WARBURG AND PROSTATE CANCER

Terrence P McGarty
White Paper No 149
January, 2018

A recent paper discusses Warburg and the lipid metabolism path and how it potentially relates to control of prostate cancer. We examine this briefly in this short note. Copyright 2018 Terrence P. McGarty, all rights reserved.

Notice

This document represents the personal opinion of the author and is not meant to be in any way the offering of medical advice or otherwise. It represents solely an analysis by the author of certain data which is generally available. The author furthermore makes no representations that the data available in the referenced papers is free from error. The Author also does not represent in any manner or fashion that the documents and information contained herein can be used other than for expressing the opinions of the Author. Any use made and actions resulting directly or otherwise from any of the documents, information, analyses, or data or otherwise is the sole responsibility of the user and The Author expressly takes no liability for any direct or indirect losses, harm, damage or otherwise resulting from the use or reliance upon any of the Author's opinions as herein expressed. There is no representation by The Author, express or otherwise, that the materials contained herein are investment advice, business advice, legal advice, medical advice or in any way should be relied upon by anyone for any purpose. The Author does not provide any financial, investment, medical, legal or similar advice in this document or in its publications on any related Internet sites.

Furthermore, this document contains references to and quotes from papers and documents under the premise of Fair Use in order to present ideas and understandings in context. The Author has attempted to make any and all references to such material separate from those of the author per se and has referenced the source expressly in all cases. These documents are for the dissemination of ideas and have no commercial intent. The Author would appreciate any communications relating to these documents and these should be sent to:

tmcgarty@telmarc.com.

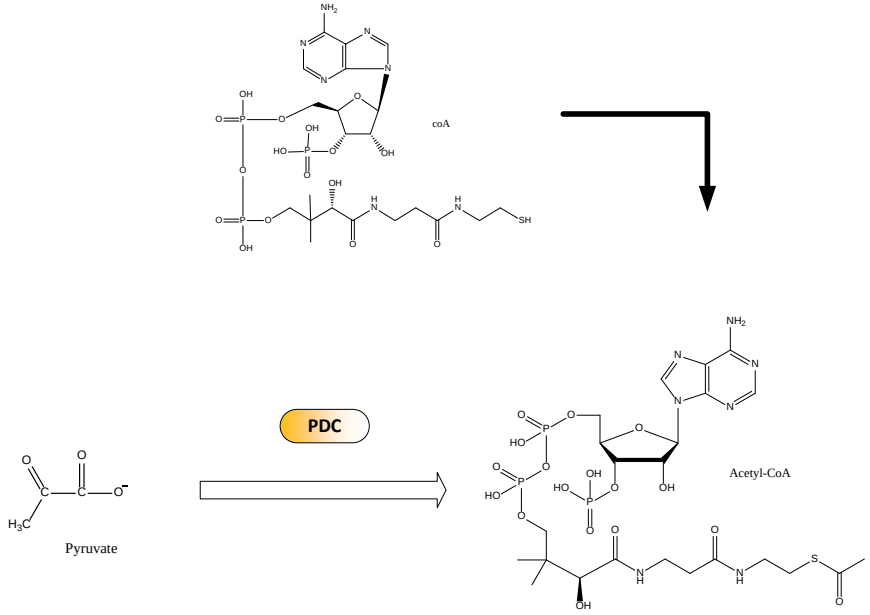
Contents

1 INTRODUCTION

We have recently discussed the Warburg effect and cancers. Recent studies have brought this effect to the fore as a possible means to control prostate cancer, PCa. This brief note is a commentary on a recently published paper addressing Warburg, and its offshoots in the lipid domain in the treatment of prostate cancer. Recall that the general process internal to the mitochondria can be written as follows.

$$Pyruvate + NAD^{+} + CoA \rightarrow AcetylCoA + NADH + H^{+} + CO_2$$

Specifically we have:



Pyruvate feeds the TCA cycle, it is a result of glycolysis. In contrast, aerobic glycolysis, the Warburg effect is a mix of both the TCA plus anaerobic glycolysis, namely no oxygen. The enzyme PDC enables this process. This we depict above. Pyruvate is the product of glycolysis and AcetylCoA is the feeder to the TCA.

