Role of the Intracellular pH in the Metabolic Switch between Oxidative Phosphorylation and Aerobic Glycolysis - Relevance to Cancer

Author(s): Mr. Jose Manuel Calderon-Montano, Ms. Estefania Burgos-Moron, Dr. Concepcion Perez-Guerrero, Dr. Javier Salvador, Dr. Antonio Robles, Dr. Miguel Lopez-Lazaro

Corresponding Author:
Dr. Miguel Lopez-Lazaro,
Associate Professor, Department of Pharmacology, Faculty of Pharmacy, University of Seville - Spain

Submitting Author:
Dr. Miguel Lopez-Lazaro,
Associate Professor, Department of Pharmacology, Faculty of Pharmacy, University of Seville - Spain

Role of the Intracellular pH in the Metabolic Switch between Oxidative Phosphorylation and Aerobic Glycolysis - Relevance to Cancer

Abstract

Cellular energy in the form of ATP can be produced through oxidative phosphorylation and through glycolysis. Since oxidative phosphorylation requires oxygen and generates ATP more efficiently than glycolysis, it has been assumed for many years that the presence or absence of oxygen determines that cells generate energy through oxidative phosphorylation or through glycolysis. Although cells must activate glycolysis in the absence of oxygen to produce ATP, it is now accepted that they can activate both glycolysis and oxidative phosphorylation in the presence of oxygen. In fact, normal proliferating cells and tumor cells are known to have a high glycolytic activity in the presence of adequate oxygen levels, a phenomenon known as aerobic glycolysis or the Warburg effect. Recent observations have demonstrated that the activation of aerobic glycolysis plays a major role in carcinogenesis and tumor growth. Understanding the mechanisms involved in the metabolic switch between oxidative phosphorylation and aerobic glycolysis may therefore be important for the development of potential preventive and therapeutic interventions. In this article, we discuss the role of the intracellular pH in the metabolic switch between oxidative phosphorylation and aerobic glycolysis. We propose that, in the presence of adequate oxygen levels, the intracellular pH may play a key role in determining the way cells obtain energy, an alkaline pH driving aerobic glycolysis and an acidic pH driving oxidative phosphorylation.

Introduction

Cells require energy in the form of adenosine-5-triphosphate (ATP) to carry out numerous cellular processes. Most ATP molecules are produced in the mitochondria through oxidative phosphorylation (oxphos), an oxygen-dependent process that couples the oxidation of NADH and $FADH_2$ with the phosphorylation of ADP to form ATP. Oxphos requires oxygen because the electrons resulting from the oxidation of NADH and $FADH_2$ need to be ultimately accepted by oxygen. Cells can also produce ATP through glycolysis, which takes place in the cytosol and does not require oxygen. In the glycolytic process, one molecule of glucose is transformed into two molecules of pyruvate resulting in the production of ATP. This transformation consumes NAD^+, which can be regenerated by the conversion of pyruvate to lactate. Because oxphos is over ten times more efficient than glycolysis in generating ATP, it is comprehensible that cells generate ATP through oxphos when the oxygen levels are adequate. This was first noted by Louis Pasteur in the late 19th century, who observed that the generation of ATP shifted from oxphos to glycolysis when the oxygen levels decreased (Pasteur effect) (1-3).

Several decades later the biochemist Otto Warburg first observed that cancer cells had increased glycolytic activity despite the presence of an adequate oxygen supply (4). This phenomenon, called aerobic glycolysis or Warburg effect, has repeatedly been observed and is currently used worldwide as a diagnostic tool to detect malignant tumors (Fluorodeoxyglucose - Positron Emission Tomography: FdG-PET) (5). It is important to mention that the metabolic switch from oxphos to aerobic glycolysis is not a unique feature of tumor cells, because it has also been observed in non-transformed proliferating cells (6-8). It is also important to note that, although normal proliferating cells and tumor cells activate aerobic glycolysis, these cells also rely on oxphos for their ATP production (4,9).

