HIGH SCHOOL EDITION
Journal of Student Research
Volume 14 Issue 1 (2025)

The Warburg Effect and Its Role in Tumorigenesis

Angela Peddapalli

Lambert High School, USA

ABSTRACT

The second leading cause of death in the United States, cancer is the rapid proliferation of abnormal cells that grow and spread beyond their usual boundaries in the body. A significant part of cancer research has focused on elucidating the nature of tumorigenic cells to determine viable treatment therapies and prevention methods for cancer development and metastasis. One of the major hallmarks of cancer is tumor cell glycometabolism, where cancer cells rewire their metabolism to promote their growth, survival, maintenance, and proliferation. This aberrant metabolism is characterized by increased glycolysis even in the presence of oxygen, a phenomenon known as the 'Warburg Effect'. Several prominent characteristics of the Warburg Effect have been observed over the last century, including increased glucose uptake, lactate accumulation, and induced acidosis in the tumor microenvironment. This review examines the Warburg Effect, its role in tumorigenesis, and current anticancer therapeutics that have arisen from related studies.

Introduction

Awareness that the metabolic phenotype of tumorigenic cells differs from that of non-tumorigenic cells has grown in the last decade. Tumors reprogram their nutrient acquisition and glycometabolism pathways to meet the bioenergetic, biosynthetic, metabolic, and redox needs of malignant tumor cells. Cancer cells generally metabolize glucose, lactate, pyruvate, and fatty acids at significantly higher rates than their nontumor counterparts [1]. Recent experimental evidence suggests that tumorigenic metabolic activity can induce cancer progression and metastasis. Therefore, targeting the metabolic differences between tumor and non-tumor cells may be a promising anticancer strategy.

In 1924, German scientist Otto Warburg observed that cancer cells consumed greater amounts of glucose when compared to normal cells [2]. His subsequent research discovered that cancer cells metabolize glucose substrate in an unusual manner and accelerated rate compared to cells in normal, healthy tissue [3]. Unlike non-tumorigenic cells, tumorigenic cells were found to produce ATP (adenosine triphosphate) by fermenting glucose into lactate with overactive glycolysis despite having sufficient oxygen [4]. This unusual shift from respiration to fermentation became known as the Warburg Effect. Although characteristics of the Warburg Effect can be found in many cancers, different tumor types have different bioenergetic alterations that enable them to meet their specific energy requirements [5]. Therefore, although the Warburg Effect is not consistent across all cancer types, it is still a valuable model to discover potential anticancer therapeutic candidates by studying its primary characteristics: increased glucose uptake, lactate production and accumulation, and induced acidosis [6].

Characteristics Of the Warburg Effect

Glucose Uptake

The basis of cancer cell metabolism highlights an increased glucose uptake in tumorigenic cells due to increased protein production and membrane translocation of facilitative glucose transporters (GLUTs) that allow cancer cells to take up extracellular glucose [7,8]. To produce sufficient ATP through glycolysis, cancer cells upregulate GLUTs, increasing intracellular glucose uptake [9]. Out of these transporters, GLUT1, GLUT3, GLUT4, and GLUT6 are

upregulated in cancer tissue [10,11]. GLUT expression and localization changes in response to nutrient deprivation. In non-cancer cells, nutrient deprivation causes GLUT1 to internalize and undergo lysosomal degradation to decrease metabolism before the induction of apoptosis. However, cancer cells overexpress GLUT1 after nutrient deprivation to maintain glucose metabolism. As a result, the cancer cell becomes resistant to induced apoptosis [12]. GLUT1 overexpression is also activated during hypoxia conditions resulting from overactive glycolysis and encourages the expression of several cell survival genes, including vascular endothelial growth factor (VEGF) [13,14]. GLUT1 overexpression has been discovered in several cancer types, including breast, brain, pancreatic, prostate, renal, lung, and endometrial. Increased GLUT1 expression has also been associated with unfavorable prognosis, poorly differentiated tumors, larger tumor size, and positive lymph node metastasis [15,16,17].