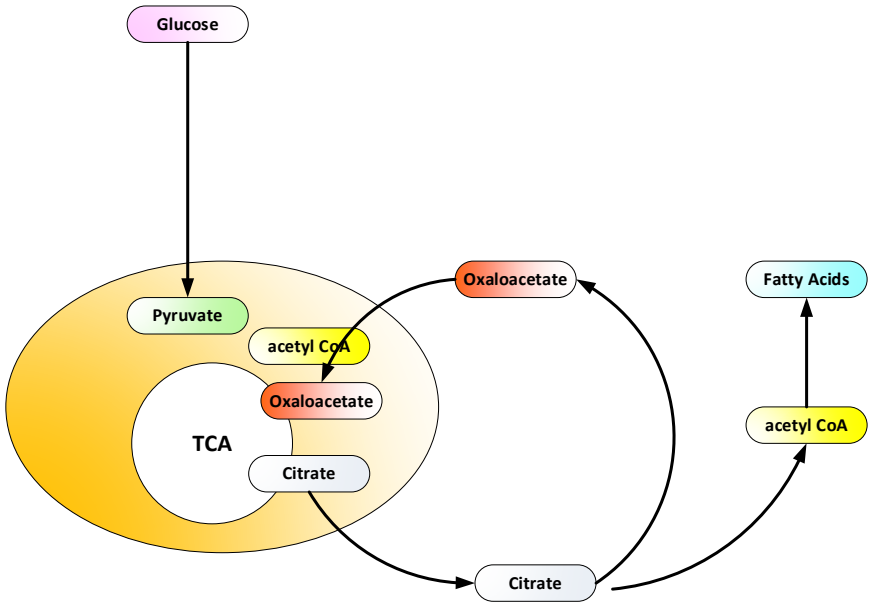
2 LIPIDS, WARBURG AND PCA

We know that lipids play a role in carcinogenesis. As Hsu and Sabatini note:

Although studies in cancer metabolism have largely been energy-centric, rapidly dividing cells have diverse requirements. Proliferating cells require not only ATP but also nucleotides, fatty acids, membrane lipids, and proteins, and a reprogrammed metabolism may serve to support synthesis of macromolecules. Recent studies have shown that several steps in lipid synthesis are required for and may even actively promote tumorigenesis. Inhibition of ATP citrate lyase, the distal enzyme that converts mitochondrial-derived citrate into cytosolic acetyl coenzyme A, the precursor for many lipid species, prevents cancer cell proliferation and tumor growth (Hatzivassiliou et al., 2005).

Fatty acid synthase, expressed at low levels in normal tissues, is upregulated in cancer and may also be required for tumorigenesis (reviewed in Menendez and Lupu, 2007). Furthermore, cancer cells may also enhance their biosynthetic capabilities by expressing a tumor-specific form of pyruvate kinase (PK), M2-PK. Pyruvate kinase catalyzes the third irreversible reaction of glycolysis, the conversion of phosphoenolpyruvate (PEP) to pyruvate. Surprisingly, the M2-PK of cancer cells is thought to be less active in the conversion of PEP to pyruvate and thus less efficient at ATP production.

A simplified version of this in Bauer et al is shown below:



Flavin et al also noted:

Cancer cells synthesize de novo large amounts of fatty acids and cholesterol, irrespective of the circulating lipid levels and benefit from this increased lipid synthesis in terms of growth

advantage, self-survival and drug resistance. Key lipogenic alterations that commonly occur in prostate cancer include over-expression of the enzyme fatty acid synthase (FASN) and deregulation of the 5-AMP-activated protein kinase (AMPK). FASN is a key metabolic enzyme that catalyses the synthesis of palmitate from the condensation of malonyl-CoA and acetyl-CoA de novo and plays a central role in energy homeostasis, by converting excess carbon intake into fatty acids for storage. AMPK functions as a central metabolic switch that governs glucose and lipid metabolism. Recent interest has focused on the potential of targeting metabolic pathways that may be altered during prostate tumorigenesis and progression. Several small molecule inhibitors of FASN have now been described or in development for therapeutic use; in addition, drugs that directly or indirectly induce AMPK activation have potential benefit in prostate cancer prevention and treatment[12].

Prostate cancer and lipid metabolism has been studied extensively. Now in a recent report in Science Daily it states[3]:

For years, attempts have been made to understand the mechanism behind the proliferation of cancer cells: they need metabolites to grow and proliferate as much as a vehicle needs gasoline or electricity to move. However, until now it was not known which metabolites cancer cells actually need. A team of researchers from the Institute of Oncology Research (IOR) at the Università della Svizzera Italiana (USI, Faculty of Biomedical Sciences) led by Prof. Andrea Alimonti has identified one of the mechanisms behind this process, as published in a recent article in the journal Nature Genetics.

