Anticancer Efficacy of the Metabolic Blocker 3-Bromopyruvate: Specific Molecular Targeting

SHANMUGASUNDARAM GANAPATHY-KANNIAPPAN,
RANI KUNJITHAPATHAM and JEAN-FRANCOIS GESCHWIND

Department of Radiology and Radiological Sciences, Interventional Radiology Center, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A.

Abstract. *The anticancer efficacy of the pyruvate analog 3-bromopyruvate has been demonstrated in multiple tumor models. The chief principle underlying the antitumor effects of 3-bromopyruvate is its ability to effectively target the energy metabolism of cancer cells. Biochemically, the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been identified as the primary target of 3-bromopyruvate. Its inhibition results in the depletion of intracellular ATP, causing cell death. Several reports have also demonstrated that in addition to GAPDH inhibition, the induction of cellular stress also contributes to 3-bromopyruvate treatment-dependent apoptosis. Furthermore, recent evidence shows that 3-bromopyruvate is taken up selectively by tumor cells via the monocarboxylate transporters (MCTs) that are frequently overexpressed in cancer cells (for the export of lactate produced during aerobic glycolysis). The preferential uptake of 3-bromopyruvate via MCTs facilitates selective targeting of tumor cells while leaving healthy and non-malignant tissue untouched. Taken together, the specificity of molecular (GAPDH) targeting and selective uptake by tumor cells, underscore the potential of 3-bromopyruvate as a potent and promising anticancer agent. In this review, we highlight the mechanistic characteristics of 3-bromopyruvate and discuss its potential for translation into the clinic.*

Tumors are characterized by a profound dependency on glucose metabolism for energy generation. This increased demand for glucose is the basis for tumor imaging with ^{18}F-fluorodeoxyglucose (an analog of glucose) positron-emission tomography (FDG-PET), which is clinically widely used in the diagnosis of human cancer. When combined with computed tomography (CT), PET/CT imaging is a powerful diagnostic tool with a high sensitivity and specificity (>90%) (1). The success of PET imaging as a cancer diagnostic tool has opened the door to the possibility of exploiting glucose metabolism as a target for therapy. Published pre-clinical data and reviews have revealed the therapeutic potential of targeting glucose metabolism in cancer (2-4). However, despite tremendous progress in our understanding over the molecular mechanisms regulating tumor metabolism which has led to the development of several antiglycolytic agents, the translation of such agents to the clinic has not yet been successful.

The glucose analog 2-deoxyglucose was one of the first known inhibitors of glucose metabolism tested for anticancer effects (5). Its mechanisms of action is rather simple; 2-deoxyglucose competes with glucose in the first step of glucose metabolism for phosphorylation by the enzyme type II hexokinase (HK II) resulting in the conversion of glucose to glucose-6-phosphate. 2-Deoxyglucose is converted to deoxyglucose-6-phosphate, a molecule that cannot be metabolized any further in the glycolytic pathway, thus causing an inhibition of glycolysis. 2-Deoxyglucose has been shown to inhibit glycolysis and cause cancer cell death *in vitro*. However, further testing in pre-clinical animal studies and human clinical trials showed no significant tumor response to 2-deoxyglucose used as a single anticancer agent. This could be due to two reasons. Firstly, 2-deoxyglucose does not chemically bind with the target enzyme to inactivate it; therefore, the enzyme is able to continue catalyzing any glucose available in an *in vivo* system. Secondly, the existence of feeder pathways into the glycolytic pathway enables carbohydrate molecules such as sucrose, maltose and lactose to enter the pathway subsequent to the step catalyzed by hexokinase, and keeps the energy-producing ability of the cell patent. In addition, the possibility of the emergence of a chemoresistant phenotype also exists (6). In particular, aggressive or

Correspondence to: Jean-Francois Geschwind, MD, Professor of Radiology, Surgery and Oncology, Director, Vascular and Interventional Radiology, Director, Interventional Radiology Center, Johns Hopkins University School of Medicine, Sheikh Zayed Tower, Suite 7203, The Johns Hopkins Hospital, 1800 Orleans Street, Baltimore, MD 21287, U.S.A. Tel: +1 4106146597, Fax: +1 4109550233, e-mail: jfg@jhmi.edu

Key Words: 3-Bromopyruvate, GAPDH, glucose metabolism, glycolysis, HCC, locoregional therapy, molecular targeting, review.

