et al., 2019). Many experiments have been conducted to study how gene expression correlates with different aspects of breast cancer (Waks & Winer, 2019).

Defining the genomic signature of the parous breast

The risk of women developing breast cancer at menopause is reduced by a full-term pregnancy in early life (Peri et al., 2012). The hormones associated with the creation of the placenta and fetus could affect the development and differentiation of the breasts (Peri et al., 2012). This could result in a genomic profile imprinted in the mammary epithelium (Peri et al., 2012). Biopsies using Affymetrix Human Genome U133 Plus 2.0 arrays were taken from post-menopausal women between the ages of 50-69 (Peri et al., 2012). 71 women were pregnant and delivered one or more children (parous) and 42

women were never pregnant (nulliparous) (Peri et al., 2012). Transcriptomes were compared indicating that pregnancy causes a genomic imprint in the mRNA processing reactome which acts as protection after transcription (Peri et al., 2012).

SETER/PR: a robust 18-gene predictor for sensitivity to endocrine therapy for metastatic breast cancer

Endocrine therapy is the primary treatment for metastatic hormone receptor-positive and HER2-negative (HR+/HER2-) breast cancer (Sinn et al., 2019). A decrease in expression of the estrogen and progesterone receptors (ER and PR) creates a resistance to endocrine treatment (Sinn et al., 2019). To predict sensitivity to endocrine therapy (SET) in HR+/HER2- metastatic breast cancer eighteen transcripts linked to estrogen and progesterone receptors (*ESR1* and *PGR*), but not proliferation, and ten reference transcripts were measured (Sinn et al., 2019). Longer progression-free (PFS) and overall survival (OS) increased with a higher SETER/PR index in metastatic samples when undergoing endocrine therapy (Sinn et al., 2019).

Expression data from primary breast tumors 2

Breast cancer commonly metastasizes to the liver and being able to identify patients at a higher risk for liver metastasis could improve the understanding of tumor biology and treatment management (Smid, 2014). Gene expression was measured for 210 liver relapse patients and compared to patients with breast cancer that metastasized to other locations (Smid, 2014). Using quantitative RT-PCR it was found that ERBB2-positive tumors may be at an increased risk of liver metastasis (Smid, 2014). Targeted

therapy for overexpression of ERBB2 can be used to help reduce liver metastasis (Smid, 2014).

Glycolysis and the citric acid cycle are key processes for cells (Chandel, 2021). They use enzymes to catalyze these reactions but when a cell becomes cancerous the level of gene expression for those enzymes changes (Chen et al., 2007). This is due to the Warburg effect where cancer cells favor and show an increased rate of glycolysis (Chen et al., 2007). Many experiments involve gene expression. Using three breast cancer experiments that measured gene expression; nulliparous breast tissue, metastatic breast cancer, and relapsed breast cancer, statistical analysis can be used to compare the expression of enzymes associated with cellular respiration. Being able to measure and compare specific gene expressions allows for a better understanding and specific treatment of breast cancers.

METHODS

Using R2 genomics analysis and visualization platform (Koster) the gene expression of enzymes associated with glycolysis and the citric acid cycle were obtained from normal breast tissue, metastatic cancer tissue, and relapsed cancer tissue to run a quantitative analysis. Breast (nulli-parous) included 113 individuals, metastatic included 1103 individuals, and relapsed included 210 individuals. nine enzymes were measured for glycolysis; HK1, GPI, PFKM, ALDOA, TPI1, PGK1, PGAM1, ENO1, PKM. Seven enzymes were measured for the citric acid cycle; GOT1, CS, IDH1, SUCLA2, SDHA, FH, MDH2. GAPDH and ACTB were used as loading controls to ensure accurate quantification of gene expression levels. For each type of breast tissue, the gene

expression was normalized by dividing each enzyme level by the average of ACTB to compare the three tissue types. Once normalized, box plot overlays were created to visualize relative gene expression. To determine if the difference between gene expression is significant, a T-test and an ANOVA were completed in Prism GraphPad. P-values were obtained for each enzyme from the ANOVA which compared the mean of each group with the mean of the other groups. For the T-test p-values were obtained for three different comparisons; Normal vs Metastatic, Normal vs Relapse, and Metastatic vs Relapse.

