HIF1A Expression is a Common Cause for the Warburg Effect in Lung Cancer Tumors, Cervical Cancer Tumors, and Brain Cancer Tumors

Abstract

This paper focuses on the relationship between HIF1A expression and the warburg effect. Considering that the warburg effect is a hallmark of cancer, it is possible that it could be caused by a common mechanism within tumor microenvironments. This paper considered the common cause of that to be hypoxia and HIF1A overexpression. By experimenting on mouse models and cancer cell lines for lung tumors, brain tumors, and cervical tumors, we investigated to find out whether HIF1A overexpression is, in fact, a common cause for the warburg effect among the tested cancer types. The results were very promising and with this understanding, treatments can be developed to block HIF1A expression which would cause the inhibition of the warburg effect and decrease cancer cell proliferation rate.

Key Words: HIF1A expression; warburg effect; Lung Cancer; Cervical Cancer. Brain Cancer: Adenosine Triphosphate; Glycolysis; Krebs cycle; electron transport; electron transport; choose your own key words.

Introduction

Write your introduction with references supporting the statements ater each statement you need to put in bracket the reference number.

All cells require energy in the form of Adenosine Triphosphate(ATP) to perform a multitude of cellular functions. ATP is a product of cellular respiration which consists of three separate parts. The first step is glycolysis followed by the citric acid cycle (krebs cycle) and then the electron transport chain. Glycolysis oxidized glucose to create intermediates called pyruvates and 4 ATP per glucose (net gain of 2 ATP). Pyruvates are used in the following steps to create 36 ATP per glucose by the end of the electron transport chain. This metabolic pathway requires oxygen, but in the absence of oxygen, cells use an alternate pathway called lactic acid fermentation. Instead of going to the mitochondria to be oxidized in the citric acid cycle, the pyruvate produced in glycolysis goes to the cytoplasm and is then reduced to lactate using lactate dehydrogenase. By producing lactate, Nicotinamide adenine dinucleotide hydrogen (NADH) is reduced to Nicotinamide adenine dinucleotide (NAD+) which allows for glycolysis to occur again. Glycolysis relies on NAD+ to act as a reducing agent and accept an electron to split glucose into 2 pyruvate molecules. This would allow glycolysis to keep running so that ATP can be produced despite the absence of oxygen. This would be used when you need to generate ATP rapidly during, for example, an intense workout.

The warburg effect, introduced by Otto Warburg, is a hallmark of cancer and follows a similar pathway to lactic acid fermentation despite the presence of oxygen giving it the name aerobic glycolysis. While it may seem inefficient, aerobic glycolysis requires fewer steps than the traditional metabolic pathway, which allows tumor cells to produce ATP 10-100[1] times faster. This rate of production compensates for the inefficiency of ATP production from aerobic glycolysis[1]. The quick rate of ATP production is also why tumor cells prefer this metabolic pathway to support their rapid cell proliferation.

Due to the efficiency of oxidative phosphorylation, many multicellular organisms evolved to utilize aerobic respiration as their primary strategy of ATP production. This created a heavy dependence upon oxygen for ATP production and exposure to hypoxic environments caused organisms to evolve mechanisms to survive in a hypoxic environment. One of these adaptations was the introduction of Hypoxia Inducible Factors (HIF)[4] which would assist in mediating cellular metabolism during a state of hypoxia to allow for cell and tissue survival. HIF1 is made up of HIFα(HIF1α, HIF2α, HIF3α) and HIF1ß[3]. During a state of hypoxia, HIFα forms a heterodimer with HIF1ß which causes enhanced expression of metabolic enzymes. This increases the expression of pyruvate dehydrogenase kinase1(PDK1) which phosphorylates pyruvate dehydrogenase (PDH)[3]. PDH is necessary for the conversion of pyruvate into acetyl Coenzyme A(acetyl CoA)[3]. This prevents the citric acid cycle from following and decreases the need for oxygen. This causes an overall decrease in cellular metabolism. By enhancing the glycolytic enzyme expression, HIF1 enhances the rate of glycolysis to make more ATP.