From a theory dating back to the early 20th century by Nobel Prize laureate Otto Warburg, it has been believed that, in order to support their growth, cancer cells needed to increase their glucose consumption, without using mitochondrial metabolism. The mitochondrion is an organelle that produces the energy needed for the cell survival, operating as a sort of power station. "Contrary to what was believed for almost a century -- says Prof. Alimonti -- we have discovered that cells in prostate cancer need the mitochondrion, not to produce energy, rather to regulate a specific metabolic process.

[1] FASN from NCBI: The enzyme encoded by this gene is a multifunctional protein. Its main function is to catalyze the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of NADPH, into long-chain saturated fatty acids. In some cancer cell lines, this protein has been found to be fused with estrogen receptor-alpha (ER-alpha), in which the N-terminus of FAS is fused in-frame with the C-terminus of ER-alpha. https://www.ncbi.nlm.nih.gov/gene/2194

[2] AMPK from NCBI: The protein encoded by this gene belongs to the ser/thr protein kinase family. It is the catalytic subunit of the 5'-prime-AMP-activated protein kinase (AMPK). AMPK is a cellular energy sensor conserved in all eukaryotic cells. The kinase activity of AMPK is activated by the stimuli that increase the cellular AMP/ATP ratio. AMPK regulates the activities of a number of key metabolic enzymes through phosphorylation. It protects cells from stresses that cause ATP depletion by switching off ATP-consuming biosynthetic pathways. Alternatively spliced transcript variants encoding distinct isoforms have been observed. https://www.ncbi.nlm.nih.gov/gene/5562

[3] https://www.sciencedaily.com/releases/2018/01/180115121635.htm

Specifically, the mitochondrion is able to regulate fat synthesis (lipids) through an enzyme complex called PDC.

Thus the glycolysis is but one part. Lipid metabolism is a second, and we have discussed this earlier as well. PDC is used on the classic glycolysis and TCA. Here they argue PDC is used in the mitochondria to regulate lipid development.

The study published by Nature Genetics shows that without the ability to efficiently produce lipids, prostate cancer cells are not able to grow and metastasize, even in the presence of increased glycolysis. "We noticed -- continues Alimonti -- that in prostate cancer cells the activity of the enzyme complex PDC is 10 times that of a normal proliferating cell, and that as a result the cells store several lipids."

As noted above, PDC is the enzyme in the connection between glycolysis and the TCA.

It is known that a diet rich in fat can increase the risk of developing prostate cancer, and that obese people are more prone to develop this type of tumour. However, the fact that the metabolism of lipids acts as a fuel to support the tumour has never been clarified in detail and this discovery opens up new and unexpected scenarios in cancer therapy.

"We have identified a number of pharmaceutical compounds that selectively inhibit -- in different experimental models -- the mitochondrial enzyme responsible for the tumour growth, thus limiting fat synthesis and without harming normal cells." "I would like to point out, however -- concludes Alimonti -- that our discovery does not imply that cancer patients must undergo a strict dietary regime, which might in fact hurt them: a reduction of fat in cancer cells can only be obtained by blocking the cancer cells metabolism through specific drugs."

The authors of the above references paper, Chen et al, note:

The mechanisms by which mitochondrial metabolism supports cancer anabolism remain unclear. Here, we found that genetic and pharmacological inactivation of pyruvate dehydrogenase A1 (PDHA1), a subunit of the pyruvate dehydrogenase complex (PDC), inhibits prostate cancer development in mouse and human xenograft tumor models by affecting lipid biosynthesis. Mechanistically, we show that in prostate cancer, PDC localizes in both the mitochondria and the nucleus.

Whereas nuclear PDC controls the expression of sterol regulatory element-binding transcription factor (SREBF)-target genes by mediating histone acetylation, mitochondrial PDC provides cytosolic citrate for lipid synthesis in a coordinated manner, thereby sustaining anabolism. Additionally, we found that PDHA1 and the PDC activator pyruvate dehydrogenase phosphatase 1 (PDP1) are frequently amplified and overexpressed at both the gene and protein levels in prostate tumors.

Together, these findings demonstrate that both mitochondrial and nuclear PDC sustain prostate tumorigenesis by controlling lipid biosynthesis, thus suggesting this complex as a potential target for cancer therapy.