Its prominent function in supporting cancer metabolism implies that GLUT1 may be an ideal prognostic biomarker and potential therapeutic target in various cancers. Resveratrol (RSV) is a natural compound that has gained much attention in the cancer field with its anticarcinogenic, anti-inflammatory, cardioprotective, and antiproliferative properties [18,19]. RSV has also been found to inhibit glucose uptake in human leukemic cell lines by directly interacting with GLUT1 [20,21]. RSV has been an especially attractive candidate for cancer therapy because of its ability to wield concentrated short-term effects of metabolism through the mTOR/AMPK signaling pathway [20]. One study concluded that elevated levels of RSV lead to tumor regression and widespread cancer cell death in human neuroblastoma [22]. RSV can also reverse multidrug resistance in cancer cells and sensitize cancer cells to standard chemotherapeutic drugs when combined with clinically used pharmaceuticals [23]. However, there have been several controversial reports of RSV due to its photosensitivity. Therefore, it must be handled carefully and used at precise dosages according to cell type and metabolic state. Moreover, while all cells need glucose to survive, partial inhibition of GLUT1 as monotherapy has been unsuccessful [20]. Thus, combinatorial strategies that use GLUT1 inhibitors like RSV with anticancer conventional drugs seem more promising. Other GLUT1 inhibitors like SMI277 and BAY-876 have also shown promising results and are undergoing further testing [24,25,26,27].

PI3K/Akt signaling regulates glucose uptake by facilitating GLUT1 membrane localization, thus augmenting the rate of glycolysis. Many studies have revealed a gain of function of PI3Ks and hyperactive PI3K/Akt signaling several cancer types [28]. Hence, reducing activity along the PI3K/Akt signaling pathway poses a new approach for targeted cancer therapy [29]. Various PI3K isoform-specific inhibitors have undergone clinical testing, including ACP-319, BYL719, and Serabelisib. However, most of the monotherapies have failed to produce promising results. Thus, recent clinical trials are attempting to use a combination of two inhibitors of different signaling transduction pathways to target parallel pathways. So far, a combination of 7-hydroxystaurosporine and topotecan hydrochloride has been used to treat patients with small-cell lung cancer, and ONC201 is being studied in phase II clinical trials for metastasized breast cancer and advanced endometrial carcinoma [30].

Lactate Production

Lactate is the product metabolite of glycolysis. Therefore, overactive glycolysis in tumorigenic cells leads to increased lactate production and accumulation in cancer tissue. Cancer cells export lactic acid out of the cell to prevent intracellular acidification. The subsequent accumulation of extracellular lactate can contribute to several effects and hallmarks of cancer. First, lactate stimulates hyaluronan production and CD44 expression, reducing cell adherence and contributing to malignant progression and metastasis in cancer tissue [31,32]. Lactate also acts as a signaling molecule for the G-protein-coupled receptor GPR81. Excessive lactate production activates GPR81 on cancer cells and immune cells, which promotes angiogenesis and chemoresistance in tumors. Moreover, lactic acid acts as a pro-inflammatory and immunosuppressive molecule that dampens the immune response to cancer in a concentration-dependent manner [33,34]. In this process, lactic acid dissociates into lactate and H+ ions that are exported into the extracellular tumor microenvironment (TME). The exported H+ ions lower the pH in the TME and consequently undermine the T-cell response to cancer, allowing immune escape to occur [35]. Tumor-derived lactate has also been shown to promote T-

cell apoptosis by inhibiting FIP200 protein [36]. Finally, tumor-derived lactate contributes to inhibiting the anticancer functions of natural killer cells (specialized white blood cells that destroy infected and cancerous cells) [37,38].

As demonstrated by a number of experimental studies, the overproduction of lactate and its entry into the TME by transporter complexes contributes greatly to several cancerous mechanisms. Although it has proven difficult to directly reduce lactate production in cancer cells without endangering cancer patients, it has been speculated that targeting lactic acid transporter complexes may be a potential therapeutic strategy in cancer trials. MCT4, known for its high affinity for lactate, is a lactic acid transporter that is primarily expressed in highly glycolytic cells. MCT4 expression is upregulated in hypoxia conditions and in many cancer types. Additionally, MCT4 expression is strongly associated with lymph node metastasis, distant metastasis, and for colorectal and hepatic cancers [39,40,41,42]. Therefore, selective inhibition of MCT4 may effectively undermine cancer progression to metastasis. One study in 2018 demonstrated that selective inhibition of MCT4 decreased cell growth and reduced induction of apoptosis in invasive urothelial carcinoma [43]. Another study in 2015 showed that MCT4 depletion caused an increased dependence of cancer cells on mitochondrial respiration and glutamine metabolism, effectively undermining their capacity to proliferate in a 3D matrix or as multilayered spheroids [41]. Another lactate transporter of the same protein family, MCT1, has also been considered as an anticancer therapeutic candidate. AZD3965 is an MCT1 inhibitor that is currently undergoing Phase I clinical trials [44]. Syrosingopine, an anti-hypertensive drug, is a dual MCT1 and MCT4 inhibitor (with a 60-fold higher potency on MCT4) that prevents lactate and H+ efflux [45]. Syrosingopine also sensitizes cancer cells to killing by metformin and phenformin. Therefore, combining syrosingopine with other codrugs seems to be a promising therapeutic strategy for cancer treatment [46].