Tumor microenvironments tend to exist in a state of hypoxia which causes HIF1 to be overexpressed[5]. Considering that the warburg effect is a hallmark of cancer it's possible that the HIF1 overexpression is a primary and common cause of the warburg effect in cancer. Understanding the relationship between the warburg effect and HIF1 expression is critical to creating a new therapeutic approach to cancer treatment. Finding a way to prevent the instigation of the warburg effect by blocking HIF1 expression would decrease the rate of cancer progression and could prove useful to cancer therapy.
[support your statements in the introduction by references]

Aim of the study ------ Document your aims

Materials and Methods

1. The Cancer Genome Atlas Program (TCGA) database from the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN) (https://ualcan.path.uab.edu/analysis.html) was used to obtain the expression of HIF1A expression in lung, brain, and cervical cancer tumors compared to normal tissue.
2. Mouse models for lung cancer (LSL-K-ras-G12D), cervical cancer (K14E7), and brain cancer (GL261) were purchased from Jackson Laboratory. By using oxygen microsensors, we were able to determine the partial pressure of oxygen and then compare that to normal levels of oxygen. We then measured mRNA levels by using a quantitative polymerase chain reaction method multiple times over a 48 hour time period while simultaneously increasing the oxygen levels in a linear manner. To increase oxygen levels, we used oxygen filled microbubbles.
3. A549, LN229, and SiHa were purchased from the American Type Culture Collection. Add 100µM of CoCl2(Cobalt (II) Chloride to the cells and incubate in a conventional incubator for 24 hours. This creates a state of hypoxia. A state of hypoxia will increase HIF1A expression which we measure. After 24 hours, lactate was measured using nuclear magnetic resonance.

What about a bit of detail of your methodology how you carried out the study of the expression of HIFIA step by step in the laboratory?
You need to write the step-by-step way you undertook the study in the laboratory for every scientist to follow your methodology properly step by step

Results

HIF1A overexpression is caused by hypoxia in Lung Adenocarcinoma, Esophageal Carcinoma, and Glioblastoma Multiforme

To understand the relationship between HIF1A and tumor environments, data from the cancer genome atlas can be used to compare the levels of expression between cancer cells and normal cells. We can then compare it to the levels of oxygen within tumor environments to confirm that a state of hypoxia is what causes overexpression of HIF1A.