Now PDHA1 is a part of the PDC. From NCBI[4]:

The pyruvate dehydrogenase (PDH) complex is a nuclear-encoded mitochondrial multienzyme complex that catalyzes the overall conversion of pyruvate to acetyl-CoA and CO(2), and provides the primary link between glycolysis and the tricarboxylic acid (TCA) cycle. The PDH complex is composed of multiple copies of three enzymatic components: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2) and lipoamide dehydrogenase (E3). The E1 enzyme is a heterotetramer of two alpha and two beta subunits. This gene encodes the E1 alpha 1 subunit containing the E1 active site, and plays a key role in the function of the PDH complex. Mutations in this gene are associated with pyruvate dehydrogenase E1-alpha deficiency and X-linked Leigh syndrome.

There are two SREBF genes. Again from NCBI:

SREBF1[5](SREBP1): *This gene encodes a basic helix-loop-helix-leucine zipper (bHLH-Zip) transcription factor that binds to the sterol regulatory element-1 (SRE1), which is a motif that is found in the promoter of the low density lipoprotein receptor gene and other genes involved in sterol biosynthesis. The encoded protein is synthesized as a precursor that is initially attached to the nuclear membrane and endoplasmic reticulum. Following cleavage, the mature protein translocates to the nucleus and activates transcription. This cleaveage is inhibited by sterols. This gene is located within the Smith-Magenis syndrome region on chromosome 17. Alternative promoter usage and splicing result in multiple transcript variants, including SREBP-1a and SREBP-1c, which correspond to RefSeq transcript variants 2 and 3, respectively.*

and

SREBF2[6] (SREBP2): *This gene encodes a member of the a ubiquitously expressed transcription factor that controls cholesterol homeostasis by regulating transcription of sterol-regulated genes. The encoded protein contains a basic helix-loop-helix-leucine zipper (bHLH-Zip) domain and binds the sterol regulatory element 1 motif. Alternate splicing results in multiple transcript variants.*

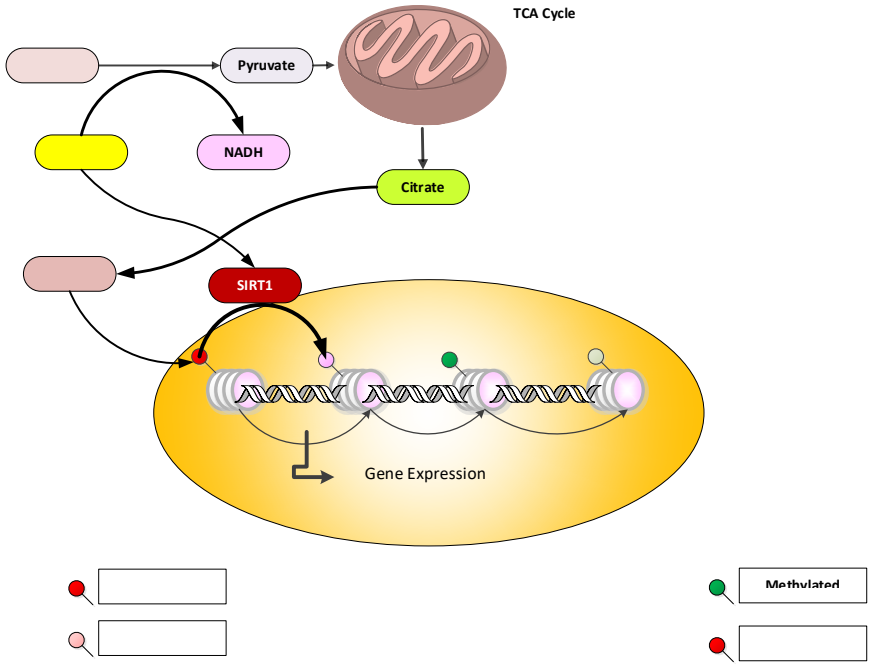
[4] https://www.ncbi.nlm.nih.gov/gene/5160