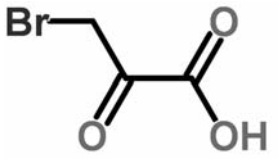

Figure 1. *The molecular structure of the pyruvate analog 3-bromopyruvate.*

hypoxic (known to be more glycolytic) tumors exhibited resistance to 2-deoxyglucose. Recent data showed that the effect of 2-deoxyglucose-mediated cancer cell death is primarily due to the inhibition of glycosylation, a process that adds glycol moieties to proteins, thus necessitating further investigation to understand the principal molecular mechanisms underlying the therapeutic efficacy of 2-deoxyglucose (6).

Lactate dehydrogenase (LDH), another glycolytic enzyme, has attracted considerable interest as the production of lactate during glycolysis essentially relies on LDH activity. Compelling *in vitro* and *in vivo* data demonstrated that LDH could indeed be a potential therapeutic target (7, 8). However, the challenge possibly limiting the usefulness of LDH as a therapeutic target lies in the fact that tumor cells can switch to oxidative phosphorylation (mitochondrial respiration), thereby evading the effect of LDH inhibition. Perhaps the recent developments targeting glutamine metabolism along with glucose metabolism could profoundly affect cancer cells (9, 10).

Chemoresistance and systemic toxicities are two of the several factors that can affect the successful translation of drugs to the clinic. Thus, from a clinical perspective, in order to be successful, a potential drug candidate (inhibitor of a target) must possess key attributes, such as (i) the ability to selectively target tumor cells and (ii) molecular specificity resulting in the direct potent inhibition of the target. One such agent, the pyruvate analog, 3-bromopyruvate fulfills these requirements and has generated significant interest due to its remarkable anticancer effects in various tumor types. During the past decade, since its discovery as an anticancer agent, impressive data have emerged providing tremendous insight into the therapeutic efficacy and mechanistic aspects of 3-bromopyruvate action.

3-Bromopyruvate: Selective Targeting of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

The first reports on the cytotoxic effects of 3-bromopyruvate (Figure 1) in cancer cells demonstrated a massive depletion of intra-cellular ATP in a dose-dependent manner (11, 12). Further analysis of 3-bromopyruvate-treated cells revealed

