Exploring Lipid Reprogramming in Hypoxic Cancer Cells: Targeting Lipid Metabolism for Therapeutic Innovation

Renad Shady Sayed

1. Abstract

The rapid proliferation of cancer cells results in hypoxia (low oxygen levels). They undergo metabolic reprogramming, including lipid metabolism to survive and grow in response to hypoxia. This study investigates the specific enzymes and processes that occur in response to low oxygen levels, including fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC), which are upregulated in response to hypoxia. In addition, this study indicates the accumulation of lipid droplets under hypoxia. The role of hypoxia-inducible factors (HIFs) under hypoxia is investigated. Gene expression analysis, metabolite profiling, and enzyme activity assays are employed to identify the changes in lipid metabolism that facilitate tumor progression under hypoxia and vivo techniques by using mice as models. The findings provide the development of lipid-targeting therapies that may enhance the current cancer treatment by targeting the upregulated enzymes because they help cancer cells to proliferate and survive in the case of low-oxygen, therefore targeting these enzymes may significantly slow tumor progression, reduce proliferation, and inhibit metastasis.

Keywords: Hypoxia, Lipid Metabolism, Cancer Cell Adaptation, Fatty Acid Synthase (FASN), Acetyl-CoA Carboxylase (ACC), Therapeutic Targeting

2. Introduction

Hypoxia or low oxygen availability is an essential hallmark in the tumor microenvironment (TME)—the condition that influences the progression and the survival of the cancer cells and provides support for them, which results from the rapid proliferation of cancer cells exceeding the blood supply. The lack of oxygen levels forces the cancer cells to alter metabolic pathways to maintain the proliferation and survival of the cancer cells. This adaptation provides critical components for energy storage, membrane synthesis, and the production of signaling molecules. Research indicated that hypoxia-inducible factor 1 (HIF-1) is a pivotal modulator of the metabolic reprogramming of hypoxic cancer cells (Infantino et al., 2021). Even with these advancements, the key enzymes and specific pathways involved in metabolic reprogramming are still poorly understood. This study aims to explain the specific processes and enzymes involved in the case of hypoxia and to find new therapeutic ways for cancer progression.

Hypoxia-inducible factors (HIFs) are proteins that act as transcriptional factors activating

in response to hypoxia to help the cell survive. HIFs are classified into three main types: HIF1, which includes HIF-1α and HIF-1β, which regulates processes such as metabolism and formation of new blood vessels; HIF2, which includes HIF-2α and HIF-2β and plays an essential role in red blood cell production as well as influencing cancer progression; and HIF3, which modulates the activity of HIF1 and HIF2. Together, these factors enable cancer cells to adapt to low oxygen levels.

The HIFs play a critical role in promoting angiogenesis, the process by which new blood vessels form from pre-existing ones under normoxic conditions (normal oxygen levels), HIF-α subunits analyzed by prolyl hydroxylase domain (PHD) enzymes. However, under hypoxic conditions (low oxygen levels), the PHDs are inhibited, so the HIF-α subunits are stabilized and accumulated. The stabilized HIF-α subunits dimerize with HIF-β subunits to form the HIF-1 complex, which then translocates to the nucleus. This complex binds to hypoxia-response elements (HREs), specific DNA sequences in genes activated by hypoxia. HREs work with specific proteins to turn on genes to help the cell adapt by promoting the formation of new blood vessels. HIF1 activates various genes, including vascular endothelial growth factor (VEGF), which promotes the growth of new blood vessels.

In response to hypoxia, cells undergo a metabolic change known as the Warburg effect. These alterations include a shift from oxidative phosphorylation to aerobic glycolysis to support the increased need for ATP (Schiliro & Firestein, 2021) through anaerobic glycolysis. HIF-1α is responsible for this metabolic reprogramming. HIF-1α promotes glycolysis by regulating genes encoding key glycolytic enzymes such as lactate dehydrogenase and hexokinase. The Warburg effect supports tumor growth by providing ATP through anaerobic glycolysis.