RESULTS

Gene expression box plot overlays for glycolysis and citric acid cycle enzymes

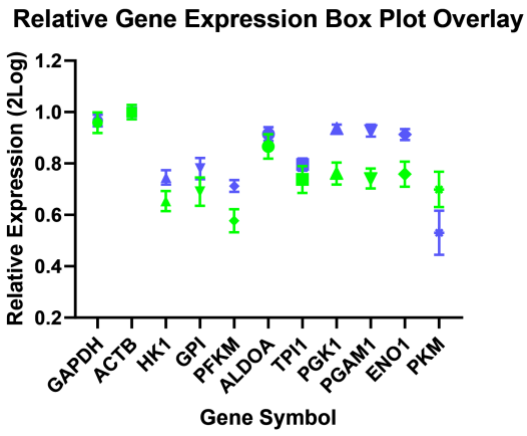

Figure 1: Normal breast tissue vs metastatic breast cancer relative gene expression for glycolysis enzymes

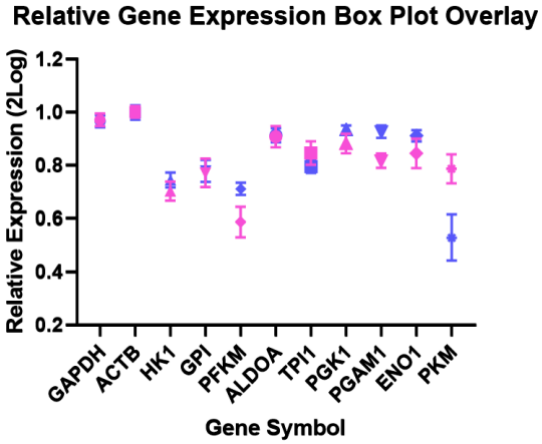

Figure 2: Normal breast tissue vs relapsed breast cancer relative gene expression for glycolysis enzymes

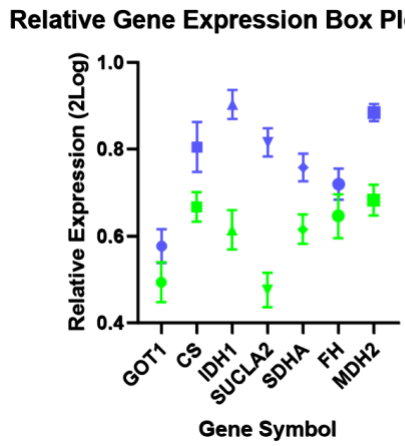

Figure 3: Normal breast tissue vs metastatic breast cancer relative gene expression for citric acid cycle enzymes

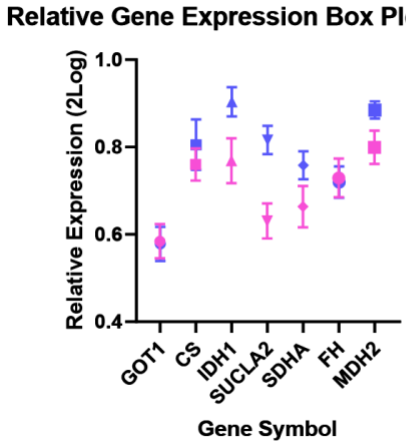

Figure 4: Normal breast tissue vs relapsed breast cancer relative gene expression for citric acid cycle enzymes

Key

Color	**Tissue**
Purple	Breast (nulli-parous)
Green	Metastatic breast cancer
Pink	Relapsed breast cancer

T-test - Glycolysis

Table 1: P-values from glycolysis enzyme t-test. * indicates statistical significance.

Enzyme	P-Value: Normal to Metastatic	P-Value: Normal to Relapse	P-Value: Metastatic to Relapse
GAPDH	0.0608	0.8986	0.0002*
ACTB	>0.9999	>0.9999	>0.9999
HK1	<0.0001*	<0.0001*	<0.0001*
GPI	<0.0001*	0.2191	<0.0001*
PFKM	<0.0001*	<0.0001*	0.0303
ALDOA	<0.0001*	0.2437	<0.0001*

TPI1	<0.0001*	<0.0001*	<0.0001*
PGK1	<0.0001*	<0.0001*	<0.0001*
PGAM1	<0.0001*	<0.0001*	<0.0001*
ENO1	<0.0001*	<0.0001*	<0.0001*
PKM	<0.0001*	<0.0001*	<0.0001*

T-test - Citric acid cycle

Table 2: P-values from citric acid cycle enzyme t-test. * indicates statistical significance.

Enzyme	P-Value: Normal to Metastatic	P-Value: Normal to Relapse	P-Value: Metastatic to Relapse
GOT1	<0.0001*	0.4794	<0.0001*
CS	<0.0001*	<0.0001*	<0.0001*
IDH1	<0.0001*	<0.0001*	<0.0001*
SUCLA2	<0.0001*	<0.0001*	<0.0001*
SDHA	<0.0001*	<0.0001*	<0.0001*
FH	<0.0001*	0.1971	<0.0001*
MDH2	<0.0001*	<0.0001*	<0.0001*

Ordinary one-way ANOVA

Table 3: P-values from ANOVA. * indicates statistical significance.