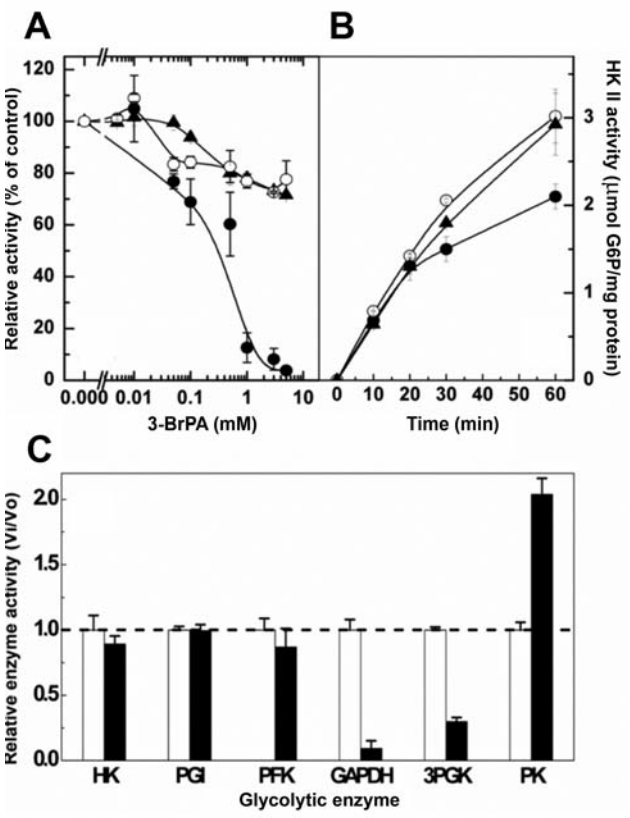

Figure 2. *Effect of 3-bromopyruvate on mitochondrial (mt) HK type II and other glycolytic enzymes in HepG2 cells. A: Activities of mt-HK type I from mouse brain (▲), type II from HepG2 cells (○) and glucokinase (GK) from mouse liver (●) were measured in the presence of different concentrations of 3-bromopyruvate (0.01–5 mM). B: Time course of mt-HK type II activity in the absence (○), and presence of 150 μM (▲) or 5 mM (●) 3-bromopyruvate. C: Cells in culture were pre-incubated with 150 μM 3-bromopyruvate for 30 min and the activities of HK, phosphoglucose isomerase (PGI), phosphofructo kinase (PFK), glyceraldehyde-3 -phosphate dehydrogenase (GAPDH), 3-phosphoglycerate kinase (3PGK) and pyruvate kinase (PK) were measured. Open bars, control cells; closed bars, cells pre-incubated with 3-bromopyruvate. The activity of each enzyme is expressed relative to that under the control conditions. The maximal activities (m-units) of controls were: HK=33±4; PGI=1040±27; PFK=35±3; GAPDH=515±41; 3PGK=637±15; and PK=654±39. Values represent the mean±SEM (n=4). Vi, enzyme activity in the presence of 3-bromopyruvate, Vo, in the absence of 3-bromopyruvate. Reproduced with permission (13).*

the release of cytochrome *c*, a well-studied apoptotic marker, thereby suggesting disruption of the mitochondrial membrane. The combination of ATP depletion, synonymous with impaired glucose metabolism, and disruption or depolarization of the mitochondrial membrane made HK II the likely target since it is a mitochondrial-membrane bound enzyme. Treatment of cancer cells with 3-bromopyruvate at 5 mM resulted in the inhibition of HK II activity, implying that HK II was indeed the target of 3-bromopyruvate (11). In addition, since cancer cells are also characterized by up-

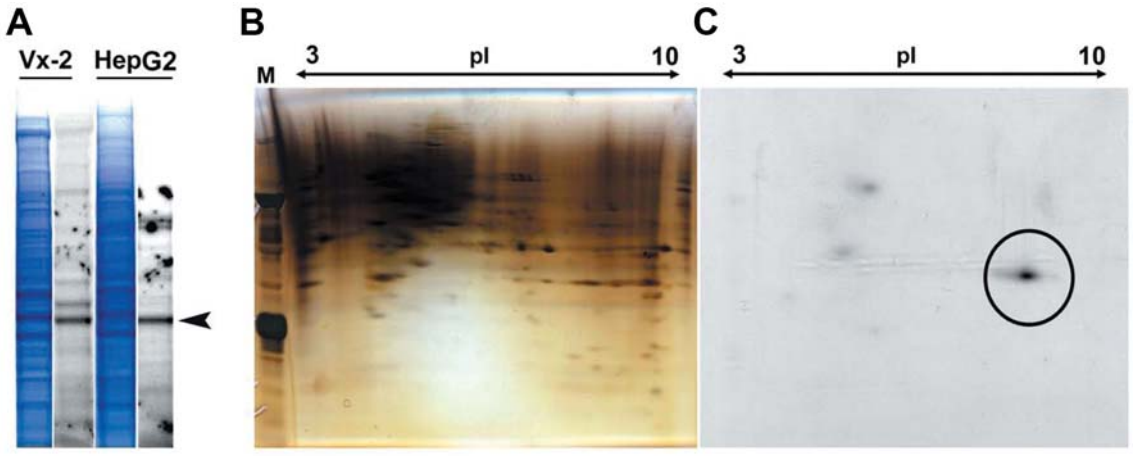

Figure 3. *Selective binding of 3-bromopyruvate to intracellular proteins. A: Coomassie blue-stained gels and their corresponding autoradiograms are shown for HepG2 and Vx-2 cell lines treated with ^{14}C-3-bromopyruvate. The arrowhead indicates the gel band excised and subjected to mass spectrometric characterization. B: Silver-stained 2D gel of whole cell lysate from SK-Hep1 cells treated with ^{14}C-3-bromopyruvate and its corresponding autoradiogram showing ^{14}C-3-bromopyruvate incorporation. The circle indicates the intense gel spot excised and characterized by mass spectrometry. Reproduced with permission (15).*

regulation of HK II, it was thought that inhibiting HK II underlies 3-bromopyruvate-mediated cytotoxicity in cancer cells. However, recent studies in several laboratories demonstrated that at a cytotoxic dose, 3-bromopyruvate did not affect HK II activity (Figure 2A and B) and that the dose required for HK II inhibition was much higher. For example in HepG2 cells, 3-bromopyruvate was able to induce cell death at a concentration of 100 μM, whereas HK II activity remained unaffected at a dose 10- to 50-times higher (13), confirming that HK II inhibition is unlikely to be the prime target of 3-bromopyruvate. On the other hand, what these results revealed was the fact that another or possibly several other molecular targets were involved.

