3-bromopyruvate as a Promising Treatment for Hematological Cancer

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Abstract Many biological differences exist between cancer cells and normal cells that can act as potential targets in targeted cancer therapy. Hematological cancers e.g. lymphoma, leukemia and myeloma exhibit drug-resistance that ultimately results in deteriorated patients' conditions and high mortality rates. Resistance of hematological malignancy to conventional chemotherapy is attributed in part to upregulation of glucose oxidation (glycolysis) genes evidenced by gaining a promising chemosensitization effect upon adding a glycolysis inhibitor to chemotherapeutics. The promising anticancer agent 3-bromopyruvate (3BP) is a structural analog of both pyruvate and lactate. 3BP was reported to antagonize the Warburg effect (malignant phenotype where cancer cells utilize cytoplasmic glucose oxidation to produce ATP and lactate even in the presence of oxygen without making benefit of the generous ATP provision from glucose oxidation via mitochondrial pathways). Warburg effect deprives cancer cells from the high energetic yield achieved through utilizing mitochondrial pathways. 3BP is a promising antiglycolytic agent that targets major glycolysis enzymes (hexokinase II and glyceraldehyde-3-phosphate dehydrogenase. In this article, 3BP promising anticancer effects in treating lymphoma, leukemia and myeloma are discussed in addition to the mode of inhibition of Warburg effect using 3BP. In conclusion, 3BP is a promising anticancer drug (that will be more powerful upon proper pharmaceutical formulations) for treating hematological malignancies. 3BP is advisable to be included in treatment protocols in hematological cancers as a chemosensitizer or as a sole anticancer agent.

Keywords: 3-bromopyruvate, lymphoma, leukemia, myeloma, Warburg effect

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1. Introduction

Treatment of hematological malignancy still needs a lot of advances due to the diverse biological differences that characterize leukemias from lymphomas or myeloma. However, many major differences between normal cells and cancer cells as regard energy generating pathways exist. Among the critical differences that distinguish cancer cells from normal cells are Warburg effect (conversion of glucose into lactate even in the presence of oxygen that is called aerobic glycolysis), dependence on glycolysis for energy production, hostile cancer microenvironment, mitochondrial impairment, high endogenous steady-state ROS conditions in cancer cells and persistently high energy demands. In addition to that, pyruvate is the end product of glycolysis in normal cells, while lactate is the end glycolytic end product in aggressive cancer cells [1]. Better understanding to cancer biology helps in designing better effective cancer therapeutics. Although the pioneering work of Warburg was reported early last century, useful applications based on it started recently and further work seems necessary as regard glycolysis inhibition as a monotherapy or adjuvant therapy for cancer.

2. 3-bromopyruvate as a Promising General Drug for Treating Cancer

Hexokinase II is a key enzyme of glycolysis and is widely over-expressed in so many cancer cells. However, Hexokinase II levels and its roles in ATP production and ATP-dependent cellular process have not been well studied in hematopoietic malignant cells including multiple myeloma cells. 3-bromopyruvate (3BP) is a potent inhibitor of hexokinase II and effectively inhibits glycolysis [2,3]. This compound is effective in killing liver cancer cells in the rabbit VX2 tumor implantation animal tumor model when given by local infusion [4].