Although it is now accepted that the Warburg effect plays an important role in carcinogenesis and tumor growth, it is not clear why and how this phenomenon occurs. We have proposed previously that normal proliferating cells and tumor cells need to activate glycolysis despite the presence of oxygen in order to proliferate (10-12). Cell proliferation requires the synthesis of new macromolecules (e.g., nucleic acids, lipids, proteins) and glycolysis provides building blocks (e.g. glucose 6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, phosphoenolpyruvate,

pyruvate) that participate in the synthesis of these macromolecules. This means that cell proliferation would be compromised if glycolysis were always inhibited in the presence of oxygen, and can explain why tumor cells and normal proliferating cells activate glycolysis under aerobic conditions (10-12). As to how cells switch from oxphos to aerobic glycolysis, several mechanisms have been proposed (9,11). Less focus has been placed on how cells come back from aerobic glycolysis to oxphos, probably because it has been considered for some time that the Warburg effect was caused by irreversible damages to oxphos (4,13). Because aerobic glycolysis plays a key role in cancer development, understanding how cells switch from aerobic glycolysis to oxphos could be important for the development of potential preventive and therapeutic interventions. In this article, we discuss evidence that the cytosolic concentration of protons (intracellular pH) may play a key role in the metabolic switch between oxidative phosphorylation and aerobic glycolysis, an alkaline pH driving aerobic glycolysis and an acidic pH driving oxidative phosphorylation.

An alkaline intracellular pH may drive the metabolic switch from oxidative phosphorylation to aerobic glycolysis

In the process of oxphos, high-energy electrons from NADH and $FADH_2$ are passed along the electron-transport chain, located in the inner mitochondrial membrane, to oxygen (illustration 1). This electron transport drives protons (H^+) pumping from the mitochondrial matrix to the intermembrane space, which generates an electrochemical H^+ gradient across the inner mitochondrial membrane. This electrochemical H^+ gradient (or proton motive force) drives H^+ entry into the mitochondrial matrix through ATP synthase, a transmembrane protein complex that uses the energy of the H^+ flow to synthesize ATP from ADP and inorganic phosphate (P_i). Therefore, as shown in illustration 1, ATP generation through oxphos ultimately depends on an electrochemical H^+ gradient across the inner mitochondrial membrane. This H^+ gradient also drives pyruvate and P_i transport into the mitochondrial matrix (2).

The outer mitochondrial membrane contains transport proteins called voltage dependent anion channels (VDAC), or mitochondrial porins, which form large channels through the lipid bilayer (2,14-16). When VDACs are open, the intermembrane space and the cytosol are supposed to be chemically equivalent with respect to the small molecules they contain, including H^+ (2). This means that a decrease in the cytosolic concentration of H^+ (intracellular alkalinization) would

decrease the concentration of H^+ in the mitochondrial intermembrane space. This would reduce the electrochemical H^+ gradient across the inner mitochondrial membrane and would repress oxphos even in the presence of adequate oxygen levels (illustration 1) (2,17).

It is important to note that, when VDACs are closed, the concentration of protons in the mitochondrial intermembrane space may be rather different than that in the cytosol. Indeed, pH differences across the outer mitochondrial membrane have been measured in tumorigenic ECV304 cells (18,19). Evidence indicates that hexokinase can repress oxphos in tumor cells by binding to VDAC and inducing its closure (9,14-16,20). Interestingly, hexokinase binding to mitochondria is strongly dependent on the intracellular pH; an alkaline pH would increase hexokinase binding and activity (21), and would lead to VDAC closure and repression of mitochondrial activity (14). This supports the idea that intracellular alkalinization can repress oxphos.