Induced Acidosis

We have discussed that the tumorigenic microenvironment of a cancer cell is acidic due to the cell's high glycolic rate and subsequent increase in lactate that accumulates in the interstitial space to lower the extracellular pH. This phenomenon occurs because cytotoxic T lymphocytes co-transport H+ and lactate to the extracellular space to maintain intracellular pH while producing cytokines [47]. Moreover, since the cancerous overproduction of lactate decreases intracellular pH, PFK-1 (6-phosphofructo-1-kinase) – a rate-limiting enzyme in glycolysis – is inhibited. This process encourages glycolysis, making lactate production a positive feedback control mechanism in aberrant cancer metabolism [10].

The acidic microenvironment is a potent driver of cancer development, progression, and metastasis as it encourages cell proliferation, acid-induced cell motility, extracellular matrix degradation, attenuated immune responses, modified cellular and intracellular signaling, and – as previously mentioned – further glycolysis. The low pH facilitates local invasion, where H+ ions are transported into adjacent non-cancerous tissue via the concentration gradient. The H+ ions cause tissue remodeling that permits local invasion [48]. This proton concentration gradient can also act as a driving force for proton-coupled transporters in cancer cells to maintain the supply of selective nutrients that the cell requires for additional growth and proliferation [33]. Non-tumourigenic cells cannot tolerate the acid microenvironment of tumourigenic cells. So when non-tumorigenic cells are close or adjacent to tumorigenic cells, their surrounding extracellular matrix is eventually degraded due to the activity of proteinases, an increase in VEGF, and the inhibition of immune response to tumor antigens from cell migration and metastasis [10,49]. Studies have also revealed that the acidic TME in cancer tissue promotes tumorigenesis and tumor cell dormancy as the low pH increases angiogenesis, promotes tumor cell invasion, inhibits T-cell mediated immune surveillance, and increases cell resistance to the induction of apoptosis and autophagy [50,51]. Finally, the acidic TME decreases the intracellular concentration of chemotherapy drugs -- including anthraquinones and vinca alkaloids -- by ion trapping mechanisms, resulting in drug resistance [52].

Genetic instability and epigenetic changes represent another prominent driver in the development of most cancers as well as key determining factors in the transition from normal, healthy tissue to preneoplastic tissue (a critical stage in cancer development) [53,54]. Recent studies have suggested that a strongly acidic microenvironment

can be clastogenic and cause double-stranded breaks in DNA through acid-induced damage on topoisomerase II [55,56]. Repairing sublethal DNA damage is also inhibited at acidic pH levels, resulting in the accumulation of chromosomal aberrations [57]. Taken together, acidic stress in a preneoplastic setting may augment genetic instability in precancerous conditions and increase a patient's risk of transitioning to cancer.

Investigating the detrimental effects that TME pH dysregulation has on cancer development and prognosis has given scientists new paths of exploration in anticancer therapeutic drug development. Numerous experiments have proven that neutralizing the pH in the acidic TME can restore immune-cell function and improve antitumor responses to immunotherapy [58,59,60,61]. The most notable therapeutic strategy developed from such experiments seems to be oral buffer therapy. Neutralizing tumor microenvironment acidity in mice with bicarbonate monotherapy impaired the growth of cancers associated with increased T-cell infiltration. Combining bicarbonate therapy with anti-CTLA-4, anti-PD1, or adoptive T-cell transfer also improved antitumor responses [59]. Several other buffers have decreased tumor acidity and inhibited invasion and metastasis, including PDAC, L-DOS47, and imidazole effectively. However, despite the experimental promise of some of these buffer therapies, translating them into clinical trials has been challenging. Phase I/II trials for PDAC failed to escalate beyond the second dose levels, leading to poor compliance. On the other hand, L-DOS47 was well-tolerated and dose escalated in a phase I/II trial in non-small cell lung cancer. Some tumor models show that metastasis is not inhibited by buffers, regardless of the buffer used. Murine melanoma, murine lung, and human colon tumors are resistant to treatment with lysine buffer therapy, whereas metastasis is effectively inhibited by lysine buffers in human breast and prostate tumors. The observed buffer-resistant cell lines displayed constitutive secretion of matrix-degrading proteases without elevated glycolysis, and further characterization of these models is required for future therapeutic development [62]. The field is currently exploring alternatives to some of these anticancer buffer therapeutics that may indirectly neutralize acidosis by targeting proton transport mechanisms that contribute to lowering TME pH. Such alternatives include carbonic anhydrase-9 (CA-IX) and monocarboxylate transporters (MCT1/4) [63].