Tracer studies using radiolabeled (^{14}C)-3-bromopyruvate clearly demonstrated that 3-bromopyruvate primarily inhibits the enzyme GAPDH (Figure 2C) (14, 15). Other investigators have also confirmed this finding, thus establishing once and for all that the antiglycolytic effect of 3-bromopyruvate is primarily mediated through direct inhibition of GAPDH (Figures 3-5). A recent report showed that the propyl ester derivative of 3-bromopyruvate also primarily targets GAPDH (16). In addition to directly inhibiting GAPDH, it appears that 3-bromopyruvate also significantly increased the level of intracellular reactive oxygen species (ROS) (17). In turn, the disruption of glycolysis and/or increase in intracellular ROS caused endoplasmic reticulum (ER) stress and translation inhibition (18) that directly led to cancer cell death. It has therefore become abundantly clear that 3-bromopyruvate inflicts a multipronged lethal assault on cancer cells leading to apoptotic cell death. While it is evident that ER stress and the accumulation of ROS are established consequences of the interaction of 3-bromopyruvate and its target GAPDH, what remains unknown is whether the sole blockade of GAPDH and resultant inhibition of glycolysis is sufficient to induce tumor cell death. Or is it possible that all these elements, *i.e.* increased level of ROS, chronic ER stress and finally glycolysis inhibition, are required to be present for the cancer cells to die? Recent data using shRNA specifically targeting GAPDH showed that the GAPDH-targeting property of 3-bromopyruvate is sufficient to promote apoptosis. This was true both *in vitro* and *in vivo* (19). GAPDH is therefore the selective preferred target of 3-bromopyruvate and its blockade by 3-bromopyruvate is sufficient to promote antitumor effects.

3-Bromopyruvate: Beyond Molecular Specificity

Drug-related toxicity constitutes one of the major challenges of drug therapy. This is also true when considering a drug candidate that inhibits a specific target. After having demonstrated the ability of 3-bromopyruvate to selectively bind GAPDH, it became critical to understand whether it would bind to similar targets in the blood and plasma, since such interactions could easily be responsible for major systemic toxicities. Unpublished data from our laboratory demonstrate that 3-bromopyruvate exhibits strikingly selective molecular targeting *in vivo*. Although antineoplastic, alkylating agents such as metallo-drugs (*e.g.* cisplatin and oxaliplatin) have been known to bind with albumin (20, 21), systemic administration of 3-bromopyruvate to Sprague-Dawley rats showed no evidence of albumin as the primary