HIF-1α is a critical component of the HIF pathway as it enhances the storage and synthesis of lipids by regulating genes involved in de novo lipogenesis—a process through which the body converts the non-fat sources into new fats, such as carbohydrates, and turns the excess sugars in the body and other nutrients into fats. Among the genes involved in de novo lipogenesis are fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC), which are important for fatty acids and triglycerides. This adaptation supports tumor growth and aggressiveness by providing necessary lipids and energy, as ACC is widely used in macromolecule biosynthesis and energy production to support cell growth and proliferation (Gao et al., 2016).

The metabolic reprogramming resulted in the upregulation of FASN and ACC and increased the activity of processes of lipid syntheses that could support the survival and proliferation of cancer cells.

3. Literature Review

3.1 Comprehensive Understanding of Lipid Metabolism Pathways: Although recent research has outlined some aspects of lipid metabolism in hypoxic cancer cells, a significant deficiency exists in studies that integrate the entire hypoxic lipid metabolic pathway. Numerous studies concentrate on specific elements, such as FASN or ACC, but they don't offer a comprehensive picture of how these elements interact with one another within the larger metabolic network (Seo et al., 2022). Currently, no

comprehensive investigation shows the whole pathway of lipid metabolism in response to hypoxia.

3.2 Mechanistic Insights into Enzyme Regulation: Although recent studies have highlighted the function of enzymes such as ACC and FASN in hypoxic tumor metabolism, there is a lack of specific mechanistic information regarding the molecular regulation of these enzymes (Gao et al., 2023). Targeted therapy approaches may be improved by having a better understanding of the particular regulatory mechanisms and connections between these enzymes and other metabolic pathways.
3.3 Clinical Settings: A large portion of current research is based on short-term in vivo and in vitro models, which may not adequately represent the long-term consequences of hypoxia-induced lipid metabolic alterations in human cancers. To validate these results and determine their significance for patient outcomes, longitudinal studies conducted in clinical settings are required.

3.4 Lack of Comprehensive Research: One major research gap is the lack of studies covering the whole metabolic cycle in hypoxic cancer cells, from lipid synthesis to use. Studies that have already been conducted usually focus on specific elements of lipid metabolism rather than offering a comprehensive and integrative view of how lipid metabolism aids in cancer cells' ability to adapt and survive in low-oxygen settings.

This work offers a thorough examination of the whole lipid metabolic network as opposed to concentrating on individual components to fill in the knowledge gaps regarding lipid metabolism in hypoxic cancer cells. It provides thorough mechanistic insights into the regulation of important enzymes like FASN and ACC by hypoxia-inducible factors (HIFs), highlighting their upregulation in low-oxygen environments. Furthermore, this study provides a more precise and practically applicable viewpoint by capturing the long-term impacts of hypoxia on lipid metabolism using both in vitro and in vivo models. These results indicate possible directions for therapeutic intervention and fill in some of the existing gaps.

4. **Methods**

The methodology focuses on understanding the affected lipid metabolism done by hypoxia in the cancer cells using vitro (cell culture) and vivo (animal models) techniques, moreover, this methodology is based on a lot of experimental papers.

The key tools that have been used include Lipidomic: To measure lipid changes. In addition to qPCR and Western Blotting: To assess gene and protein expression. Moreover, Enzyme Activity Assays to evaluate the functionality of metabolic enzymes.

4.1 Cell Culture

4.1.1 Purpose: This step aims to create controlled normoxic and hypoxic environments to detect alterations in the

cancer cell metabolism and behavior in response to low oxygen levels.
4.1.2 Procedure: -Cell Line Selection: Lung and breast cancer cell lines are examples of human cancer cell lines. They are chosen because these types are known to experience hypoxic conditions in their tumor microenvironment (Gilreath et al., 2021). Examples include MCF-7 (breast cancer) and A549 (lung cancer) cell lines.
4.1.3 Culturing Condition: Cells are grown under two types of conditions which are normoxic and hypoxic.
-Normoxic: This refers to a normal level of oxygen, as it is argued that normoxia (20% oxygen) (McKeown, 2014). This is what is found in most tissues in the body.
-Hypoxia**:** This refers to a low oxygen level (approximately 1% oxygen). Expressing the oxygen level found in the solid tumors.
After exposing the cells to these conditions, the lipid content can be detected by using lipidomics –a technique that detects various types of lipids (fats) in the cells. Mass spectrometry is employed to quantify lipid molecules.