[5] https://www.ncbi.nlm.nih.gov/gene/6720

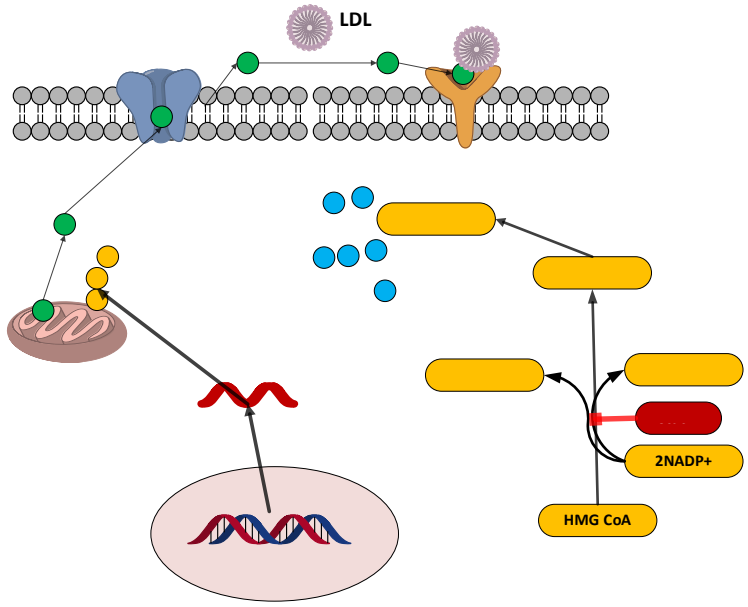
[6] https://www.ncbi.nlm.nih.gov/gene/6721

3 EXTENSIONS

We do know the impact of the TCA on androgen receptor as shown below:



Alaso as detailed below we have:



As McFate et al note:

High lactate generation and low glucose oxidation, despite normal oxygen conditions, are commonly seen in cancer cells and tumors. Historically known as the Warburg effect, this altered metabolic phenotype has long been correlated with malignant progression and poor clinical outcome.

However, the mechanistic relationship between altered glucose metabolism and malignancy remains poorly understood.

Here we show that inhibition of pyruvate dehydrogenase complex (PDC) activity contributes to the Warburg metabolic and malignant phenotype in human head and neck squamous cell carcinoma. PDC inhibition occurs via enhanced expression of pyruvate dehydrogenase kinase-1 (PDK-1), which results in inhibitory phosphorylation of the pyruvate dehydrogenase (PDH) subunit. We also demonstrate that PDC inhibition in cancer cells is associated with normoxic stabilization of the malignancy-promoting transcription factor hypoxia-inducible factor-1 (HIF-1) by glycolytic metabolites.

Knockdown of PDK-1 via short hairpin RNA lowers PDH phosphorylation, restores PDC activity, reverts the Warburg metabolic phenotype, decreases normoxic HIF-1 expression, lowers hypoxic cell survival, decreases invasiveness, and inhibits tumor growth. PDK-1 is an HIF-1-regulated gene, and these data suggest that the buildup of glycolytic metabolites, resulting from high PDK-1 expression, may in turn promote HIF-1 activation, thus sustaining a feed-forward loop for malignant progression.

In addition to providing anabolic support for cancer cells, altered fuel metabolism thus supports a malignant phenotype. Correction of metabolic abnormalities offers unique opportunities for cancer treatment and may potentially synergize with other cancer therapies.

As Fan et al note:

The mitochondrial pyruvate dehydrogenase complex (PDC) plays a crucial role in regulation of glucose homoeostasis in mammalian cells.

PDC flux depends on catalytic activity of the most important enzyme component pyruvate dehydrogenase (PDH).PDH kinase inactivates PDC by phosphorylating PDH at specific serine residues, including Ser-293,whereasdephosphorylation of PDH by PDH phosphatase restores PDC activity. The current understanding suggests that Ser-293 phosphorylation of PDH impedes active site accessibility to its substrate pyruvate.

Here, we report that phosphorylation of a tyrosine residue Tyr-301 also inhibits PDH 1 (PDHA1) by blocking pyruvate binding through a novel mechanism in addition to Ser-293 phosphorylation. In addition, we found that multiple oncogenic tyrosine kinases directly phosphorylate PDHA1 at Tyr-301, and Tyr-301 phosphorylation of PDHA1 is common in EGF-stimulated cells as well as diverse human cancer cells and primary leukemia cells from human patients.

Moreover, expression of a phosphorylation-deficient PDHA1 Y301F mutant in cancer cells resulted in increased oxidative phosphorylation, decreased cell proliferation under hypoxia, and reduced tumor growth in mice. Together, our findings suggest that phosphorylation at distinct serine and tyrosine residues inhibits PDHA1 through distinct mechanisms to impact active site accessibility, which act in concert to regulate PDC activity and promote the Warburg effect.