Conclusions

When it comes to developing viable pharmaceuticals for anticancer therapies and treatments, the Warburg Effect has been a major tool and influence in cancer research for the past century and continues to be applicable to experimental models today. Although still in its exploratory stage, targeting the Warburg effect has already led to promising results and several successful clinical trial phases in drug development. By aiming to reach a full understanding of tumor cell metabolism, effective research can be conducted to target those mechanisms to antagonize tumor cell pathways and improve chemotherapeutic differential targeting strategies.

Acknowledgments

I would like to thank my advisor for the valuable insight provided to me on this topic.

References

[1] Martinez-Outschoorn, U. E., Peiris-Pagés, M., Pestell, R. G., Sotgia, F., & Lisanti, M. P. (2016). Cancer metabolism: A therapeutic perspective. *Nature Reviews Clinical Oncology*, *14*(1), 11–31. doi:10.1038/nrclinonc.2016.60

[2] Lu, J., Tan, M., & Cai, Q. (2015). The Warburg effect in tumor progression: Mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Letters*, *356*(2), 156–164. doi:10.1016/j.canlet.2014.04.001

[3] Ostroukhova, M., Goplen, N., Karim, M. Z., Michalec, L., Guo, L., Liang, Q., & Alam, R. (2012). The role of low-level lactate production in airway inflammation in asthma. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *302*(3). doi:10.1152/ajplung.00221.2011

[4] DeBerardinis, R. J., Lum, J. J., Hatzivassiliou, G., & Thompson, C. B. (2008). The biology of cancer: Metabolic reprogramming fuels cell growth and proliferation. *Cell Metabolism*, *7*(1), 11–20. doi:10.1016/j.cmet.2007.10.002

[5] Potter, M., Newport, E., & Morten, K. J. (2016). The warburg effect: 80 years on. *Biochemical Society Transactions*, *44*(5), 1499–1505. doi:10.1042/bst20160094

[6] Ferreira L. M. (2010). Cancer metabolism: the Warburg effect today. *Experimental and molecular pathology*, *89*(3), 372–380. https://doi.org/10.1016/j.yexmp.2010.08.006

[7] Pauwels, E. K., Sturm, E. J., Bombardieri, E., Cleton, F. J., & Stokkel, M. P. (2000). Positron-emission tomography with [18F]fluorodeoxyglucose. Part I. Biochemical uptake mechanism and its implication for clinical studies. *Journal of cancer research and clinical oncology*, *126*(10), 549–559. https://doi.org/10.1007/pl00008465

[8] Gonzalez-Menendez, P., Hevia, D., Alonso-Arias, R., Alvarez-Artime, A., Rodriguez-Garcia, A., Kinet, S., … Sainz, R. M. (2018). GLUT1 protects prostate cancer cells from glucose deprivation-induced oxidative stress. *Redox Biology*, *17*, 112–127. doi:10.1016/j.redox.2018.03.017

[9] Cazzaniga, M., & Bonanni, B. (2015). Relationship Between Metabolic Reprogramming and Mitochondrial Activity in Cancer Cells. Understanding The Anticancer Effect of Metformin and Its Clinical Implications. *Anticancer research*, *35*(11), 5789–5796.

[10] Lebelo, M. T., Joubert, A. M., & Visagie, M. H. (2019). Warburg effect and its role in tumourigenesis. *Archives of Pharmacal Research*, *42*(10), 833–847. doi:10.1007/s12272-019-01185-2

[11] Macheda, M. L., Rogers, S., & Best, J. D. (2004). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *Journal of Cellular Physiology*, *202*(3), 654–662. doi:10.1002/jcp.20166

[12] Gonzalez-Menendez, P., Hevia, D., Alonso-Arias, R., Alvarez-Artime, A., Rodriguez-Garcia, A., Kinet, S., … Sainz, R. M. (2018a). GLUT1 protects prostate cancer cells from glucose deprivation-induced oxidative stress. *Redox Biology*, *17*, 112–127. doi:10.1016/j.redox.2018.03.017

[13] Aquino-Gálvez, A., González-Ávila, G., Delgado-Tello, J., Castillejos-López, M., Mendoza-Milla, C., Zúñiga, J., Checa, M., Maldonado-Martínez, H. A., Trinidad-López, A., Cisneros, J., Torres-Espíndola, L. M., Hernández-Jiménez, C., Sommer, B., Cabello-Gutiérrez, C., & Gutiérrez-González, L. H. (2016). Effects of 2-methoxyestradiol on apoptosis and HIF-1α and HIF-2α expression in lung cancer cells under normoxia and hypoxia. *Oncology reports*, *35*(1), 577–583. https://doi.org/10.3892/or.2015.4399