In addition to repressing oxphos, a decrease in the cytosolic concentration of H^+ can stimulate glycolysis. The key glycolytic enzyme phosphofructokinase (PFK) is well-known to be repressed by ATP (2). Intracellular alkalinization-induced oxphos repression would decrease cellular ATP levels; this would release PFK inhibition by ATP and would increase glycolysis to compensate such ATP deficit. In addition, evidence indicates that intracellular alkalinization activates glycolysis by directly increasing the activity of PFK. The enzyme PFK is extremely sensitive to small changes in pH in the physiological range, a high pH increasing its activity (22,23). In fact, an increase in pH of 0.1-0.3 units can change the activity of PFK from an inactive form to a saturated state (22).

Although the activation of the enzyme PFK is fundamental for the activation of glycolysis, cells need to increase the expression of glucose transporters (e.g. GLUTs) and glycolytic enzymes (e.g. hexokinase, PFK, pyruvate kinase) to keep sustained glycolytic rates. Hypoxia-inducible factor 1 (HIF-1) plays a key role in the transcription of genes that code for glucose transporters and glycolytic enzymes (24-27). Interestingly, it has been demonstrated that the glucose metabolite pyruvate increases HIF-1 activity under aerobic conditions by preventing HIF-1 degradation (28,29). As represented in illustration 2, the activation of glycolysis would increase the cellular levels of pyruvate. The reduction in the electrochemical H^+ gradient across the inner mitochondrial membrane induced by intracellular alkalinization would decrease pyruvate entry into the mithochondrial matrix, which would increase the

cytosolic concentrations of pyruvate further (illustration 1). The increased pyruvate levels in the cytosol would increase HIF-1 activity and the expression of glucose transporters and glycolytic enzymes, which would keep sustained glycolytic rates (28,29). Interestingly, HIF-1 activation can also induce oxphos repression (13,30). HIF-1 mediates the expression of pyruvate dehydrogenase kinase; this results in pyruvate dehydrogenase inhibition, decreased conversion of pyruvate to acetyl-CoA, reduced activity of the tricarboxylic acid cycle and subsequent oxphos repression (13,30).

Additional evidence supports the view that intracellular alkalinization can activate glycolysis in the presence of adequate oxygen levels (31-33). Experimental data have revealed that cancer cells, which are known to have high rates of aerobic glycolysis, have an increase in their intracellular pH of 0.13-0.45 units (7.12-7.65 compared with 6.99-7.20 in normal cells) (33,34). Growth factor-induced proliferation of normal cells has also been associated with intracellular alkalinization (mediated by the Na^+/H^+ antiporter) and with increased rates of aerobic glycolysis (31,35,36).

An acidic intracellular pH may drive the metabolic switch from aerobic glycolysis to oxidative phosphorylation: relevance to cancer

Since a decrease in the cytosolic concentration of H^+ (intracellular alkalinization) may drive the metabolic switch from oxphos to aerobic glycolysis, one can expect that an increase in the cytosolic levels of H^+ (intracellular acidification) can reverse this metabolic switch (illustration 2). It is well known that the activation of glycolysis produces H^+ in the cytosol. Therefore, cells would come back from aerobic glycolysis to oxphos when the activation of glycolysis would lead to a concentration of H^+ high enough to reverse the intracellular alkalinization. This increase in the cytosolic concentration of H^+ would raise the electrochemical H^+ gradient across the inner mitochondrial membrane, which would drive pyruvate and P_i entry into de matrix and would activate oxphos (illustration 1). This reduction in the intracellular pH would also inhibit glycolysis by directly and indirectly inhibiting PFK activity, and by reducing the expression of glucose transporters and glycolytic enzymes induced by pyruvate and mediated by HIF-1 (illustration 2).