3. 3BP Is Promising in Treating Lymphoma

Systemic administration of 3BP was reported to inhibit aggressiveness of disseminated aggressive lymphoma. In mice treated with a daily dose of 10 mg/kg 3BP for 7 days, a significant reduction in lymphoma tumor activity was
3BP was promising for treatment of systemic metastatic cancers [5]. 3BP-induced anticancer effects were evident against the murine transplantable Dalton's lymphoma (DL). 3BP treatment suppressed cellular metabolism and viability while apoptosis and necrosis were marked. 3-BP treatment inhibited glucose utilization e.g. lactate release, glucose uptake, pH homeostasis that ultimately acted as a chemosensitizer. 3BP diminished the expression of survival molecules, energetic molecules e.g. Hexokinase-2, glyceraldehyde-3-phosphate dehydrogenase, LDH, hypoxia-inducible factor-1α, multidrug resistance-1 & glucose transporter-1 and cytokine repertoire of interferon-γ, IL-6, IL-10, & vascular endothelial growth factor. Inhibiting monocarboxylate-1 receptors using β-remission and partial remission. The expression of hypoxia inducible factor -γ, IL-6, IL-10, & vascular endothelial growth factor increased in AML patients having no remission, compared with the expression in the complete remission group. Treatment of drug-resistant AML cells decreased compared with the expression in the complete remission group. Treatment of prednisolone-resistant AML cells exhibited sensitivity to glucocorticoids i.e. glycolysis inhibitors sensitized glucocorticoid-resistant ALL cells to many antileukemic drugs e.g. vincristine and daunorubicin. Interestingly, targeting the glycolysis enzyme GAPDH by RNA interference sensitized ALL cells to prednisolone.

Based on that, glycolytic pathway seems to be vital for steroid resistant ALL that can be utilized for future leukemia treatment [12].

4. BP Is Promising for Treating Leukemia

An interesting recent report revealed that treatment of mesenchymal embryonic stem cells with 3BP enhanced cellular maturation even in the presence of leukemia-inducing factor [7]. The human leukemia cell lines K562 and THP-1 were killed upon 3BP treatment by membrane depolarization and increased ROS [8]. Both mouse and human drug-resistant leukemia exhibit upregulated glucose oxidation (glycolysis). The sorafenib-resistant cells exhibit increased expression of a majority of glycolytic enzymes, including hexokinase 2, which is also highly expressed in the mitochondrial fraction and is associated with resistance to apoptotic cell death. The sorafenib-resistant leukemia cells exhibited sensitivity to the glycolysis inhibitor 3-bromopyruvate [9].

Glucose metabolism plays a major part in inducing drug-resistance in acute myeloid leukemia (AML) cells. In an interesting recent study, bone marrow and serum samples were obtained from patients with AML that were newly diagnosed or had relapsed. The messenger RNA expression of hypoxia inducible factor-1α, glucose transporter-1, and hexokinase-II was measured by quantitative polymerase chain reaction. The levels of LDH and β subunit of human F1-F0 adenosine triphosphate synthase (β-F1-ATPase) were detected by ELISA and western blot assays. Leukemia cell lines HL-60 and HL-60/ADR judge glycolytic activity and effect of glycolysis inhibition on cellular proliferation and apoptosis. Drug-resistant HL-60/ADR cells exhibited a significantly increased level of glycolysis compared with the drug-sensitive HL-60 cell line. The expression of hypoxia inducible factor-1α, hexokinase-II, glucose transporter-1 and LDH were increased in AML patients having no remission, compared to healthy control individuals and patients with complete remission and partial remission. The expression of β-F1-ATPase in patients having no remission was decreased compared with the expression in the complete remission group. Treatment of drug-resistant AML cells with 3-bromopyruvate increased in vitro sensitivity to Adriamycin (ADR). Interestingly, glycolytic inhibitors in combination with ADR increased AML necrosis [10]. 3BP-induced AML cell death may be attributed to chemosensitizing, energy depletion, oxidative stress, and protein kinase activity modulation [11].

Treatment failure in pediatric acute lymphoblastic leukemia (ALL) i.e. leukemia resistance to steroids e.g. prednisolone was reported to be attributed to enhanced glycolysis in steroid-resistant ALL cell lines. Genes enhancing glucose metabolism are overexpressed in steroid-resistant ALL more than in steroid-sensitive B-lineage ALL patients. It was reported that prednisolone resistance is strongly related to increased glucose oxidation, a mechanism that may be utilized as a future strategy for treating ALL. Treatment of prednisolone-resistant cells with glycolysis inhibitors e.g. 3BP increased the in vitro sensitivity to glucocorticoids i.e. glycolysis inhibitors sensitized glucocorticoid-resistant ALL cells to many antileukemic drugs e.g. vincristine and daunorubicin. Interestingly, targeting the glycolysis enzyme GAPDH by RNA interference sensitized ALL cells to prednisolone.