The Warburg effect is clear in the above. Its presence disappears when examining lipid metabolism, however. This point is further emphasized as Zhong et al note:

Cells generate adenosine-5'-triphosphate (ATP), the major currency for energy consuming reactions, through mitochondrial oxidative phosphorylation (OXPHOS) and glycolysis. One of the remarkable features of cancer cells is aerobic glycolysis, also known as the "Warburg Effect", in which cancer cells rely preferentially on glycolysis instead of mitochondrial OXPHOS as the main energy source even in the presence of high oxygen tension.

One of the main players in controlling OXPHOS is the mitochondrial gatekeeper pyruvate dehydrogenase complex (PDHc) and its major subunit is E1α (PDHA1). To further analyze the function of PDHA1 in cancer cells, it was knock out (KO) in the human prostate cancer cell line LnCap and a stable KO cell line was established. We demonstrated that PDHA1 gene KO significantly decreased mitochondrial OXPHOS and promoted anaerobic glycolysis, accompanied with higher stemness phenotype including resistance to chemotherapy, enhanced migration ability and increased expression of cancer stem cell markers. We also examined PDHA1 protein expression in prostate cancer tissues by immunohistochemistry and observed that reduced PDHA1 protein expression in clinical prostate carcinomas was significantly correlated with poor prognosis.

Collectively, our results show that negative PDHA1 gene expression is associated with significantly higher cell stemness in prostate cancer cells and reduced protein expression of this gene is associated with shorter clinical outcome in prostate cancers…..

We herein demonstrated that PDHA1 gene knockout resulted in dysfunctional mitochondrial OXPHOS and enhanced glycolysis. We previously reported that impartial mitochondrial OXPHOS by using mitochondrial pyruvate carrier (MPC) blocker enhanced stemness phenotype of prostate cancer cells. In keeping with our previously study, the mitochondrial gatekeeper PDHA1 gene knockout also leads to dysfunctional mitochondrial and enhanced glycolysis, as well as higher cell stemness phenotype. And by immuno-histochemical examination of PDHA1 protein expression in prostate cancer samples, it was revealed that negative PDHA1 protein expression was related with poor clinical outcome in patients with prostate cancer.

As Li et al note:

Alternative pathways of metabolism endowed cancer cells with metabolic stress. Inhibiting the related compensatory pathways might achieve synergistic anticancer results. This study demonstrated that pyruvate dehydrogenase E1α gene knockout (PDHA1 KO) resulted in alterations in tumor cell metabolism by rendering the cells with increased expression of glutaminase1 (GLS1) and glutamate dehydrogenase1 (GLUD1), leading to an increase in

glutamine-dependent cell survival. Deprivation of glutamine induced cell growth inhibition, increased reactive oxygen species and decreased ATP production.

Pharmacological blockade of the glutaminolysis pathway resulted in massive tumor cells apoptosis and dysfunction of ROS scavenge in the LNCaP PDHA1 KO cells. Further examination of the key glutaminolysis enzymes in human prostate cancer samples also revealed that higher levels of GLS1 and GLUD1 expression were significantly associated with aggressive clinicopathological features and poor clinical outcome. These insights supply evidence that glutaminolysis plays a compensatory role for cell survival upon alternative energy metabolism and targeting the glutamine anaplerosis of energy metabolism via GLS1 and GLUD1 in cancer cells may offer a potential novel therapeutic strategy.

As Justus et al note:

There are several molecular mechanisms whereby acidosis may alter tumor cell metabolism. p53 is an important regulator of the metabolic response to acidosis. The ability of acidosis to activate p53 and stimulate the TCA cycle through inhibition of glycolysis has been demonstrated. For example, acidosis induced p53 expression may transcriptionally inhibit the expression of glucose transporters GLUT1 and GLUT4 in specific tissues, thereby effectively reducing glucose availability for glycolysis. In addition, acidosis is reported to activate p53 and increase expression of glucose 6 phosphate dehydrogenase (G6PD) and glutaminase 2.

This is suggested to direct glucose towards the pentose phosphate pathway (PPP) as well as increase glutaminolysis. This may also drive the TCA cycle through the production of metabolic intermediates and increase the amount of NADPH in the cell to counteract ROS production. p53 activation may also induce the expression of Parkin (PARK2), a Parkinson disease-associated gene, to reduce glycolytic activity.