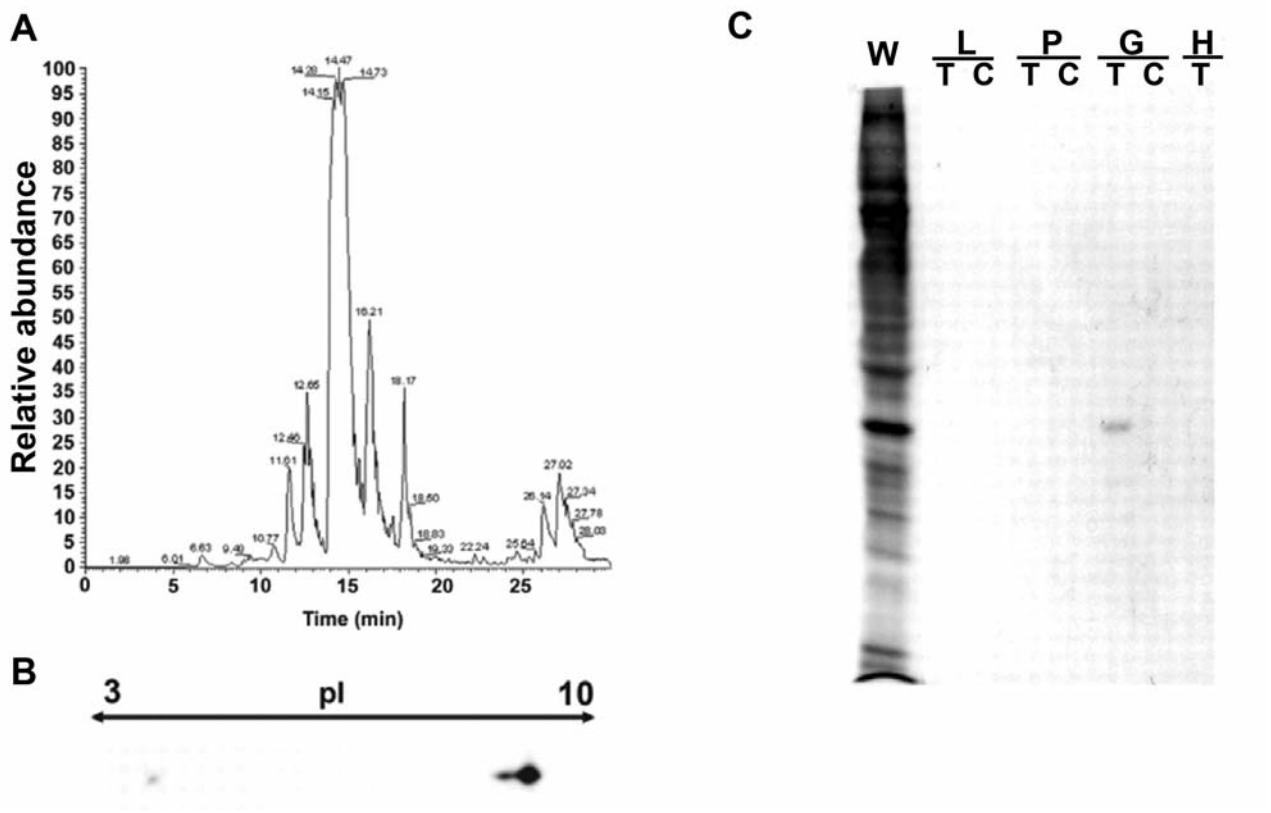

Figure 4. *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) pyruvylation on 3-bromopyruvate treatment. A: Liquid chromatographic-mass spectrometry chromatogram showing GAPDH to be the peptide identified from autoradiogram spot of the 2D gel shown in Figure 3B. B: Immunoblot of a 2D gel showing GAPDH as the spot identified on 2D autoradiogram. C: Autoradiogram of immunoprecipitates from SK-Hep1 cells treated with ^{14}C-3-bromopyruvate, showing the ^{14}C incorporation. Lanes: W, whole cell lysate; lanes L, P, G and H correspond to reactions to specific antibodies against the targets lactate dehydrogenase (L), pyruvate dehydrogenase (P), glyceraldehyde-3-phosphate dehydrogenase (G) and hexokinase type II (H), respectively. T, Target-specific antibody and C, Control IgG for the respective target. Reproduced with permission (15).*

target of 3-bromopyruvate (unpublished data). Thus, the guiding principle underlying the selective and specific binding of 3-bromopyruvate with its molecular target remains to be characterized.

Unique Opportunities

Safety and selective uptake. Selectivity is the critical element required for the successful developmental outcome of compounds that bind irreversibly to their target. 3-Bromopyruvate, a highly reactive alkylating agent selectively binds with GAPDH. Intriguingly, similar alkylators currently in clinical practice, shown to bind with genomic DNA and mitochondria have been known to cause neurotoxicity, nephrotoxicity and cardiotoxicity (22, 23). Fortunately, and surprisingly, 3-bromopyruvate has so far demonstrated a safer toxicity profile than any of these agents, at least in various pre-clinical animal models. In a rabbit model of liver tumor, intravenous administration of 3-bromopyruvate (at 1.75 mM and higher doses) resulted in no considerable accumulation in major vital organs or toxicity (24). Similarly, toxicology studies on rats showed that intravenous delivery of therapeutic dose of 3-bromopyruvate (1.75 mM) did not cross the blood-brain barrier, thus preventing any potential brain toxicity (unpublished data). The findings, to date, reveal that a locoregional approach for delivering 3-bromopyruvate to tumors is the preferred method because it is therapeutically highly effective, without causing any systemic toxicity. Even if the drug were to escape into the systemic circulation during locoregional administration, it would not be a significant problem since no systemic toxicity was evident when the drug was given systemically.

As already mentioned, specific inhibition of the target is clearly a desirable property of any anticancer agent, but unfortunately it alone is not sufficient for therapeutic success in the clinical setting. Indeed, other essential characteristics are required for successful translation of any potential therapeutic agent. These are (a) selective targeting of tumors and (b)