Based on that, glycolytic pathway seems to be vital for steroid resistant ALL that can be utilized for future leukemia treatment [12].

5. 3BP Is Promising for Treating Multiple Myeloma

Relatively low concentrations of 3BP (25, 50, 100 μM) were effective for treating human multiple myeloma cells) where 3BP caused a significant decrease in glutathione concentration in tumor cells. 3BP-induced myeloma cell death was attributed to a dramatic decrease in cellular ATP levels and GSH concentration in addition to increase oxidative stress (ROS)-mediated cytotoxicity [13].

Human MM cells were reported to start losing viability significantly 8 h after addition of 3BP while peripheral blood mononuclear cells did not. The monocarboxylate transporters that transport 3BP inside myeloma cells are highly expressed in myeloma cells compared with control cells. 3BP-induced myeloma cell death was reported to be enhanced via inhibiting glutathione synthesis using buthionine sulfoximine [13]. Hexokinase II is over-expressed in multiple myeloma cells. 3BP treatment to myeloma cells induced ATP depletion and cell death. In a wonderful Japanese report, 3BP depleted ATP-dependent ATP-binding cassette transporter activity. That strongly restored drug retention in myeloma cells. That strongly suggested that glycolysis inhibition using 3BP is promising in killing myeloma cells and restoring drug sensitivity [14].

6. Targeting the Warburg Effect by 3BP

Decreasing the production of lactate is expected from all antiglycolytic agents working upstream of LDH step (the lactate formation step) in the glycolysis pathway. In a tumor exhibiting the glycolytic phenotype, lactate is continuously extruded through MCT to the interstitial space helping in creating the tumor microenvironment
which plays a role in resistance of cancer cells to current therapeutics [15]. Targeting lactate as a key metabolite for cancer cells breaks the cascade which starts with glucose and ends with lactate (Warburg effect). Targeting Warburg effect can be induced at 3 important levels: targeting the formation of lactate, targeting the extrusion of lactate through MCT and targeting the effects of lactate while being in the extracellular space. Targeting the formation of lactate can be achieved simply by dietary treatment in the form of carbohydrate restriction or treatment using antiglycolytic agents. Feeding cancer patients a diet poor in carbohydrates, rich in proteins and lipids starves tumors exhibiting the glycolytic phenotype in which dietary glucose is catabolized into lactate. Interestingly, glucose restriction extended life span of normal fibroblast cells (WI-380), while growth inhibition and apoptosis occurred in their malignant counterpart (WI-38/S) cells as a result of glucose restriction [16]. In cancer cells, glucose restriction decreased the expression of human telomerase reverse transcriptase (up-regulated in most malignant tumors e.g. renal cell carcinoma and is rarely detectable in normal cells) [17,18] and increased expression of p16INK4a (important for suppression of tumor growth and induction of cellular senescence [19,20], while the reverse occurs in normal cells [16]. The fate of glucose catabolism in normal cells ends by formation of pyruvate which is channeled to the mitochondria [21] to be oxidatively decarboxylated to acetyl CoA to initiate Krebs cycle. The difference in glucose metabolism in normal cells from cancer cells can be a promising target for cancer treatment and prevention [22,23,24]. When glucose is restricted, many signaling pathways can start and affect cellular survival [25]. Ketonic diet rich in ketone bodies e.g. acetoacetate and β-hydroxybutyrate and poor in glucose proved effective in decreasing significantly the viability of neuroblastoma cells [26]. All antiglycolytic agents e.g. 3BP and 2-deoxyglucose can decrease lactate levels in tumors by decreasing formation of lactate. 3BP was recently reported to decrease glucose consumption (source of lactate) by cancer cells [12]. 3BP decreased significantly the production of lactate in different cancer cell lines [14,27].