Enzyme	P-Value
GAPDH	<0.0001*
ACTB	>0.9999
HK1	<0.0001*
GPI	<0.0001*
PFKM	<0.0001*
ALDOA	<0.0001*

TPI1	<0.0001*
PGK1	<0.0001*
PGAM1	<0.0001*
ENO1	<0.0001*
PKM	<0.0001*
GOT1	<0.0001*
CS	<0.0001*
IDH1	<0.0001*
SUCLA2	<0.0001*
SDHA	<0.0001*
FH	<0.0001*
MDH2	<0.0001*

DISCUSSION

Cancer cells favor aerobic glycolysis over oxidative phosphorylation. An increase in gene expression for enzymes associated with glycolysis and a decrease in gene expression associated with the citric acid cycle may be observed in metastatic and relapsed cancer compared to normal breast tissue. Graphs one and two compare the relative gene expression of metastatic and relapsed glycolysis enzymes. For these graphs, except PKM enzymes, the metastatic and relapsed relative gene expression is approximately the same or a bit lower than the normal breast tissue. Graphs three and four compare the relative gene expression of metastatic and relapsed citric acid cycle enzymes. For these graphs, the metastatic and relapsed relative gene expression is approximately the same or lower than the normal breast tissue. According to the t-test, for

all three comparisons, and the ANOVA the difference in gene expression for most enzymes is statistically significant. Although cancer cells favor aerobic glycolysis an increase in the expression of glycolytic enzymes is not observed but a decrease of enzymes associated with the citric acid cycle is. The obtained p-values indicate that the difference in gene expression is statistically significant but not if the metastatic or relapsed expression has increased or decreased compared to normal expression.

This change in gene expression is impacted and or targeted by many drugs and other interventions such as Metformin, Semaglutide, nutrition, and exercise.

Metformin is commonly used in treating type 2 diabetes but can potentially impact cancer metabolism by altering the Warburg effect (Zepeda, 2022). Metformin may inhibit glycolysis by reducing glucose uptake and impairing glycolytic enzymes (Zepeda, 2022). AMP-activated protein kinases (AMPK) is a sensor that regulates cellular energy balance. Metformin activates AMPK which could alter metabolism, counteract the Warburg effect, and interfere with energy requirements having an anti-proliferation effect on cancer cells (Zepeda, 2022).

Semaglutide is a glucagon-like peptide (GLP-1) receptor agonist (Akl, 2024). It is a class of incretin mimetics and is commonly used as a treatment for type 2 diabetes (Akl, 2024). It typically causes an increase in insulin secretion, a reduction of glucagon levels, and regulation of blood sugars (Akl, 2024). Insulin has growth-promoting effects and by influencing insulin and insulin-like growth factor (IGF) pathways it may indirectly impact the proliferation and development of cancer cells (Akl, 2024).

Maintaining a healthy weight/lifestyle such as a balanced diet and regular exercise can contribute to a lower cancer risk (Tran et al., 2020). Nutrition and exercise affect

many metabolic processes, including the Warburg effect (Tran et al., 2020). The ketogenic diet which is high in fats and low in carbohydrates reduces the amount of glucose available (Tran et al., 2020). Compared to high glycemic or pro-inflammatory diets that are high in processed or sugar foods, it can contribute to chronic inflammation and increased blood glucose levels which could support the Warburg effect (Tran et al., 2020). Exercise may influence insulin, metabolism, and has an anti-angiogenic effect. Insulin sensitivity refers to how effectively cells respond to the hormone insulin which is produced by the pancreas (Hofmann, 2018). Exercise may help reframe insulin resistance and elevate insulin levels (Hofmann, 2018). With insulin functioning properly, it can impede growth-promoting effects and excess blood glucose (Hofmann, 2018). Exercise increases oxygen delivered to tissues and may promote a shift towards oxidative metabolism by enhancing oxidative phosphorylation and mitochondrial function impacting cancer metabolism which relies heavily on glycolysis (Hofmann, 2018). Angiogenesis is the formation of new blood vessels which tumors require for growth. Exercise stimulates the production of nitric oxide (NO) which inhibits endothelial cell proliferation and migration producing an anti-angiogenic effect (Hofmann, 2018). With continued research, using the difference in gene expression for cellular respiration enzymes between cancerous and normal cells drugs and lifestyle interventions could be used as a cancer treatment targeting the Warburg effect.

CONCLUSION

Cellular respiration is a set of metabolic processes; glycolysis, citric acid cycle, and oxidative phosphorylation. Cancer cells undergo a phenomenon known as the Warburg effect which causes cancer cells to favor aerobic glycolysis over oxidative

phosphorylations in aerobic conditions. A statical significant change in the gene expression of enzymes involved in glycolysis and the citric acid cycle is observed in metastatic cancer tissue and relapsed cancer tissue when compared to normal breast tissue. Multiple cancer drugs target specific characteristics of cancer cells. If cancer cells undergo the Warburg phenomenon and change their gene expression enough to differentiate them from normal breast cells drugs can be developed to target cancerous breast cells while not affecting normal breast cells.

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