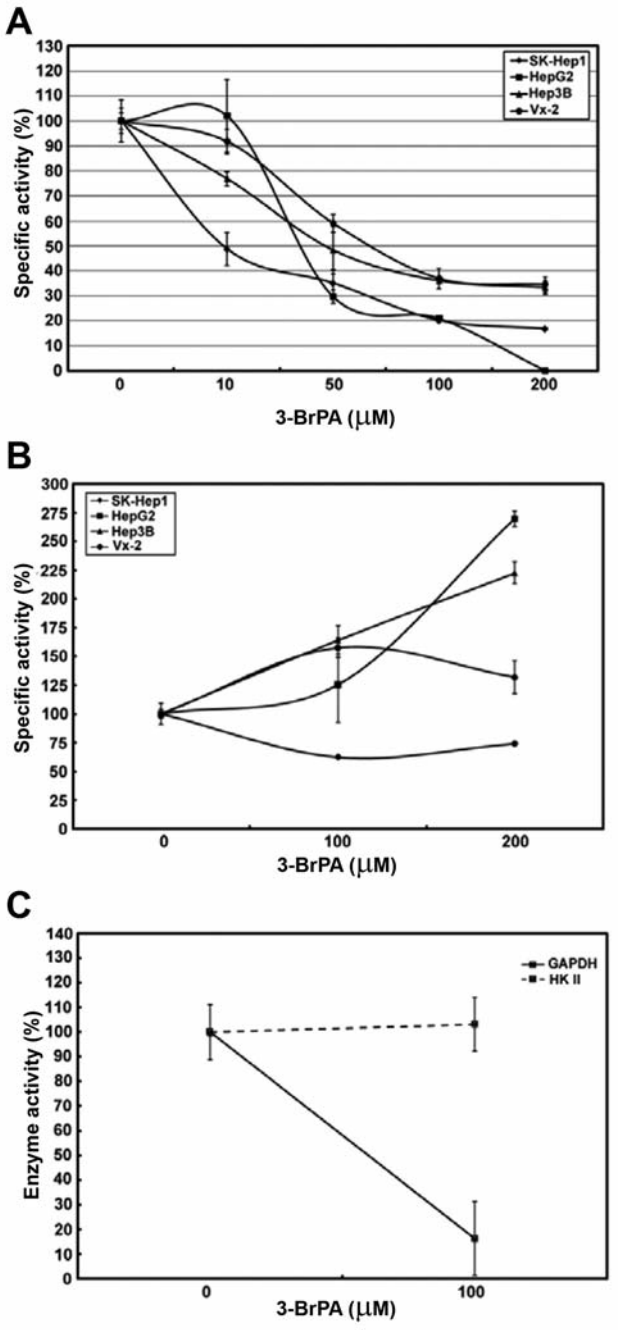

Figure 5. *Effect of pyruvylation on enzymatic function. A: Dose-dependent decrease in the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity of 3-bromopyruvate-treated cell lines. B: Hexokinase type II (HK II) activity after 3-bromopyruvate treatment of various cell lines. C: Activity of purified GAPDH and HK II. Equal quantities of enzymes (enzyme units) were used for these assays. Reproduced with permission (15).*

minimal or absence of systemic toxicity. 3-Bromopyruvate, despite being a small molecule (molecular mass=166.96), shows marked selectivity in targeting tumors because of the preferential and specific uptake of 3-bromopyruvate by tumor cells. Recent research has shed light on the process of tumor selectivity, which appears to be dependent on the expression of monocarboxylate transporters (MCTs) that are overexpressed in the majority of cancer types (25, 26). Thangaraju *et al*. (27) and Matsumoto *et al*. (28) have demonstrated that MCT-1 is the primary transporter used by 3-bromopyruvate to penetrate inside cancer cells, thereby facilitating the initiation of its anticancer effects. Thus, the cancer-related overexpression of MCTs and the selective uptake of 3-bromopyruvate through them provide a novel paradigm to specifically target cancer (Figure 6).

Combination therapy. Emerging data also indicate that resistance to chemotherapy can be overcome by combining current anticancer agents with 3-bromopyruvate. Studies of several *in vivo* and *in vitro* models of different tumor types have shown that combination therapy with 3-bromopyruvate enhances tumor response to therapy better than the response obtained with the use of single agent alone. Moreover, the therapeutic efficacy of combination therapy with 3-bromopyruvate has been proven to be valid for different classes of anticancer agents (29-35). It is noteworthy that 3-bromopyruvate-dependent increased efficacy of other anticancer agents involves various mechanistic principles depending upon the nature of the drug combined with 3-bromopyruvate. In a recent report, Nakano *et al*. (36) showed that the anticancer effects of daunorubicin were improved greatly in the presence of 3-bromopyruvate. The underlying mechanism for this enhanced effect in the presence of 3-bromopyruvate has been attributed to the increased retention of daunorubicin by 3-bromopyruvate-mediated inhibition of ATP-binding cassette transporters, eventually blocking the drug efflux. Furthermore, as 3-bromopyruvate causes ATP depletion, and the intracellular ATP level directly affects chemoresistance (37), 3-bromopyruvate can therefore promote the retention within cancer cells of chemotherapeutic agents that are frequently pumped-out *via* efflux mechanisms during chemoresistance. On the other hand, when combined with platinum-based drugs, 5-fluorouracil, daunorubicin and mitoxantrone, the increase in therapeutic efficacy has been found to be due to the simultaneous inhibition of proliferation (by drug) and the induction of oxidative stress, and energy depletion (by 3-bromopyruvate) (17, 36). Thus, combining 3-bromopyruvate with other agents certainly demonstrates increased therapeutic efficacy and is valid for multiple agents targeting different tumor types.