4.2 Gene Expression Analysis

4.2.1 Purpose: To identify the effects of hypoxia on gene expression, specifically identifying which genes and enzymes are regulated.
4.2.2 Procedure: -Quantitative PCR (qPCR): A common application of qPCR is gene expression analysis, e.g., comparing the mRNA concentrations of a gene of interest between control and treated samples (Quantitative PCR Basics, n.d.). Specifically, genes encoding enzymes such as fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) will be measured.

-Western Blotting: Western blotting is a laboratory technique used to detect a specific protein in a blood or tissue sample (*Western Blot*, n.d.). This includes proteins such as FASN, ACC, and other enzymes involved in lipid metabolism.

4.3 Enzyme Activity Assays

4.3.1 Purpose: To evaluate the functional activity of enzymes and understand how metabolic pathways are reprogrammed under hypoxic conditions.
4.3.2 Procedure: Activity Measurement: Enzyme assays are laboratory methods for measuring enzymatic activity (*Enzyme Activity Assays - Creative Biolabs*, n.d.).
-Comparison between the two conditions: Enzyme activities will be compared between normoxic and hypoxic conditions to determine the effect on the enzyme function under hypoxia.

4.4 In Vivo Studies:

4.4.1 Purpose: To validate in vitro findings in a living organism, providing more details about lipid metabolism under hypoxia.
4.4.2 Mouse Models:
This *mouse* model *mimics* the *human tumor* through genetic manipulation of the desired gene (Khuda-Bukhsh et al., 2023). Hypoxic tumors will be induced either by decreasing oxygen supply or by genetically engineering mice to have tumors with poor blood supply.

-Lipid Analysis: Tumors will be harvested from the mice and lipid content will be detected by mass spectrometry in vitro method.
-Gene Expression and protein analysis: Using qPCR and Western blotting, which are used in vitro to measure gene and protein expression levels related to lipid metabolism.

5. Results

Our findings show that hypoxia significantly impacts the lipid metabolism of cancer cells. In particular, we found that hypoxia cells produced and stored more triglycerides and fatty acids than normoxic controls. Upregulation of FASN and ACC and increased activity of pathways involved in lipid synthesis accompanied this metabolic change, as the protein level of FAS was also strongly increased in hypoxic conditions (Furuta et al., 2008); moreover, malonyl-CoA levels increase in the cells by 7 h of hypoxia (Wang et al., 1996), suggesting that cancer cells take up more acetate under hypoxia than normoxia (Gao et al., 2016). Furthermore, there was a considerable increase in the creation of lipid droplets in hypoxic cells, suggesting an improved ability for lipid storage that could support the survival and aggressiveness of cancer cells in low-oxygen environments.

In vivo, investigations employing mouse models further supported the in vitro findings by showing that tumors growing in hypoxic environments had a significantly higher lipid content and upregulated expression of genes related to lipid metabolism, including FASN and ACC. Moreover, these hypoxic tumors showed more ability to metastasize and more aggressiveness which was most likely due to the changes in lipid metabolism that were observed. The results point to a likely connection between the enhanced aggressive activity of cancer cells and modified lipid metabolism under hypoxia. Another research has shown that they treated cancer cells with gradient acetate concentrations and found that FASN and ACC mRNA expression were upregulated in a dose-dependent manner under hypoxia (Gao et al., 2016).

These graphs are illustrative and represent theoretically anticipated results; they are not based on actual experimental data.

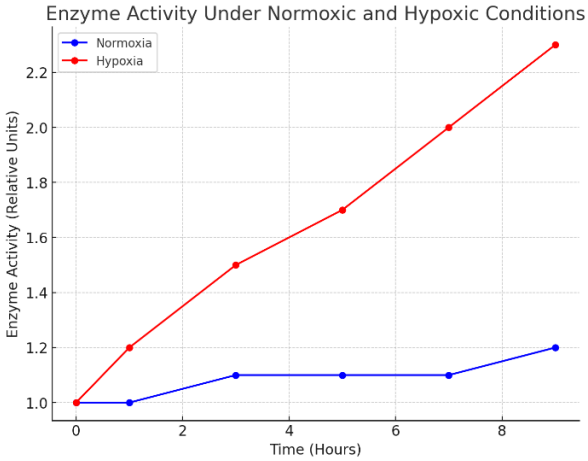

Fig. 1: This graph indicates enzyme activity under normoxic and hypoxic conditions.