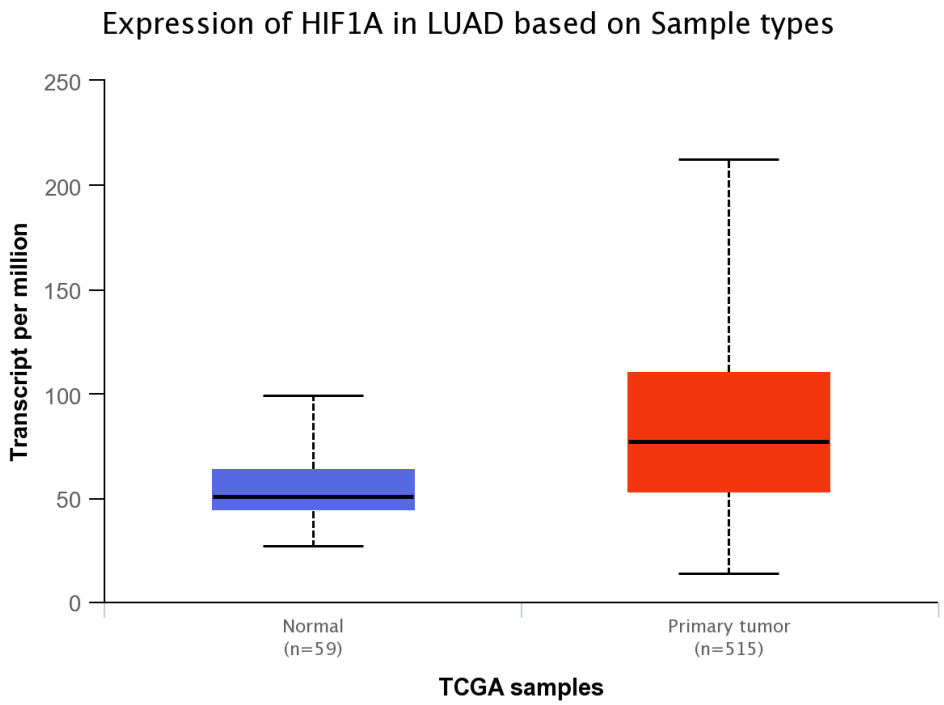

Figure 1A. HIF1A expression in Lung Adenocarcinoma compared to normal tissue sampling. HIF1A is shown to have much higher expression in tumor tissue compared to normal tissue.[6]

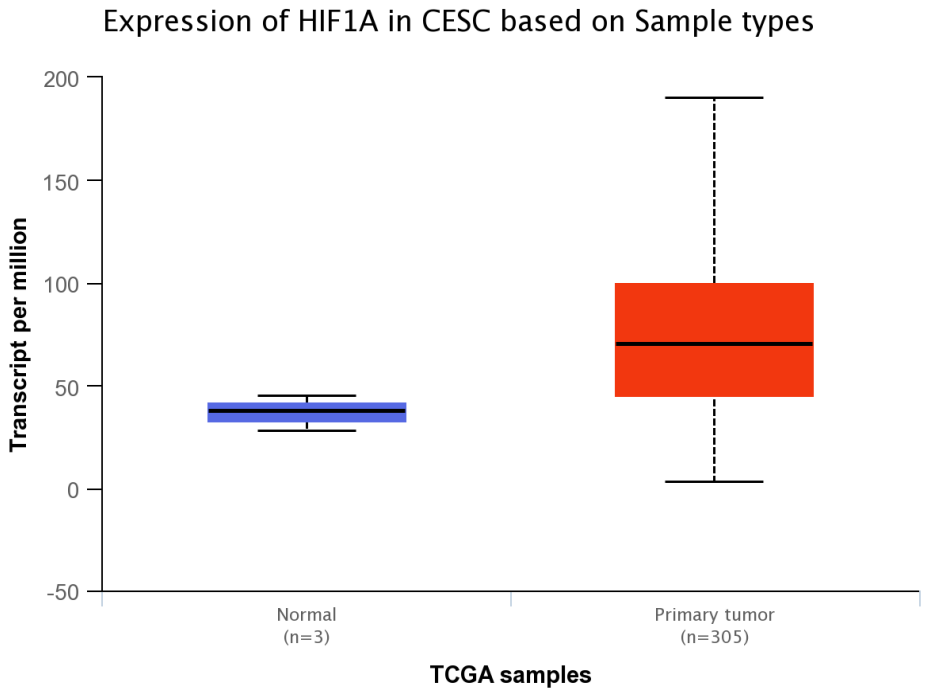

Figure 1B. HIF1A expression in Cervical squamous cell carcinoma compared to normal tissue sampling. HIF1A is shown to have a much higher level of expression in tumor samples.[7]