Cancer cells are known to have increased glycolytic rates despite the presence of adequate oxygen levels. Since an increased glycolytic activity acidifies the cytosol and an acidic intracellular pH can drive the metabolic switch from aerobic glycolysis to oxphos, one could wonder why cancer cells keep increased glycolytic activity instead of switching back to oxphos. It has recently been proven that tumor cells do not necessarily have irreversible damages to oxphos, as some cancer cells have been forced to switch from aerobic glycolysis to oxphos (13). Evidence indicates that tumor cells prevent cytosolic acidification by activating a number of transporters that extrude the protons produced during glycolysis (37). This active proton transport across the cellular membrane can explain the reversed intra-extracellular pH gradients found in cancer cells (31,37); experimental data have demonstrated that tumor cells have alkaline intracellular pH values (7.12–7.65 compared with 6.99–7.20 in normal tissues) and acidic interstitial extracellular pH values (6.2–6.9 compared with 7.3–7.4) (33,34). The increased activity of these H^+ extruders seems to play a critical role in the maintenance of high rates of aerobic glycolysis in cancer cells. The inhibition of these transporters has already been proposed as an attractive anticancer strategy that could potentially be used in a wide range of cancer types (37,38). Inhibition of the sodium pump, e.g. by cardiac glycosides, may also interfere with proton extrusion in tumor cells and induce selective anticancer activity (39,40).

Conclusion

Despite many decades of research, we still do not fully understand what makes cells choose between oxidative phosphorylation and glycolysis to produce energy. It is clear that cells must use glycolysis to produce energy when oxygen is not available. Here we have discussed that, in the presence of adequate oxygen levels, the intracellular pH may play a key role in determining the way cells obtain energy (illustration 3). This knowledge may be important for the development of potential cancer preventive and therapeutic interventions.

References

1. Pasteur, L. (1861) Experiments and novel views on the nature of fermentation. Comp. Rend. Acad. Sci., 89, 1260-1264.

2. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) Molecular biology of the cell. New York: Garland Publishing.

3. Berg J, Tymoczko J, Stryer L (2002) Biochemistry. New York: W. H. Freeman and Co.

4. Warburg, O. (1956) On the origin of cancer cells. Science, 123, 309-314.

5. Gatenby, R. A. and Gillies, R. J. (2004) Why do cancers have high aerobic glycolysis? Nat. Rev. Cancer, 4, 891-899.

6. Wang,T.,Marquardt, C.and Foker, J. (1976) Aerobic glycolysis during lymphocyte proliferation. Nature, 261, 702-705.

7. Brand, K. A. and Hermfisse, U. (1997) Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. FASEB J., 11, 388-395.

8. Fox, C. J., Hammerman, P. S. and Thompson, C. B. (2005) Fuel feeds function: energy metabolism and the T-cell response. Nat. Rev. Immunol., 5, 844-852.

9. Pedersen, P. L. (2007) Warburg, me and Hexokinase 2: Multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. J. Bioenerg. Biomembr., 39,211-222.

10. Lopez-Lazaro, M. (2006) Does hypoxia really control tumor growth? Cell Oncol., 28, 327-329.

11. Lopez-Lazaro, M. (2008) The Warburg effect: why and how do cancer cells activate glycolysis in the presence of oxygen? Anticancer Agents Med. Chem., 8, 305-312.

12. Lopez-Lazaro, M. (2010) A new view of carcinogenesis and an alternative approach to cancer therapy. Mol. Med., 16, 144-153.

13. Fantin, V. R., St Pierre, J. and Leder, P. (2006) Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell, 9, 425-434.

14. Lemasters, J. J. and Holmuhamedov, E. (2006) Voltage-dependent anion channel (VDAC) as mitochondrial governator - thinking outside the box. Biochim. Biophys. Acta, 1762, 181-190.

15. Mannella, C. A. and Kinnally, K. W. (2008) Reflections on VDAC as a voltage-gated channel and a mitochondrial regulator. J. Bioenerg. Biomembr., 40, 149-155.

16. Shoshan-Barmatz, V., De, P., V, Zweckstetter, M., Raviv, Z., Keinan, N. and Arbel, N. (2010) VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol. Aspects Med., 31, 227-285.

17. Lopez-Lazaro, M. (2006) HIF-1: Hypoxia-inducible Factor or Dysoxia-inducible Factor? FASEB J, 20, 828-832.

18. Porcelli, A. M., Ghelli, A., Zanna, C., Pinton, P., Rizzuto, R. and Rugolo, M. (2005) pH difference across the outer mitochondrial membrane measured with a green fluorescent protein mutant. Biochem. Biophys. Res. Commun., 326, 799-804.