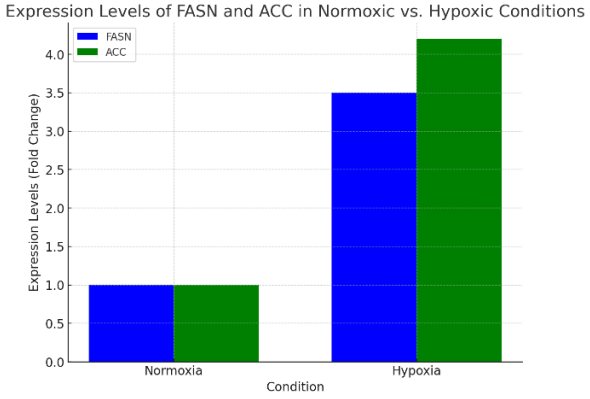

Fig. 2: This graph indicates the difference between the expression levels of FASN and ACC under normoxia and hypoxia.

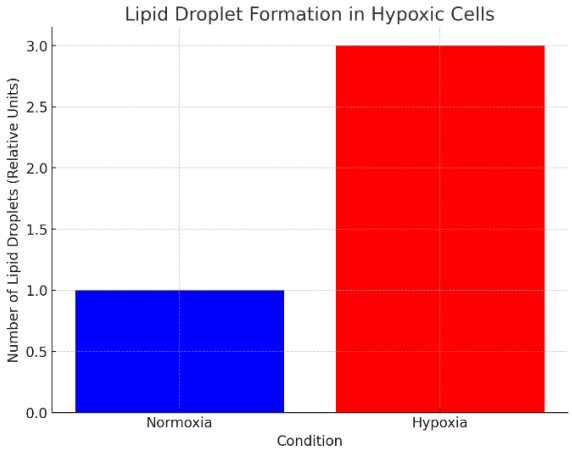

Fig. 3: This graph indicates the difference between the lipid droplet in the case of normoxia and hypoxia.

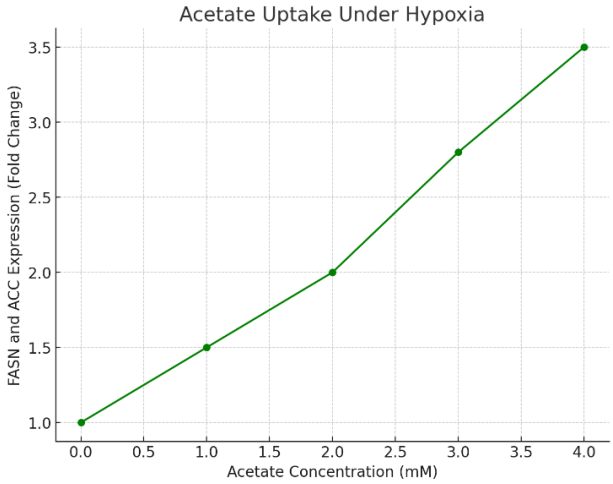

Figure 4: This graph indicates the upregulation of FASN and ACC in response to hypoxia.

6. **Discussion**

The results of this study show how lipid metabolism is essential for cancer cells' survival and proliferation to adapt to hypoxia, by the upregulation of productions and lipid storage. As shown in Figure 1, the enzyme activity under the hypoxic condition upregulated, indicating the specific enzymes in another graph. As shown in Figure 2, fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) are upregulated enzymes in response to hypoxia, which play a critical role in de novo lipogenesis, suggesting that these enzymes may be used as therapeutic targets. By blocking these enzymes, the cancer cells will not be able to the metabolic reprogramming, which will reduce the amounts of divisions and metastasis that can occur. Future research should aim to investigate the efficiency of inhibition of the enzymes under hypoxia as therapeutic ways to slow the progression of cancer cells.

As shown in Figure 3, the upregulation of FASN and ACC are essential for lipids synthesis, which is not just important in the energy reservoir for cancer cells, but they are also essential as building blocks for membrane synthesis, enabling cancer cells in their proliferation. This upregulation also has shown that it has consequences in the potential metastatic and aggressiveness. These findings offer the development of targeting therapies in cancer tumors.