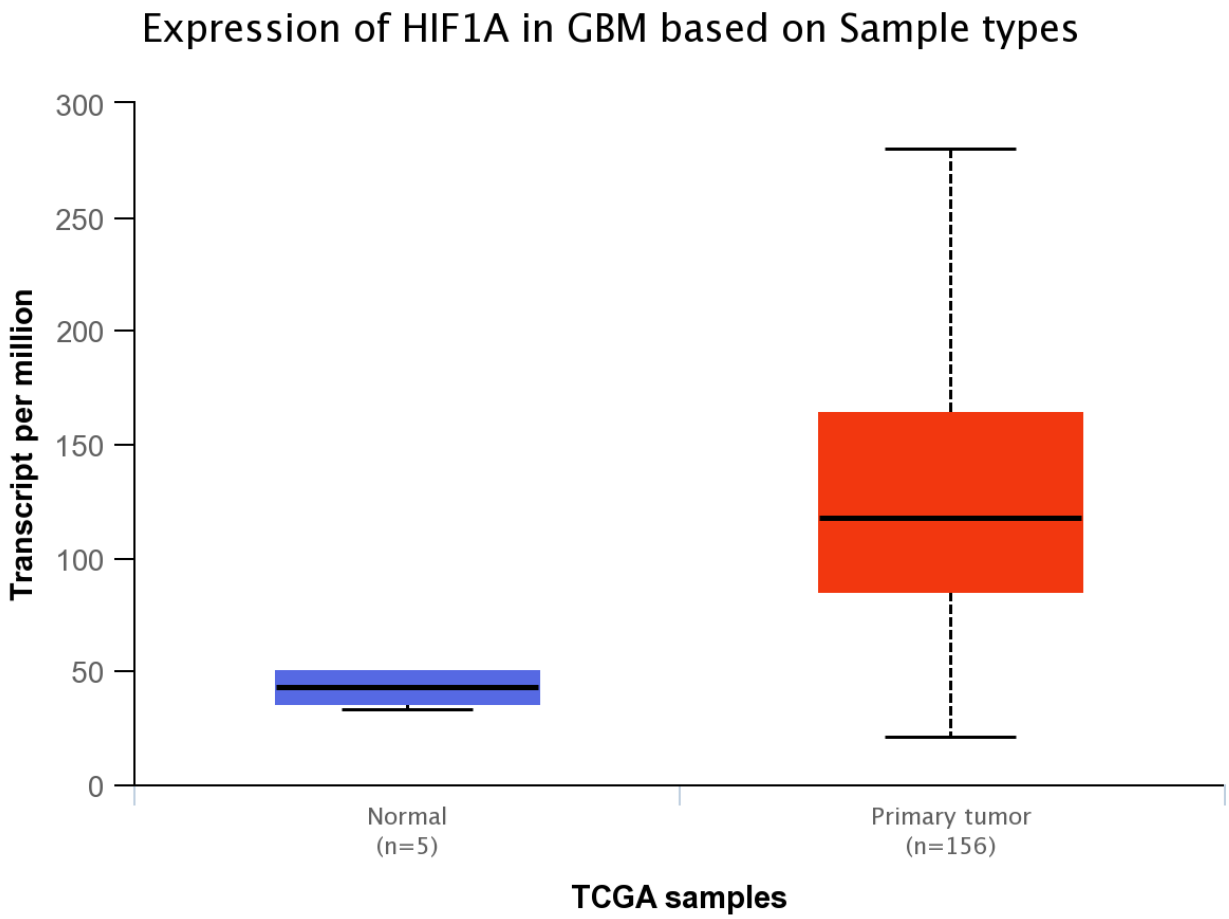

Figure 1C. HIF1A expression in Glioblastoma Multiforme. HIF1A is shown to have a much higher level of expression in tumor samples[8].

As shown in Figure 1A, in Lung Adenocarcinoma, HIF1A is shown to have increased expression compared to normal lung cells. Furthermore, data comparing HIF1A expression in cervical squamous cell carcinoma and glioblastoma multiforme shows a similar trend in HIF1A expression between normal cells and tumor cells. The increase in all of these types of cancers indicates that it's potentially caused by a common trait that exists in all the tumor microenvironments.

Tumour type	Median tumour pO_2	Median % oxygen	Median normal tissue pO_2	Median % oxygen	Fold pO_2 decrease[a]
Brain	13.0	1.7	26.0	3.4	2.0
Lung cancer	14.3	1.9	ND	5.6	3.0
	16.6	2.2	42.8	5.6	2.6
Cervical cancer	9.0	1.2	42.0	5.5	4.7

Figure 2A. Oxygen levels in each cancer type compared to the normal amount of oxygen. Mouse model GL261 was shown to have a partial pressure of 13.0 atm which is 50% normal amount. Mouse model LSL-Kras-G12D had a partial pressure of oxygen of 16.6 atm which is 38.7% of the normal amount. Mouse model K14E7 had a partial pressure of oxygen of 9.0 atm which is 18.8% of the normal amount. Oxygen was measured using oxygen pO2 microsensors. (hypothetical)[9]