19. Takahashi, K., Sawasaki, Y., Hata, J., Mukai, K. and Goto, T. (1990) Spontaneous transformation and immortalization of human endothelial cells. In Vitro Cell Dev. Biol., 26, 265-274.

20. Mathupala, S. P., Ko, Y. H. and Pedersen, P. L. (2009) Hexokinase-2 bound to mitochondria: cancer's stygian link to the "Warburg Effect" and a pivotal target for effective therapy. Semin. Cancer Biol., 19, 17-24.

21. Miccoli, L., Oudard, S., Sureau, F., Poirson, F., Dutrillaux, B. and Poupon, M. F. (1996) Intracellular pH governs the subcellular distribution of hexokinase in a glioma cell line. Biochem. J., 313, 957-962.

22. Trivedi, B. and Danforth, W. H. (1966) Effect of pH on the kinetics of frog muscle phosphofructokinase. J. Biol. Chem., 241, 4110-4112.

23. Erecinska, M., Deas, J. and Silver, I. A. (1995) The effect of pH on glycolysis and phosphofructokinase activity in cultured cells and synaptosomes. J. Neurochem., 65, 2765-2772.

24. Semenza, G. L. (2003) Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer, 3, 721-732.

25. Schofield, C. J. and Ratcliffe, P. J. (2004) Oxygen sensing by HIF hydroxylases. Nat. Rev. Mol. Cell Biol., 5, 343-354.

26. Seagroves, T. N., Ryan, H. E., Lu, H. et al. (2001) Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. Mol. Cell Biol., 21, 3436-3444.

27. Semenza, G. L. (2006) Development of novel therapeutic strategies that target HIF-1. Expert. Opin. Ther. Targets, 10, 267-280.

28. Lu, H., Forbes, R. A. and Verma, A. (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. J. Biol. Chem., 277, 23111-23115.

29. Lu, H., Dalgard, C., Mohyeldin, A., McFate, T., Tait, A. S. and Verma, A. (2005) Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. J. Biol. Chem., 280, 41928-41939.

30. Papandreou, I., Cairns, R. A., Fontana, L., Lim, A. L. and Denko, N. C. (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab., 3, 187-197.

31. Harguindey, S., Orive, G., Luis, P. J., Paradiso, A. and Reshkin, S. J. (2005) The role of pH dynamics and the Na(+)/H(+) antiporter in the etiopathogenesis and treatment of cancer. Two faces of the same coin-one single nature. Biochim. Biophys. Acta, 1756, 1-24.

32. Reshkin, S. J., Bellizzi, A., Caldeira, S. et al. (2000) Na+/H+ exchanger-dependent intracellular alkalinization is an early event in malignant transformation and plays an essential role in the development of subsequent transformation-associated phenotypes. FASEB J., 14, 2185-2197.

33. Cardone, R. A., Casavola, V. and Reshkin, S. J.

(2005) The role of disturbed pH dynamics and the Na(+)/H(+) exchanger in metastasis. Nat. Rev. Cancer, 5, 786-795.

34. Gillies, R. J., Raghunand, N., Karczmar, G. S. and Bhujwalla, Z. M. (2002) MRI of the tumor microenvironment. J. Magn Reson. Imaging., 16, 430-450.

35. Whetton, A. D., Vallance, S. J., Monk, P. N., Cragoe, E. J., Dexter, T. M. and Heyworth, C. M. (1988) Interleukin-3-stimulated haemopoietic stem cell proliferation. Evidence for activation of protein kinase C and Na+/H+ exchange without inositol lipid hydrolysis. Biochem. J., 256, 585-592.

36. Bauer, D. E., Harris, M. H., Plas, D. R. et al. (2004) Cytokine stimulation of aerobic glycolysis in hematopoietic cells exceeds proliferative demand. FASEB J., 18, 1303-1305.