Second level of targeting Warburg effect is to target lactate extrusion in cancer cells. Moreover, extrusion of lactate from the inside to the outside of cancer cells appears critical for the survival and proliferation of cancer cells at the expense of normal cells [28]. Lactate is continuously accumulating in cancer cells attaining the glycolytic phenotype due to the activity of LDH which converts pyruvate into lactate. LDH step in glycolysis carries two benefits for cancer cells: first benefit is the completion of Warburg effect and production of lactate that is beneficial to cancer cells. The second benefit is the oxidation of reduced NADH into NAD+ to catalyze the GAPDH step to allow continuous entry into the second half of glycolysis pathway in which energy gain in the form of 2 ATP molecules is produced at the pyruvate kinase step. Lactate accumulation intracellularly in cancer cells may be toxic to cancer cells and needs to be extruded [29]. Inhibition of lactate extrusion can be achieved by therapeutic agents that target MCT. Chemical agents e.g. cinnamic acid derivatives (α-cyano-4-hydroxy-cinnamic acid, CHCA) decreased lactate efflux from cancer cells [29]. CHCA may be competitive with lactate for transport through MCT based on their structural similarity to lactate [30]. MCTs are major determinants of regulation pH in cancer cells e.g. melanoma cells. MCT inhibition may improve the effectiveness of chemotherapeutic drugs that work best at low pH, such as alkylating agents and platinum-containing compounds that may be selective for cells in an acidic tumor bed [31]. Cinnamic acid derivatives e.g. CHCA caused a block of lactate efflux which resulted in a significant decrease in the pH of melanoma cells which sensitized melanoma cells to exposure to a relatively high temperature (42°C). Tumoricidal effect of hyperthermia occurs through the inhibition of expression of heat shock protein [32]. Targeting MCT using RNA interference technique was successful also in decreasing lactate efflux in targeted cells by about 85% which was reported to decrease lactate extrusion significantly [33]. Moreover, 3BP was reported to inhibit LDH. Inhibition of LDH was reported to redirect metabolic flux into mitochondria and decrease the metastatic potential of cancer cells [34]. As 3BP is a structural analog to lactate, transported through same MCT and exerts antagonistic effects to lactate [35], it seems necessary to investigate a possible competitive effect between 3BP and lactate for transport through MCT. A competitive effect between 3BP and lactate as regard transport via MCT seems reasonable in light of the higher intracellular lactate levels than extracellular induced by 3BP in HepG2 which may indicate that 3BP blocked export of lactate through MCT [27].

Third level of targeting Warburg effect is to antagonize the effects of lactate extruded in cancer microenvironment. In advanced cancers, the close structural similarity between 3BP and lactate confers many advantages to 3BP over other antiglycolytics as regard structural similarity between 3BP and lactate.

7. 3BP as a Solution for Cancer Chemoresistance and Radioresistance

Emergence of chemoresistant and radioresistant cancer cells is a common issue in clinical oncology. Resistant cancer cells lead to persistence of primary tumors, recurrence and metastasis. In light of previous reports, 3BP seems a promising general anticancer drug that proved to be efficient in killing many cancer cell lines in vitro and in vivo. 3BP radically cured experimental animals from cancer [36]. Anticancer drugs killing by necrosis e.g. doxorubicin are preferred to drugs that kill by apoptosis as necrotic cell death causes a rapid damage and killing to cancer cells and markedly inhibited their clonogenic power [37].

Origin of cancer chemoresistance may be related to the hostile cancer microenvironment in which normal cells and weak cancer cells cannot survive leading to evolution of stronger cells that can afford this aggressive atmosphere and develop new mechanisms to resist cancer therapeutics [28]. Even strong anticancer agents that induce necrotic cell death cannot exhibit their anticancer effects as extracellular acidosis may cause resistance to chemotherapeutics that are weak bases in nature e.g. doxorubicin. Other anticancer agents e.g. mitoxantrone, and daunorubicin are
all inhibited by low extracellular pH [15]. Lactate produced through Warburg effect exerts resistance against radiotherapy [29].