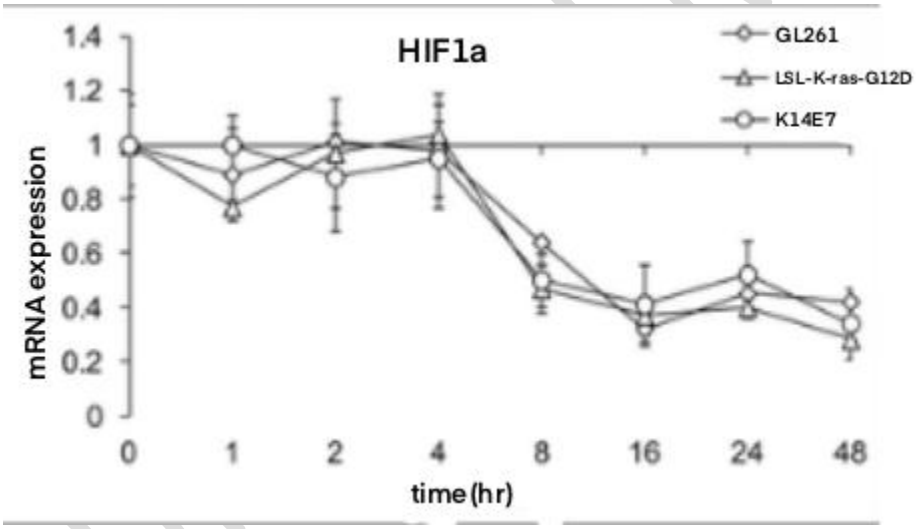

Figure 2B. mRNA expression in GL261, LSL-Kras-G12D, and K14E7 in a state of hypoxia with a linearly increasing level of oxygen through oxygen filled microbubbles. mRNA expression of HIF1A was highest during the lowest levels of oxygen presence and it decreased as oxygen levels increased indicating HIF1A expression is caused by hypoxia. mRNA of HIF1A was measured using qPCR.(hypothetical)[10]

By utilizing a mouse model for each respective cancer type, we could measure the oxygen levels within tumor microenvironments for each cancer type and compare it to normal levels of oxygen. Following that, we were able to measure the HIF1A mRNA levels and compared it to normal levels to draw a direct correlation between the two. As shown in figure 2a , the oxygen levels

were considerably lower for the tumor microenvironments in each tumor type. Tumors associated with the brain, lung, and cervical cancer were shown to have a drop in the partial pressure of oxygen. Lower partial pressure of oxygen indicated that there is less oxygen in tumors than normal. HIF1A expression is a reaction to hypoxia and considering the increased levels of HIF1A expression this showed that hypoxia is a common cause for the metabolism change that is induced by HIF1A expression which is supported by the data in figure 2b. Despite the fact that these are different types of cancers within different organs, the tumors each share a common physical property which causes the common metabolism change called the warburg effect through HIF1A expression.

Overexpression of HIF1A is a direct instigator of the Warburg effect

An indication of the warburg effect is an increased level of lactate since lactate is a byproduct of the warburg effect. By utilizing cell lines for each respective cancer type, we were able to experiment on the relationship between HIF1A and lactate. We manipulated the HIF1A expression in A549(lung cancer cells), SiHa(cervical cancer cells), and LN 229(brain cancer cells) by inducing a state of hypoxia and then measured the levels of lactate. As shown in figure 3, each type of cancer showed an increase in lactate levels when HIF1A expression was increased. Since HIF1A expression causes a greater rate of glycolysis, NAD+ has to be regenerated from NADH to keep up with the increased rate of glycolysis. To do so, pyruvate is reduced to lactate which causes an increase in the levels of lactate. Consequently, a greater level of HIF1A expression causes greater lactate production. The relationship between lactate and HIF1A shown in figure 3 indicates that HIF1A upregulation is a direct cause for the warburg effect within the studied cancer types. As previously shown, the decreased levels of oxygen for each cancer type was associated with increased HIF1A expression which was, now, correlated with increased lactate production. Considering that hypoxia is a common trait of tumor microenvironments and it causes HIF1A expression, it's possible that hypoxia is a common cause of the warburg effect in lung cancer, cervical cancer, glioblastoma multiforme, and potentially more cancer types through increases HIF1A expression.