37. Huber, V., De Milito, A., Harguindey, S. et al. (2010) Proton dynamics in cancer. J. Transl. Med., 8, 57.

38. Harguindey, S., Arranz, J. L., Wahl, M. L., Orive, G. and Reshkin, S. J. (2009) Proton transport inhibitors as potentially selective anticancer drugs. Anticancer Res., 29, 2127-2136.

39. Lopez-Lazaro, M., Pastor, N., Azrak, S. S., Ayuso, M. J., Austin, C. A. and Cortes, F. (2005) Digitoxin inhibits the growth of cancer cell lines at concentrations commonly found in cardiac patients. J Nat. Prod., 68, 1642-1645.

40. Lopez-Lazaro, M. (2007) Digitoxin as an anticancer agent with selectivity for cancer cells: possible mechanisms involved. Expert. Opin. Ther. Targets, 11, 1043-1053.

Illustrations

Illustration 1

Repression of oxidative phosphorylation (oxphos) by intracellular alkalinization.
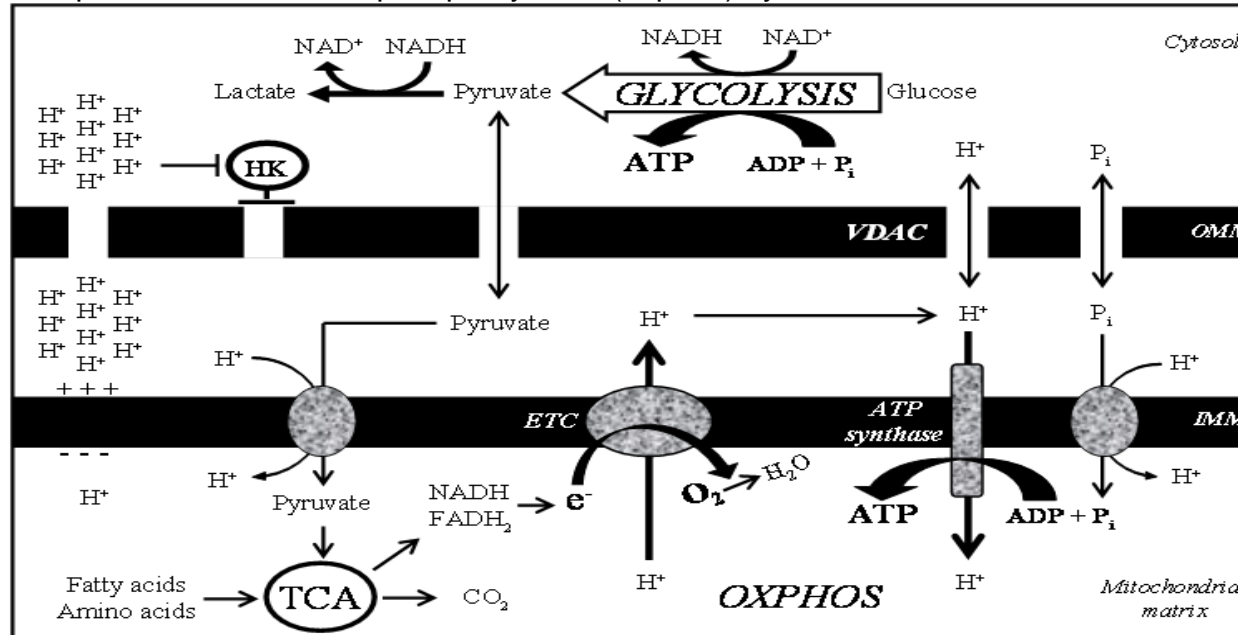
The outer mitochondrial membrane (OMM) contains voltage dependent anion channels (VDAC) that make this membrane permeable to H^+. A decrease in the cytosolic concentration of H^+ (intracellular alkalinization) results in a reduction in the concentration of H^+ in the intermembrane space. This decreases the electrochemical H^+ gradient across the inner mitochondrial membrane (IMM) and reduces H^+ entry into the matrix through ATP synthase (as well as pyruvate and P_i transport into the matrix), therefore reducing ATP synthesis through oxphos. Intracellular alkalinization can also repress oxphos by inducing hexokinase (HK) binding to VDAC, which can induce VDAC closure and mitochondrial activity repression (see text for details). ETC: electron transport chain; TCA: tricarboxylic acid cycle.