[14] Barteczek, P., Li, L., Ernst, A. S., Böhler, L. I., Marti, H. H., & Kunze, R. (2017). Neuronal HIF-1α and HIF-2α deficiency improves neuronal survival and sensorimotor function in the early acute phase after ischemic stroke. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, *37*(1), 291–306. https://doi.org/10.1177/0271678X15624933

[15] Yu M, Yongzhi H, Chen S, Luo X, Lin Y, Zhou Y, Jin H, Hou B, Deng Y, Tu L, Jian Z. The prognostic value of GLUT1 in cancers: a systematic review and meta-analysis. Oncotarget. 2017 Jun 27;8(26):43356-43367. doi: 10.18632/oncotarget.17445. PMID: 28498810; PMCID: PMC5522151.

[16] Wang, Y., Shu, Y., Gu, C., & Fan, Y. (2019). The novel sugar transporter SLC50A1 as a potential serum-based diagnostic and prognostic biomarker for breast cancer. *Cancer management and research*, *11*, 865–876. https://doi.org/10.2147/CMAR.S190591

[17] Bellance, N., Benard, G., Furt, F., Begueret, H., Smolková, K., Passerieux, E., Delage, J. P., Baste, J. M., Moreau, P., & Rossignol, R. (2009). Bioenergetics of lung tumors: alteration of mitochondrial biogenesis and respiratory capacity. *The international journal of biochemistry & cell biology*, *41*(12), 2566–2577. https://doi.org/10.1016/j.biocel.2009.08.012

[18] Hsieh, T. C., & Wu, J. M. (2010). Resveratrol: Biological and pharmaceutical properties as anticancer molecule. *BioFactors (Oxford, England)*, *36*(5), 360–369. https://doi.org/10.1002/biof.105

[19] Carter, L. G., D'Orazio, J. A., & Pearson, K. J. (2014). Resveratrol and cancer: focus on in vivo evidence. *Endocrine-related cancer*, *21*(3), R209–R225. https://doi.org/10.1530/ERC-13-0171

[20] Zambrano, A., Molt, M., Uribe, E., & Salas, M. (2019). Glut 1 in cancer cells and the inhibitory action of resveratrol as a potential therapeutic strategy. *International Journal of Molecular Sciences*, *20*(13), 3374. doi:10.3390/ijms20133374

[21] Chen, S., Zhao, Z., Ke, L., Li, Z., Li, W., Zhang, Z., … Zhu, W. (2018). Resveratrol improves glucose uptake in insulin-resistant adipocytes via SIRT1. *The Journal of Nutritional Biochemistry*, *55*, 209–218. doi:10.1016/j.jnutbio.2018.02.007

[22] van Ginkel, P. R., Sareen, D., Subramanian, L., Walker, Q., Darjatmoko, S. R., Lindstrom, M. J., Kulkarni, A., Albert, D. M., & Polans, A. S. (2007). Resveratrol inhibits tumor growth of human neuroblastoma and mediates apoptosis by directly targeting mitochondria. *Clinical cancer research : an official journal of the American Association for Cancer Research*, *13*(17), 5162–5169. https://doi.org/10.1158/1078-0432.CCR-07-0347

[23] Ko, J. H., Sethi, G., Um, J. Y., Shanmugam, M. K., Arfuso, F., Kumar, A. P., Bishayee, A., & Ahn, K. S. (2017). The Role of Resveratrol in Cancer Therapy. *International journal of molecular sciences*, *18*(12), 2589. https://doi.org/10.3390/ijms18122589

[24] Chen, Z., Vaeth, M., Eckstein, M., Delgobo, M., Ramos, G., Frantz, S., Hofmann, U., & Gladow, N. (2023). Characterization of the effect of the GLUT-1 inhibitor BAY-876 on T cells and macrophages. *European journal of pharmacology*, *945*, 175552. https://doi.org/10.1016/j.ejphar.2023.175552

[25] Chen, X., Zhao, Y., He, C., Gao, G., Li, J., Qiu, L., Wang, X., Gao, Y., Qi, Y., Sun, K., & Du, J. (2022). Identification of a novel GLUT1 inhibitor with in vitro and in vivo anti-tumor activity. *International journal of biological macromolecules*, *216*, 768–778. https://doi.org/10.1016/j.ijbiomac.2022.07.123