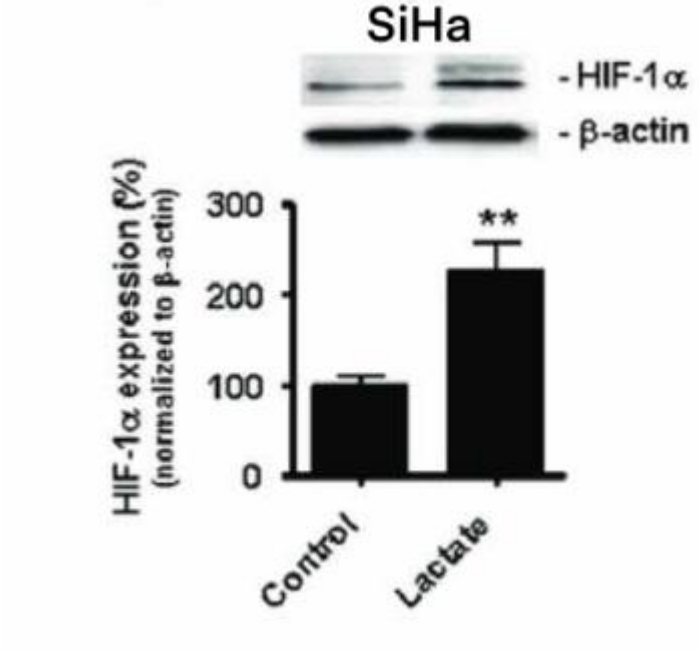

Figure 3A. SiHa was placed into a state of hypoxia to cause HIF1A overexpression and then the lactate levels were measured using bioluminescence imaging. As HIF1A expression increased, the lactate levels increased which indicates a direct relationship between the two.(hypothetical)[11]

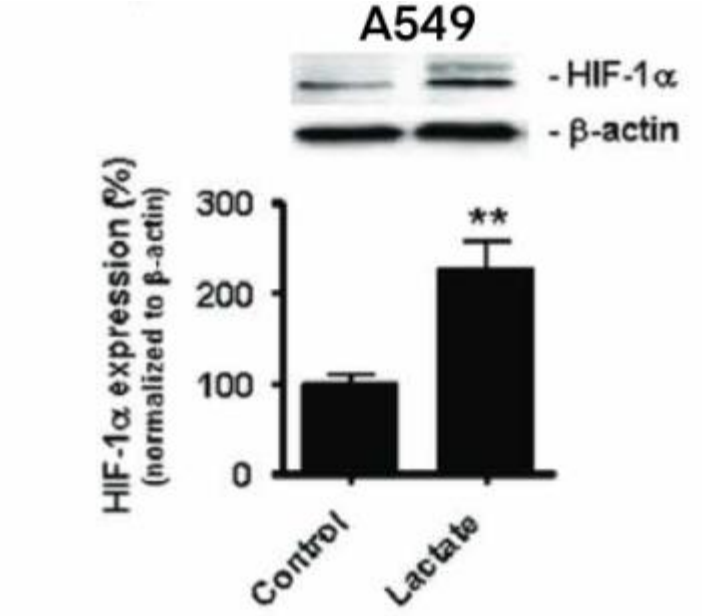

Figure 3B. A549 was placed into a state of hypoxia to cause HIF1A overexpression and then the lactate levels were measured using bioluminescence imaging. As HIF1A expression increased, the lactate levels increased which indicates a direct relationship between the two.(hypothetical)[11]