Glycolysis Inhibition using 3BP could overcome drug resistance to some chemotherapeutics [38]. Cancer microenvironment may play a role in development of resistance to chemotherapy. Lactate produced through Warburg effect is a participating factor in establishment of cancer microenvironment. 3BP targeted Warburg effect at level of lactate production [14] and lactate function [35] and further studies seem necessary to investigate the effect of 3BP on lactate transport through MCT. Chemoresistance of cancer cells may occur via the expression of ABC transporters which are drug-expelling transporters that may participate in the emergence of chemoresistance [14,39]. 3BP was reported to inactivate ABC transporters [14]. 3BP sensitized prednisolone-resistant acute lymphoblastic leukemia cells to prednisolone [12]. 3BP depleted intracellular ATP levels that are responsible for chemoresistance in cancer cells [40].

3BP potentiated cytotoxic effects of platinum chemotherapeutics e.g. cisplatin and oxaliplatin [41]. Interestingly, precursor drugs that are metabolized into 3BP e.g. 3-bromo-2-oxopropionate-1-propyl ester (3-BrOP) exerted a significant antileukemia effect in acute leukemia cell lines and patient samples. 3-BrOP was powerful in treating chemoresistant leukemia cell lines and had a synergistic effect with antimycin A and rapamycin [42].

8. Glycolysis Double and Triple Inhibition

Targeting more than one glycolytic enzyme seems attractive to combat cancer. Hexokinase II was reported to be the facilitator and gatekeeper of malignancy [43]. Other glycolytic enzymes seem important also for cancer cells for the fact that glycolysis in cancer starts with glucose phosphorylation and ends with lactate formation (Warburg effect). However, intermediates of glycolysis e.g. G6P and F6P are also intermediates of another metabolic pathways e.g. gluconeogenesis and hexose monophosphate shunt etc. Targeting more than one point of glycolysis downstream of HK together with HK II itself may carry the benefits of better therapeutic effects, lower doses used and lower side effects. Citrate is an inhibitor of the second key enzyme of glycolysis (Phosphofructokinase, PFK). Citrate is abundant in citrus fruits e.g. orange and lemon. Citrate is a safe natural organic acid that is present as a major intermediate of citric acid cycle described by sir Krebs H.A [44]. Although little attention was paid to its role as an anticancer agent, citrate was reported to exert potent anticancer effects in many types of cancer e.g. citrate was reported in treating mesothelioma in which citrate caused a reduction in the expression of anti-apoptotic proteins e.g. Mcl-1. Citrate sensitized mesothelioma cells to cisplatin [45]. Citrate alone with no adjuvant chemotherapy successfully improved a patient with medullary thyroid carcinoma [46]. Citrate induced a massive apoptotic cell death in gastric cancer cell lines that was associated with a decrease in the expression of anti-apoptotic proteins [47]. Moreover, citrate was powerful in treatment of antibiotic-resistant postoperative wounds of cancer patients [48]. In leukemia and lymphoma cell lines, citrate alone or combined with chemotherapeutic drugs induced a dose-dependent lympholytic activity. Safety of citrate was reported as regard effect on human normal peripheral blood mesenchymal cells [49].

As a future prospect for this kind of studies targeting glycolysis, it is seems necessary to include inhibitors of HK II and PFK. 3BP is highly recommended with citrate in light of the strong synergistic effect of their combinations [35]. Understanding the biology of malignant metastasis and its sequential steps [50] helps in improving current treatment outcomes in cancer. 3BP is an antagonist of lactate (facilitator of metastasis) [35]. 3BP deprives cancer cells of the essential requirement for the process of metastasis e.g. ATP, lactate and G6P (product of HK II). 3BP targeted critical steps of malignant metastasis e.g. cancer cell survival, proliferation, migratory power and clonogenic power.