7. **Conclusion**

This study is about looking into the alterations in lipid metabolism under sharp oxygen (hypoxia) conditions focusing on specific needed enzymes for this reprogramming like fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC). The results revealed that cancer celís under lipid reprogramming make for survivability

and growth when hypoxic, thus, allowing the door for targeted therapeutics. Cancer cells aren't solely using glycolysis (the so-called Warburg effect) when hypoxia is present. They come to the point of condensing their lipid production.

Besides that, it has been demonstrated that the hypoxia-inducible factors (HIFs), and most importantly HIF-1, are the things that bring about the control of lipid metabolism in the process. Besides metabolic processes regulation, HIF-1a is also accountable for new blood vessel formation, and cancer cell survival in hypoxic conditions.

Our findings demonstrate the importance of learning more about cancer metabolism via the use of live animal models as well as cell culture methods. Although variables like oxygen levels can be very precisely controlled in cell culture and in animal models like mice. We can decrease how fast tumors grow and learn how to make other treatments more effective by inhibiting these metabolic enzymes.

8. Acknowledgement

I would like to express my sincere gratitude to the Minerva Research Program for their generous support, which was essential to successfully completing this research. Additionally, I am deeply thankful to all the researchers and colleagues who provided constructive feedback and insightful discussions that greatly enhanced the quality of this paper.

9. References

1- Ackerman, D., & Simon, M. C. (2014). Hypoxia, lipids, and cancer: Surviving the harsh tumor microenvironment. *Trends in Cell Biology, 24*(8), 472–478. https://doi.org/10.1016/j.tcb.2014.06.001
2- Calabrese, B. (2019). Experimental platforms for extracting biological data: Mass spectrometry, microarray, next-generation sequencing. In *Elsevier eBooks* (pp. 126–129). https://doi.org/10.1016/b978-0-12-809633-8.20412-3
3- Chan, D. A., & Giaccia, A. J. (2010). PHD2 in tumour angiogenesis. *British Journal of Cancer, 103*(1), 1–5. https://doi.org/10.1038/sj.bjc.6605682
4- Enzyme Activity Assays - Creative Biolabs. (n.d.). https://www.creative-biolabs.com/enzyme-activity-assays.html
5- Furuta, E., Pai, S. K., Zhan, R., Bandyopadhyay, S., Watabe, M., Mo, Y., Hirota, S., Hosobe, S., Tsukada, T., Miura, K., Kamada, S., Saito, K., Iiizumi, M., Liu, W., Ericsson, J., & Watabe, K. (2008). Fatty acid synthase gene is up-regulated by hypoxia via activation of AKT and sterol regulatory element binding protein-1. *Cancer Research, 68*(4), 1003–1011. https://doi.org/10.1158/0008-5472.can-07-2489
6- 6. Gao, X., Lin, S., Ren, F., Li, J. T., Chen, J. J., Yao, C. B., Yang, H. B., Jiang, S. X., Yan, G. Q., Wang, D., Wang, Y., Liu, Y., Cai, Z., Xu, Y. Y., Chen, J., Yu, W., Yang, P. Y., & Lei, Q. Y. (2016). Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. *Nature

Communications, 7*(1). https://doi.org/10.1038/ncomms11960

7- Gao, X., Lin, S., Ren, F., Li, J. T., Chen, J. J., Yao, C. B., Yang, H. B., Jiang, S. X., Yan, G. Q., Wang, D., Wang, Y., Liu, Y., Cai, Z., Xu, Y. Y., Chen, J., Yu, W., Yang, P. Y., & Lei, Q. Y. (2023). Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. *Nature Communications, 14*(1). https://doi.org/10.1038/s41467-023-41782-w

8- Gilreath, C., Boerma, M., Qin, Z., Hudson, M. K., & Wang, S. (2021). The hypoxic microenvironment of breast cancer cells promotes resistance in radiation therapy. *Frontiers in Oncology, 10*. https://doi.org/10.3389/fonc.2020.629422

9- Heck-Swain, K., & Koeppen, M. (2023). The intriguing role of hypoxia-inducible factor in myocardial ischemia and reperfusion: A comprehensive review. *Journal of Cardiovascular Development and Disease, 10*(5), 215. https://doi.org/10.3390/jcdd10050215