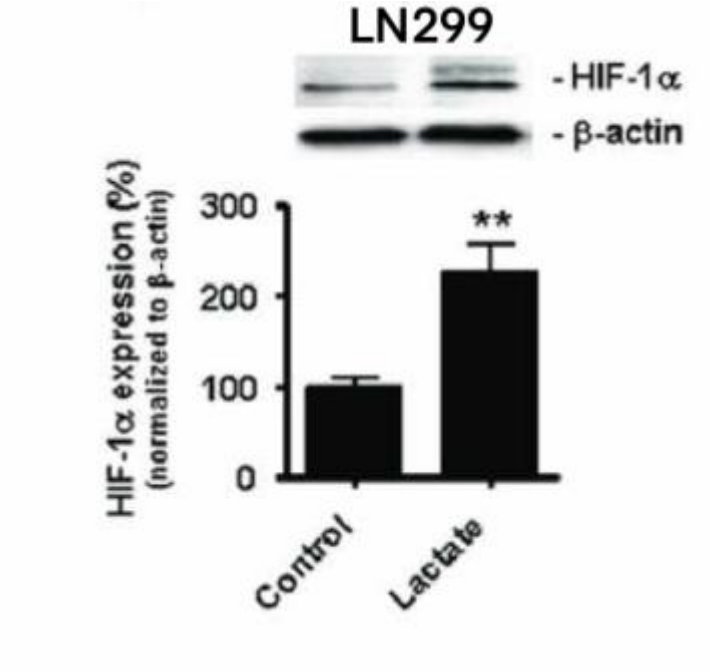

Figure 3C. A549 was placed into a state of hypoxia to cause HIF1A overexpression and then the lactate levels were measured using bioluminescence imaging. As HIF1A expression increased, the lactate levels increased which indicates a direct relationship between the two. (hypothetical)[11]

Discussion

The warburg effect is a trait of cancer cells that provide them with the adaptability to survive and proliferate at an alarming rate. This study had focused on whether HIF1A overexpression is a common cause for the warburg effect within lung, brain, and cervical tumors. HIF1A reacts to a state of hypoxia. Considering the low decreased levels of oxygen within the tumors, it is not surprising to see that HIF1A is overexpressed in the tumors. Furthermore, increased HIF1A expression was associated with greater levels of lactate which is a product of the warburg effect. This directly shows the correlation between the two and is consistent with each tumor type. This tells us that HIF1A is a common instigator of the warburg effect within the cancer types studied. But due to the difference in origin of cancer, there doesn't seem to be one gene or mechanism that causes the warburg effect throughout all cancers. For example, we see lower expression of HIF1A within pancreatic cancer compared to normal pancreatic cells[12]. This shows an inconsistency, but even this inconsistency can be questioned further.

Specifically, whether there is an external mechanism that purposefully suppresses HIF1A expression to increase metastasis and invasion and whether HIF1A still plays a role in instigating the warburg effect in pancreatic cancer. If it does still play a role in the warburg effect, then it can potentially eliminate some doubt around the inconsistencies surrounding the expression of HIF1A, because other types of cancer can follow a similar style. Another path of research could be trying to find other genes that are primary instigators of the warburg effect. Despite understanding the genetic origin of the warburg effect, trying to prevent it is still very difficult because therapy focused on this has to be localized to cancer cells. Finding other genes would help create treatments that target the proteins that are expressed by the genes in therapy. Further research could also dive into how to localize gene therapy or identify unique features about

cancer cells to create treatment targeted towards that. With our understanding of the origin of the warburg effect, we can create treatments to counteract it and potentially slow down tumor progression and cell proliferation.

Due to the nature of the warburg effect, specifically the tendency to use glucose faster, more glucose and resources tend to be sent to cancer cells for their survival. This takes away from the energy that other organs need to function and can lead to worse functioning. By stopping the warburg effect, cancer cells wouldn't get most of the glucose and resources which would lead to a healthier and more beneficial distribution of resources and energy throughout the body. Cancer cells would be forced to rely upon oxidative phosphorylation which wouldn't be very efficient or beneficial due to the lack of oxygen. The lack of energy would dramatically decrease the rate of cell proliferation.

CONCLUSIONS – You need to write your conclusions

In your write up support your statements by positioning your refence numbers at the end of each statement you are supporting within the text.

References

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