9. Conclusion

3BP is a promising antineoplastic agent for treating all types of hematological malignancy. So many anticancer mechanisms are attributed to 3BP. 3BP-induced inhibition of cancer energetics and the Warburg effect are vital for its anticancer effects. Further research is highly needed to improve 3BP delivery and release at target sites and to modify 3BP crossing at relevant biological barriers.

Conflict of Interest

The author declares that there is no conflict of interest with anyone

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References

[1] Baghdadi HH. Targeting cancer cells using 3-bromopyruvate for selective cancer treatment. Saudi J Med Med Sci. 2017, 5(1): 9-19.
[2] Geschwind JF, Georgiades CS, Ko YH, Pedersen PL. Recently elucidated energy catabolism pathways provide opportunities for novel treatments in hepatocellular carcinoma. Expert Rev Anticancer Ther 2004; 4: 449-57.
[3] Ko YH, Pedersen PL, Geschwind JF. Glucose catabolism in the rabbit VX2 model for liver cancer. Cancer Lett 2001; 173: 83-91.
[4] Geschwind JF, Ko YH, Torbenson MS, Magee C, Pedersen PL. Novel therapy for liver cancer: direct intraarterial injection of a potent inhibitor of ATP production. Cancer Res 2002; 62: 3909-13.
[5] Schaefer NG, Geschwind JF, Engles J, Buchanan JW, Wahl RL. Systemic administration of 3-bromopyruvate in treating disseminated aggressive lymphoma. Transl Res. 2012 Jan; 159(1): 51-7.
[6] Yadav S, Pandey SK, Kumar A, Kujur PK, Singh RP, Singh SM. Antitumor and chemosensitizing action of 3-bromopyruvate: Implication of deregulated metabolism. Chem Biol Interact. 2017 May 25; 270: 73-89.
Rodrigues AS, Pereira SL, Correia M, Gomes A, Perestrello T, Ramalho-Santos J. Differentiation or Die: 3-Bromopyruvate and Phosphotyrosine in BC: Embryonic Stem Cells. PLoS One. 2015 Aug 12; 10(8): e0135617

Verhoeven HA, van Giessen LJ. Flow cytometric evaluation of the effects of 3-bromopyruvate (3BP) and dichloracetate (DCA) on THP-1 cells: a multiparameter analysis. J Bioenerg Biomembr. 2012 Feb; 44(1): 91-9.

Huang A, Hu H, Liu K, Zhan G, Liu D, Wen S, Garcia-Manero G, Huang P, Hu Y. Metabolic alterations and drug sensitivity of tyrosine kinase inhibitor resistant leukemia cells with a FLT3/ITD mutation. Cancer Lett. 2016 Jul 28; 377(2): 149-57.

Song K, Li M, Xu X, Xuan LL, Huang G, Liu Q. Resistance to chemotherapy is associated with altered glucose metabolism in acute myeloid leukemia cells. Oncol Lett. 2016 Jul; 12(1): 334-342.

Calviño E, Estañ MC, Sánchez-Martín C, Brea R, de Blas E, Boyano-Adánez Mdel C, Rial E, Aller P. Regulation of death induction and chemosensitizing action of 3-bromopyruvate in myeloid leukemia cells: energy depletion, oxidative stress, and protein kinase activity modulation. J Pharmacol Exp Ther. 2014 Feb; 348(2): 324-35.

Hulleman E, Kazemier KM, Holleman A, VanderWeele JD, Rudin CM, BrochuKHS, Evans WE, Pieters R, Den Boer ML. Inhibition of glycolysis modulates prednisolone resistance in acute lymphoblastic leukemia cells. Blood. 2009 Feb 26; 113(9): 2014-21.

Niedźwiecka K, Dylag M, Augustyniak D, Majkowska-Skrobek G, Cal-Bañez-Ko Y, Ko VH, Pedersen PL, Goffeau A, Ulaszewski S. Glutathione may have implications in the design of 3-bromopyruvate for both fungal and algal protein kinase activity modulation. J Pharmacol Exp Ther. 2014 Feb; 348(2): 324-35.