The heterogeneity and versatility of tumor cells largely contribute to the differential response of tumors to therapy. Understandably, if any single pathway or mechanism is blocked or disrupted, cancer cells can compensate for this loss of function *via* alternative feeder pathways or rescue mechanisms. For example, in the case of the glycolytic

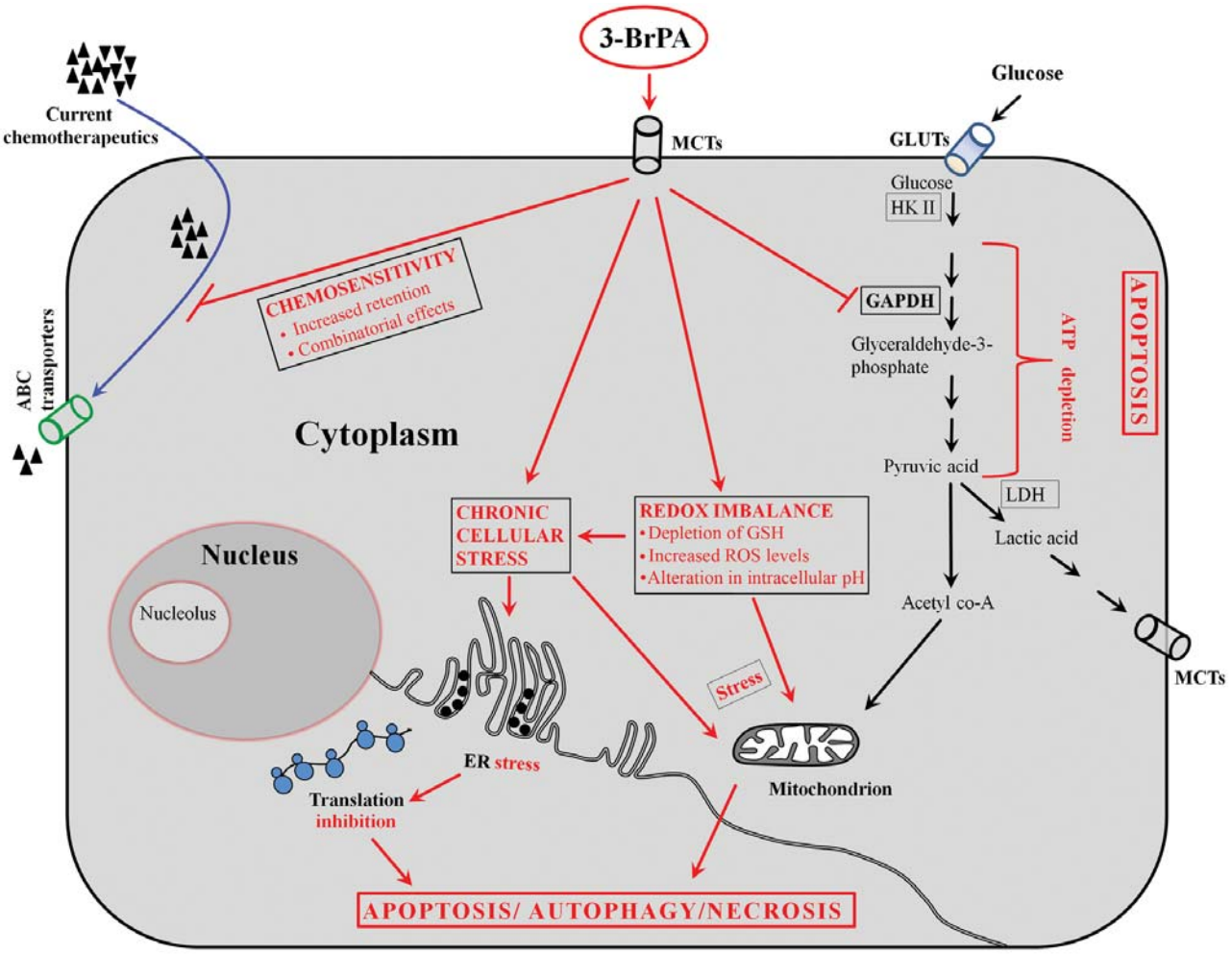

Figure 6. *A schematic representation of pathways associated with the anticancer effects of 3-bromopyruvate.*