10- Hirota, K., & Semenza, G. L. (2006). Regulation of angiogenesis by hypoxia-inducible factor 1. *Critical Reviews in Oncology/Hematology, 59*(1), 15–26. https://doi.org/10.1016/j.critrevonc.2005.12.003

11- Infantino, V., Santarsiero, A., Convertini, P., Todisco, S., & Iacobazzi, V. (2021). Cancer cell metabolism in hypoxia: Role of HIF-1 as key regulator and therapeutic target. *International Journal of Molecular Sciences, 22*(11), 5703. https://doi.org/10.3390/ijms22115703

12- Khuda-Bukhsh, A. R., Das, J., & Samadder, A. (2023). Mice as experimental models for cancer research. In *Handbook of Animal Models and Its Uses in Cancer Research* (pp. 87–109). https://doi.org/10.1007/978-981-19-3824-5_5

13- Krock, B. L., Skuli, N., & Simon, M. C. (2011). Hypoxia-induced angiogenesis: Good and evil. *Genes & Cancer, 2*(12), 1117–1133. https://doi.org/10.1177/1947601911423654

14- Lee, M. (2015). Metabolic interplay between glycolysis and mitochondrial oxidation: The reverse Warburg effect and its therapeutic implication. *World Journal of Biological Chemistry, 6*(3), 148. https://doi.org/10.4331/wjbc.v6.i3.148

15- Li, Y., Zhao, L., & Li, X. (2021). Hypoxia and the tumor microenvironment. *Technology in Cancer Research & Treatment, 20*, 153303382110363. https://doi.org/10.1177/15330338211036304

16- McKeown, S. R. (2014). Defining normoxia, physoxia, and hypoxia in tumours—implications for treatment response. *British Journal of Radiology, 87*(1035), 20130676. https://doi.org/10.1259/bjr.20130676

17- Mounier, C., Bouraoui, L., & Rassart, E. (2014). Lipogenesis in cancer progression (Review). *International Journal of Oncology, 45*(2), 485–492. https://doi.org/10.3892/ijo.2014.2441

18- Munir, R., Lisec, J., Swinnen, J. V., & Zaidi, N. (2019). Lipid metabolism in cancer cells under metabolic stress. *British Journal of Cancer, 120*(12), 1090–1098. https://doi.org/10.1038/s41416-019-0451-4

19- Mylonis, I., Simos, G., & Paraskeva, E. (2019). Hypoxia-inducible factors and the regulation of lipid metabolism. *Cells, 8*(3), 214. https://doi.org/10.3390/cells8030214
20- Peck, B., & Schulze, A. (2016). Lipid desaturation—The next step in targeting lipogenesis in cancer? *FEBS Journal, 283*(15), 2767–2778. https://doi.org/10.1111/febs.13681
21- Pavlides, S., Whitaker-Menezes, D., Castello-Cros, R., Flomenberg, N., Witkiewicz, A. K., Frank, P. G., Casimiro, M. C., Wang, C., Fortina, P., Addya, S., Pestell, R. G., Martinez-Outschoorn, U. E., Sotgia, F., & Lisanti, M. P. (2009). The reverse Warburg effect: Aerobic glycolysis in cancer-associated fibroblasts and the tumor stroma. *Cell Cycle, 8*(23), 3984–4001. https://doi.org/10.4161/cc.8.23.10238
22- Quantitative PCR Basics. (n.d.). https://www.sigmaaldrich.com/EG/en/technical-documents/technical-article/genomics/qpcr/quantitative-pcr#mrna
23- Schiliro, C., & Firestein, B. L. (2021). Mechanisms of metabolic reprogramming in cancer cells supporting enhanced growth and proliferation. *Cells, 10*(5), 1056. https://doi.org/10.3390/cells10051056
24- Seo, J., Yun, J., Kim, S. J., & Chun, Y. (2022). Lipid metabolic reprogramming by hypoxia-inducible factor-1 in the hypoxic tumour microenvironment. *Pflügers Archiv - European Journal of Physiology, 474*(6), 591–601. https://doi.org/10.1007/s00424-022-02683-x
25-Wang, D., Buja, L., & McMillin, J. B. (1996). Acetyl coenzyme A carboxylase activity in neonatal rat cardiac myocytes in culture: Citrate dependence and effects of hypoxia. *Archives of Biochemistry and Biophysics, 325*(2