Nakano A, Miki H, Nakamura S, Harada T, Oda A, Amou H, Fujii S, Kagawa K, Takeuchi K, Ozaki S, Matsumoto T, Abe M. Up-regulation of hexokinase in myeloma cells: targeting myeloma cells with 3-bromopyruvate. J Bioenerg Biomembr. 2012 Feb; 44(1): 31-38.

Cardone, R.A., Casavola, V., Reshkin, S.J. (2005). The role of disturbed EPH dynamics and the Na+/H+ exchanger in metastasis. Nature Reviews, Cancer, 5(10), 786-795.

Li, Y., Liu, L., Tollefsbol, T.O. (2010). Glucose restriction can extend normal cell lifespan and impair precancerous cell growth through epigenetic control of hTERT and p16 expression. The FASEB Journal, 24(5), 1442-1453.

Meyers SA, M., Counter, C., Eaton, E., Ellisen, L., Steiner, P., Caddie, S., et al. (1997). hESC-T, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 90, 785-795.

Kanaya, T., Kyo, S., Takakura, M., Ito, H., Namiki, M., et al. (1998). hTERT is a critical determinant of telomerase activity in renal-cell carcinoma. International Journal of Cancer 78, 539-543.

Gil, J., Peters, G. (2006). Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all. Nature Reviews, 7(11), 586-592.

Krishnamurthy, J., Torrice, C., Ramsay, M., Kohalev, G., Al-Regaiey, K., Su, L., et al. (2004). Ink4a/Arf expression is a biomarker of aging. The Journal of Clinical Investigation, 114, 1299-1307.

Robert, K., Murray, Daryl K., Granner, Peter A., Mayes, & Victor W. Rodwell. (2003). Harper's illustrated biochemistry. Medical Books/McGraw-Hill Medical Publishing Division

Robert, K., Murray, Daryl K., Granner, Peter A., Mayes, & Victor W. Rodwell. (2003). Harper's illustrated biochemistry. Medical Books/McGraw-Hill Medical Publishing Division

Thompson, C., Bauer, D., Lum, J., Hatzivasileiou, G., Zong, W., Zhao, F., et al. (2005). How do cancer cells acquire the fuel needed to support cell growth? Cold Spring Harbor Symposia on Quantitative Biology, 70, 357-362.

Garber, K. (2006). Energy deregulation: licensing tumors to grow. Science, 312, 1185-1189.

Zhu, Z., Jiang, W., McInnies, J., Price, J., Gao, B., Thompson, H. (2007). Effects of dietary energy restriction on gene regulation in mammary epithelial cells. Cancer Research, 67, 12018-12025.

Hammerman, P., Fox, C., Thompson, C. (2004). Beginnings of a signal-transduction pathway for bioenergetic control of cell survival. Trends in Biochemical Sciences, 29, 586-592.

Skinner, R., Trujillo, A., Ma, X., Beiler, E.A. (2009). Ketone bodies and pH dynamics and the viability of human neuroblastoma cells. Journal of Pediatric Surgery, 44(1), 212-216.

Pereira da Silva AP, El-Bacha, T., Kyaw, N., dos Santos, R.S., da-Silva, W.S. et al. (2009). Inhibition of energy-producing pathways of HepG2 cells by 3-bromopyruvate. The Biochemical Journal, 417(3), 717-726.

Stubb, M., McSheehy, P., Griffiths, J.R., Bushard, C.L. (2000). Causes and consequences of tumor acidity and implications for treatment. Molecular Medicine Today, 6(1), 15-19.

Colen, C.B., Shen, Y., Ghodoussi, F., Yu, P., Francis, T.B., Koch, B.J., et al. (2011). Metabolic targeting of lactate efflux by malignant glioma inhibits invasiveness and induces necrosis: an in vivo study, Neoplasia, 13(7), 620-632.