[26] Liu, Y., Zhao, Y., Song, H., Li, Y., Liu, Z., Ye, Z., Zhao, J., Wu, Y., Tang, J., & Yao, M. (2024). Metabolic reprogramming in tumor immune microenvironment: Impact on immune cell function and therapeutic implications. *Cancer letters*, *597*, 217076. https://doi.org/10.1016/j.canlet.2024.217076

[27] Tilekar, K., Upadhyay, N., Iancu, C. V., Pokrovsky, V., Choe, J. Y., & Ramaa, C. S. (2020). Power of two: combination of therapeutic approaches involving glucose transporter (GLUT) inhibitors to combat cancer. *Biochimica et biophysica acta. Reviews on cancer*, *1874*(2), 188457. https://doi.org/10.1016/j.bbcan.2020.188457

[28] Martini, M., De Santis, M. C., Braccini, L., Gulluni, F., & Hirsch, E. (2014). PI3K/AKT signaling pathway and cancer: an updated review. *Annals of medicine*, *46*(6), 372–383. https://doi.org/10.3109/07853890.2014.912836

[29] Chen, Y. L., Law, P. Y., & Loh, H. H. (2005). Inhibition of PI3K/Akt signaling: an emerging paradigm for targeted cancer therapy. *Current medicinal chemistry. Anti-cancer agents*, *5*(6), 575–589. https://doi.org/10.2174/156801105774574649

[30] Alzahrani A. S. (2019). PI3K/Akt/mTOR inhibitors in cancer: At the bench and bedside. *Seminars in cancer biology*, *59*, 125–132. https://doi.org/10.1016/j.semcancer.2019.07.009

[31] Stern, R., Shuster, S., Neudecker, B. A., & Formby, B. (2002). Lactate stimulates fibroblast expression of hyaluronan and CD44: the Warburg effect revisited. *Experimental cell research*, *276*(1), 24–31. https://doi.org/10.1006/excr.2002.5508

[32] Slomiany, M. G., Grass, G. D., Robertson, A. D., Yang, X. Y., Maria, B. L., Beeson, C., & Toole, B. P. (2009). Hyaluronan, CD44, and emmprin regulate lactate efflux and membrane localization of monocarboxylate transporters in human breast carcinoma cells. *Cancer research*, *69*(4), 1293–1301. https://doi.org/10.1158/0008-5472.CAN-08-2491

[33] Brown, T. P., & Ganapathy, V. (2020). Lactate/GPR81 signaling and proton motive force in cancer: Role in angiogenesis, immune escape, nutrition, and Warburg phenomenon. *Pharmacology & therapeutics*, *206*, 107451. https://doi.org/10.1016/j.pharmthera.2019.107451

[34] Romero-Garcia, S., Moreno-Altamirano, M. M., Prado-Garcia, H., & Sánchez-García, F. J. (2016). Lactate Contribution to the Tumor Microenvironment: Mechanisms, Effects on Immune Cells and Therapeutic Relevance. *Frontiers in immunology*, *7*, 52. https://doi.org/10.3389/fimmu.2016.00052

[35] Calcinotto, A., Filipazzi, P., Grioni, M., Iero, M., De Milito, A., Ricupito, A., Cova, A., Canese, R., Jachetti, E., Rossetti, M., Huber, V., Parmiani, G., Generoso, L., Santinami, M., Borghi, M., Fais, S., Bellone, M., & Rivoltini, L. (2012). Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. *Cancer research*, *72*(11), 2746–2756. https://doi.org/10.1158/0008-5472.CAN-11-1272

[36] Xia, H., Wang, W., Crespo, J., Kryczek, I., Li, W., Wei, S., Bian, Z., Maj, T., He, M., Liu, R. J., He, Y., Rattan, R., Munkarah, A., Guan, J. L., & Zou, W. (2017). Suppression of FIP200 and autophagy by tumor-derived lactate promotes naïve T cell apoptosis and affects tumor immunity. *Science immunology*, *2*(17), eaan4631. https://doi.org/10.1126/sciimmunol.aan4631

[37] Husain, Z., Huang, Y., Seth, P., & Sukhatme, V. P. (2013). Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *Journal of immunology (Baltimore, Md. : 1950)*, *191*(3), 1486–1495. https://doi.org/10.4049/jimmunol.1202702

[38] professional, C. C. medical. (2024). What are natural killer cells (NK cells)? Retrieved from https://my.clevelandclinic.org/health/body/24898-natural-killer-cells

[39] Ullah, M. S., Davies, A. J., & Halestrap, A. P. (2006). The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. *The Journal of biological chemistry*, *281*(14), 9030–9037. https://doi.org/10.1074/jbc.M511397200

[40] Javaeed A, Ghauri SK. MCT4 has a potential to be used as a prognostic biomarker - a systematic review and meta-analysis. Oncol Rev. 2019 Jul 22;13(2):403. doi: 10.4081/oncol.2019.403. PMID: 31410246; PMCID: PMC6661531.