PARK2 regulates the expression of pyruvate dehydrogenase alpha 1 (PDHA1), a critical component for the activity of pyruvate dehydrogenase (PDH). PDHA1 knockdown increases glucose uptake, rate of glycolysis, and lactate production, facilitating the "Warburg effect". This gives PARK2 the ability to effectively reverse the "Warburg effect" by inducing PDHA1.

Moreover, PARK2 also regulates expression of reduced glutathione (GSH), a major antioxidant and ROS scavenger in the cell. This is proposed to occur through activation of p53 and may reduce ROS when oxidative phosphorylation is increased. Furthermore, γ-irradiation-induced tumorigenesis is sensitized following the knockout of PARK2 in C57BL/6J mice, indicating the PARK2 gene as a tumor suppressor.

The ability for p53 to regulate cancer cell metabolism by reducing glycolysis and increasing oxidative phosphorylation while simultaneously mitigating ROS is crucial for understanding acidosis induced metabolic alterations in the tumor.

4 OBSERVATIONS

We can make a few observations.

1. Warburg effect generally refers to glycolysis and the reduced production of ATP. It indirectly refers to any lipid processing. Fatty acid oxidation can produce an estimated 129 ATP per oxidation[7]. This is a large producer of energy and well exceeds that of glycolysis results. Thus considering the lipid elements as conjoint is highly reasonable.

Lipids are also energy rich as noted above. Perhaps cancer cells can function in this manner if the throughput of lipids can be high.

Targets for therapeutics using the lipid control may have potential deleterious effects. If the proposal is to target the enzyme in the production of ultimately ATP from lipids then one must be aware of the significant downside regarding cross cell contamination.

This is an interesting and useful alternative and again shows that there are many options about the Warburg process.

[7] See Ferrier, pp 192-193

5 REFERENCES

1. Bauer et al, ATP citrate lyase is an important component of cell growth and transformation, Oncogene (2005) 24, 6314–6322
2. Cerniglia et al, The PI3K/Akt Pathway Regulates Oxygen Metabolism via Pyruvate Dehydrogenase (PDH)-E1a Phosphorylation, Mol Cancer Ther; 14(8) August 2015
3. Chen et al. Compartmentalized activities of the pyruvate dehydrogenase complex sustain lipogenesis in prostate cancer, Nature Genetics (2018)
4. Fan et al, Tyr-301 Phosphorylation Inhibits Pyruvate Dehydrogenase by Blocking Substrate Binding and Promotes the Warburg Effect, The Journal Of Biological Chemistry Vol. 289, No. 38, pp. 26533–26541, September 19, 2014
5. Ferreira et al, Metabolic reprogramming of the tumor, Oncogene (2012) 31, 3999–4011
6. Ferrier, Biochemistry, 6th Edition, Lippincott (New York) 2015.
7. Flavin et al, Metabolic alterations and targeted therapies in prostate cancer, J Pathol. 2011 January ; 223(2): 283–294
8. Hsu and Sabatini, Cancer Cell Metabolism: Warburg and Beyond, Cell 134, September 5, 2008
9. Jin et al, Androgen receptor genomic regulation, Transl Androl Urol 2013;2(3):158-177
10. Justus et al, Molecular Connections between Cancer Cell Metabolism and the Tumor Microenvironment, Int. J. Mol. Sci. 2015, 16, 11055-11086
11. Li et al, PDHA1 gene knockout in prostate cancer cells results in metabolic reprogramming towards greater glutamine dependence, Oncotarget, Vol. 7, No. 33 2016
12. McFate et al, Pyruvate Dehydrogenase Complex Activity Controls Metabolic and Malignant Phenotype in Cancer Cells, THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 33, pp. 22700–22708, August 15, 2008
13. McGarty, Glucose, Warburg, Cancer and Pathways, Jan 2018, http://www.telmarc.com/Documents/White%20Papers/148Warburg.pdf
14. Sugden et al, Fuel-sensing mechanisms integrating lipid and carbohydrate utilization, Biochemical Society Transactions (200 I) Volume 29, part 2
15. Wittig and Coy, The Role of Glucose Metabolism and Glucose-Associated Signalling in Cancer, Perspectives in Medicinal Chemistry 2007:1 64–82
16. Zhong et al, Pyruvate dehydrogenase expression is negatively associated with cell stemness and worse clinical outcome in prostate cancers, Oncotarget, 2017, Vol. 8, (No. 8), pp: 13344-13356