Illustration 2

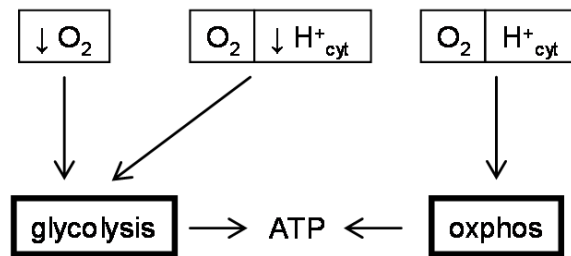Role of the intracellular pH in the metabolic switch between oxidative phosphorylation and aerobic glycolysis.
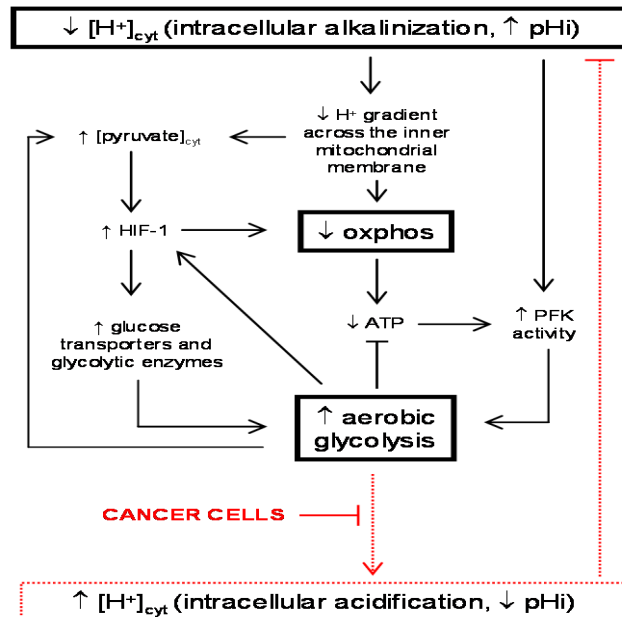
Intracellular alkalinization can activate glycolysis by directly and indirectly increasing the activity of the enzyme phosphofructokinase (PFK). An alkaline intracellular pH can also keep sustained glycolytic rates by increasing the expression of glucose transporters and glycolytic enzymes; this effect can be induced by an increase in the cytosolic concentrations of pyruvate and mediated by the activation of the hypoxia-inducible factor 1 (HIF-1). Intracellular acidification caused by accumulation of glycolytic protons in the cytosol can reverse this metabolic switch. Tumor cells keep sustained rates of aerobic glycolysis instead of coming back to oxphos because they avoid cytosolic acidification e.g. by activating a number of transporters that pump the protons produced during glycolysis out of the cell (see text for further details).

Illustration 3

The cytosolic concentration of H (intracellular pH) may play a key role in determining the way cells obtain energy in the presence of adequate oxygen levels.

$$\boxed{\downarrow O_2} \qquad \boxed{O_2 \mid \downarrow H^+_{cyt}} \qquad \boxed{O_2 \mid H^+_{cyt}}$$

$$\boxed{\text{glycolysis}} \longrightarrow \text{ATP} \longleftarrow \boxed{\text{oxphos}}$$

This figure represents in a simplified manner that cells need to activate glycolysis to generate ATP when the levels of oxygen are reduced or when the levels of oxygen are adequate but the cytosolic concentration of H^+ is reduced (intracellar alkalinization).

Disclaimer