Mulet, C., Lederer, F. (1977). Bromopyruvate as an affinity label for baker's yeast flavocytochrome b2. Kinetic study of the inactivation reaction. European Journal of Biochemistry, 73(2), 443-447.

Wahl, M.L., Owen, J.A., Burd, R., Herlands, R.A., Nagami, S.S., Rodeck, U., et al. (2002). Regulation of intracellular pH in human melanoma: potential therapeutic implications. Molecular Cancer Therapeutics, 8(1), 617-626.

Cossa R.A., Storch, C.W., Daskalakis, C., Berd, D., Wahl, M.L. (2003). Intracellular acidification abrogates the heat shock response and compromises survival of human melanoma cells. Molecular Cancer Therapeutics, 2(4), 383-388.

Mathupala, S.P., Parajuli, P., Sloan, A.E. (2004). Silencing of monocarboxylate transporters via small interfering ribonucleic acid. Molecular Cancer Therapeutics, 2(4), 383-388.

Fantin, V.R., St-Pierre, J., Leder, P. (2006). Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell, 9(6), 425-434.

El Sawy, S.M., Abou El-Magd, R.M., Shishido, Y., Chien, S.P., Diem, T.H., Sakai, T., et al. (2012). 3-Bromopyruvate antagonizes effects of lactate and pyruvate, synergizes with citrate and exerts novel anti-glioma effects. Journal of bioenergetics and biomembranes. In press.

Dean, M. (2009). ABC transporters, drug resistance, and cancer stem cells. Journal of Mammary Gland Biology and Neoplasia, 14(1), 3-9.

Zhou, Y., Tozzi, F., Chen, J., Fan, F., Xia, L., Wang, J., et al. (2011). Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells. Cancer Research, in press.

Ihrlund, L.S., Hornlund, E., Khan, O., Shoshan, M.C. (2008). 3-Bromopyruvate as inhibitor of tumour cell energy metabolism and chemopotentiator of platinum drugs. Molecular Oncology, 2(1), 94-101.

Akers, L.J., Fang, W., Levy, A.G., Franklin, A.R., Huang, P., Zweidler-McKay, P.A. (2011). Targeting glycolysis in leukemia: a novel inhibitor 3-BrOP in combination with rapamycin. Leukemia Research, 35(6), 814-820.

Mathupala, S.P., Ko, Y.H., Pedersen, P.L. (2006) Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. Oncogene, 25(54), 7477-7484.

Brosnan, J.T. (2001). Amino acids, then and now –a reflection on Sir Hans Krebs' contribution to nitrogen metabolism. IUBMB Life, 52, 265-270.

Zhang, X., Varin, E., Allouche, S., Lu, Y., Poulain, L., Icard, P. (2009). Effect of citrate on malignant pleural mesothelioma cells: a synergistic effect with cisplatin. Anticancer Research, 29(4), 1249-1254.
[46] Halabe Bucay, A. (2009). Hypothesis proved...citric acid (citrate) does improve cancer: a case of a patient suffering from medullary thyroid cancer. Medical Hypotheses, 73(2), 271.

[47] Lu, Y., Zhang, X., Zhang, H., Lan, J., Huang, G., Varin, E., et al. (2011). Citrate induces apoptotic cell death: a promising way to treat gastric carcinoma? Anticancer Research, 31(3), 797-805.

[48] Nagoba, B.S., Pulpale, A.S., Ayachit, R., Gandhi, R.C., Wadher, B.J. (2011). Citric acid treatment of postoperative wound in an operated case of synovial sarcoma of the knee. International Wound Journal, 8(4), 425-427.

[49] Yousefi, S., Owens, J.W., Cesario, T.C. (2004). Citrate shows specific, dose-dependent lympholytic activity in neoplastic cell lines. Leukemia & Lymphoma, 45(8), 1657-1665.

[50] Bogenrieder, T., Herlyn, M. (2003). Axis of evil: molecular mechanisms of cancer metastasis. Oncogene, 22(42):6524-6536.