[41] Baenke, F., Dubuis, S., Brault, C., Weigelt, B., Dankworth, B., Griffiths, B., Jiang, M., Mackay, A., Saunders, B., Spencer-Dene, B., Ros, S., Stamp, G., Reis-Filho, J. S., Howell, M., Zamboni, N., & Schulze, A. (2015). Functional screening identifies MCT4 as a key regulator of breast cancer cell metabolism and survival. *The Journal of pathology*, *237*(2), 152–165. https://doi.org/10.1002/path.4562

[42] Choi, S. Y., Xue, H., Wu, R., Fazli, L., Lin, D., Collins, C. C., Gleave, M. E., Gout, P. W., & Wang, Y. (2016). The MCT4 Gene: A Novel, Potential Target for Therapy of Advanced Prostate Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*, *22*(11), 2721–2733. https://doi.org/10.1158/1078-0432.CCR-15-1624

[43] Todenhöfer, T., Seiler, R., Stewart, C., Moskalev, I., Gao, J., Ladhar, S., Kamjabi, A., Al Nakouzi, N., Hayashi, T., Choi, S., Wang, Y., Frees, S., Daugaard, M., Oo, H. Z., Fisel, P., Schwab, M., Schaeffeler, E., Douglas, J., Hennenlotter, J., Bedke, J., … Black, P. C. (2018). Selective Inhibition of the Lactate Transporter MCT4 Reduces Growth of Invasive Bladder Cancer. *Molecular cancer therapeutics*, *17*(12), 2746–2755. https://doi.org/10.1158/1535-7163.MCT-18-0107

[44] Chen, X., Qian, Y., & Wu, S. (2015). The Warburg effect: evolving interpretations of an established concept. *Free radical biology & medicine*, *79*, 253–263. https://doi.org/10.1016/j.freeradbiomed.2014.08.027

[45] Benjamin, D., Robay, D., Hindupur, S. K., Pohlmann, J., Colombi, M., El-Shemerly, M. Y., Maira, S. M., Moroni, C., Lane, H. A., & Hall, M. N. (2018). Dual Inhibition of the Lactate Transporters MCT1 and MCT4 Is Synthetic Lethal with Metformin due to NAD+ Depletion in Cancer Cells. *Cell reports*, *25*(11), 3047–3058.e4. https://doi.org/10.1016/j.celrep.2018.11.043

[46] Benjamin D, Colombi M, Hindupur SK, Betz C, Lane HA, El-Shemerly MY, Lu M, Quagliata L, Terracciano L, Moes S, Sharpe T, Wodnar-Filipowicz A, Moroni C, Hall MN. Syrosingopine sensitizes cancer cells to killing by metformin. Sci Adv. 2016 Dec 23;2(12):e1601756. doi: 10.1126/sciadv.1601756. PMID: 28028542; PMCID: PMC5182053.

[47] Fischer, K., Hoffmann, P., Voelkl, S., Meidenbauer, N., Ammer, J., Edinger, M., Gottfried, E., Schwarz, S., Rothe, G., Hoves, S., Renner, K., Timischl, B., Mackensen, A., Kunz-Schughart, L., Andreesen, R., Krause, S. W., & Kreutz, M. (2007). Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood*, *109*(9), 3812–3819. https://doi.org/10.1182/blood-2006-07-035972

[48] Estrella, V., Chen, T., Lloyd, M., Wojtkowiak, J., Cornnell, H. H., Ibrahim-Hashim, A., Bailey, K., Balagurunathan, Y., Rothberg, J. M., Sloane, B. F., Johnson, J., Gatenby, R. A., & Gillies, R. J. (2013). Acidity generated by the tumor microenvironment drives local invasion. *Cancer research*, *73*(5), 1524–1535. https://doi.org/10.1158/0008-5472.CAN-12-2796

[49] Binker, M. G., Binker-Cosen, M. J., Binker-Cosen, A. A., & Cosen-Binker, L. I. (2014). Microenvironmental factors and extracellular matrix degradation in pancreatic cancer. *JOP : Journal of the pancreas*, *15*(4), 280–285. https://doi.org/10.6092/1590-8577/2638

[50] Halcrow, P., Datta, G., Ohm, J. E., Soliman, M. L., Chen, X., & Geiger, J. D. (2019). Role of endolysosomes and pH in the pathogenesis and treatment of glioblastoma. *Cancer reports (Hoboken, N.J.)*, *2*(6), e1177. https://doi.org/10.1002/cnr2.1177

[51] El-Kenawi, A., Gatenbee, C., Robertson-Tessi, M., Bravo, R., Dhillon, J., Balagurunathan, Y., Berglund, A., Vishvakarma, N., Ibrahim-Hashim, A., Choi, J., Luddy, K., Gatenby, R., Pilon-Thomas, S., Anderson, A., Ruffell, B., & Gillies, R. (2019). Acidity promotes tumour progression by altering macrophage phenotype in prostate cancer. *British journal of cancer*, *121*(7), 556–566. https://doi.org/10.1038/s41416-019-0542-2

[52] Peppicelli, S., Andreucci, E., Ruzzolini, J., Laurenzana, A., Margheri, F., Fibbi, G., Del Rosso, M., Bianchini, F., & Calorini, L. (2017). The acidic microenvironment as a possible niche of dormant tumor cells. *Cellular and molecular life sciences : CMLS*, *74*(15), 2761–2771. https://doi.org/10.1007/s00018-017-2496-y

[53] Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

[54] Payne, C. M., Bernstein, C., Dvorak, K., & Bernstein, H. (2008). Hydrophobic bile acids, genomic instability, Darwinian selection, and colon carcinogenesis. *Clinical and experimental gastroenterology*, *1*, 19–47. https://doi.org/10.2147/ceg.s4343

[55] Morita, T., Nagaki, T., Fukuda, I., & Okumura, K. (1992). Clastogenicity of low pH to various cultured mammalian cells. *Mutation research*, *268*(2), 297–305. https://doi.org/10.1016/0027-5107(92)90235-t

[56] Xiao, H., Li, T. K., Yang, J. M., & Liu, L. F. (2003). Acidic pH induces topoisomerase II-mediated DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(9), 5205–5210. https://doi.org/10.1073/pnas.0935978100

[57] Jayanth, V. R., Bayne, M. T., & Varnes, M. E. (1994). Effects of extracellular and intracellular pH on repair of potentially lethal damage, chromosome aberrations and DNA double-strand breaks in irradiated plateau-phase A549 cells. *Radiation research*, *139*(2), 152–162.

[58] Calcinotto, A., Filipazzi, P., Grioni, M., Iero, M., De Milito, A., Ricupito, A., Cova, A., Canese, R., Jachetti, E., Rossetti, M., Huber, V., Parmiani, G., Generoso, L., Santinami, M., Borghi, M., Fais, S., Bellone, M., & Rivoltini, L. (2012). Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. *Cancer research*, *72*(11), 2746–2756. https://doi.org/10.1158/0008-5472.CAN-11-1272

[59] Pilon-Thomas, S., Kodumudi, K. N., El-Kenawi, A. E., Russell, S., Weber, A. M., Luddy, K., Damaghi, M., Wojtkowiak, J. W., Mulé, J. J., Ibrahim-Hashim, A., & Gillies, R. J. (2016). Neutralization of Tumor

Acidity Improves Antitumor Responses to Immunotherapy. *Cancer research*, *76*(6), 1381–1390. https://doi.org/10.1158/0008-5472.CAN-15-1743

[60] Roma-Rodrigues, C., Mendes, R., Baptista, P. V., & Fernandes, A. R. (2019). Targeting Tumor Microenvironment for Cancer Therapy. *International journal of molecular sciences*, *20*(4), 840. https://doi.org/10.3390/ijms20040840

[61] Ma, S., Song, W., Xu, Y., Si, X., Zhang, D., Lv, S., Yang, C., Ma, L., Tang, Z., & Chen, X. (2020). Neutralizing tumor-promoting inflammation with polypeptide-dexamethasone conjugate for microenvironment modulation and colorectal cancer therapy. *Biomaterials*, *232*, 119676. https://doi.org/10.1016/j.biomaterials.2019.119676

[62] Bailey, K. M., Wojtkowiak, J. W., Cornnell, H. H., Ribeiro, M. C., Balagurunathan, Y., Hashim, A. I., & Gillies, R. J. (2014). Mechanisms of buffer therapy resistance. *Neoplasia (New York, N.Y.)*, *16*(4), 354–64.e643. https://doi.org/10.1016/j.neo.2014.04.005

[63] Ibrahim-Hashim, A., & Estrella, V. (2019). Acidosis and cancer: from mechanism to neutralization. *Cancer metastasis reviews*, *38*(1-2), 149–155. https://doi.org/10.1007/s10555-019-09787-4