pathway, if glycolysis is blocked, the pentose phosphate pathway can play a role in the survival and growth of tumors. Conversely, targeting such rescue mechanism(s) simultaneously while blocking glycolysis could yield better results in terms of killing cancer cells. Fortunately, as shown in Figure 6, 3-bromopyruvate-mediated anticancer effects not only involve direct targeting of glucose metabolism but also activation of chronic cellular and ER stress, thereby creating a lethal multipronged attack on tumor cells.

In summary, administration of the therapeutic dose of 3-bromopyruvate as a single agent has profound anticancer effects, and at lower doses it has been shown to enhance the efficacy of other chemotherapeutic agents. Finally, to advance the prospect of 3-bromopyruvate as a therapeutic agent for cancer, it will be necessary to develop a delivery method/vehicle for its systemic administration and characterize the optimal treatment regimen for mono- and combination therapies.

Acknowledgements

We gratefully acknowledge the support by the Charles Wallace Pratt Research Fund and Abdulrahman Abdulmalik Research Fund. The authors declare that there is no conflict of interests with regard to this article.

References

1 Tang S, Huang G, Liu J, Liu T, Treven L, Song S, Zhang C, Pan L and Zhang T: Usefulness of ^{18}F-FDG-PET, combined FDG-PET/CT and EUS in diagnosing primary pancreatic carcinoma: A meta-analysis. Eur J Radiol *78(1)*: 142-150, 2011.

2 Annibaldi A and Widmann C: Glucose metabolism in cancer cells. Curr Opin Clin Nutr Metab Care *13(4)*: 466-470, 2010.

3 Madhok BM, Yeluri S, Perry SL, Hughes TA and Jayne DG: Targeting glucose metabolism: An emerging concept for anticancer therapy. Am J Clin Oncol *34(6)*: 628-635, 2011.

4 Dang CV: Links between metabolism and cancer. Genes Dev 26(9):877-890, 2012.

5 Dwarakanath B and Jain V: Targeting glucose metabolism with 2-deoxy-D-glucose for improving cancer therapy. Future Oncol *5(5)*: 581-585, 2009.

6 Pelicano H, Martin DS, Xu RH and Huang P: Glycolysis inhibition for anticancer treatment. Oncogene *25(34)*: 4633-4646, 2006.

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

8 Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, Royer RE, Vander Jagt DL, Semenza GL and Dang CV: Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci USA *107(5)*: 2037-2042, 2010.

9 Cardaci S, Rizza S, Filomeni G, Bernardini R, Bertocchi F, Mattei M, Paci M, Rotilio G and Ciriolo MR: Glutamine deprivation enhances antitumor activity of 3-bromopyruvate through the stabilization of monocarboxylate transporter-1. Cancer Res *72(17)*: 4526-4536, 2012.

10 Hamanaka RB and Chandel NS: Targeting glucose metabolism for cancer therapy. J Exp Med *209(2)*: 211-215, 2012.

11 Ko YH, Pedersen PL and Geschwind JF: Glucose catabolism in the rabbit VX2 tumor model for liver cancer: Characterization and targeting hexokinase. Cancer Lett *173(1)*: 83-91, 2001.

12 Geschwind JF, Ko YH, Torbenson MS, Magee C and Pedersen PL: Novel therapy for liver cancer: Direct intra-arterial injection of a potent inhibitor of ATP production. Cancer Res *62(14)*: 3909-3913, 2002.

13 Pereira da Silva AP, El-Bacha T, Kyaw N, dos Santos RS, da-Silva WS, Almeida FC, Da Poian AT and Galina A: Inhibition of energy-producing pathways of HepG2 cells by 3-bromopyruvate. Biochem J *417(3)*: 717-726, 2009.

14 Ganapathy-Kanniappan S, Geschwind J, Kunjithapatham R, Buijs M, Vossen JA, Tchernyshyov I, Torbenson MS, Cole RN, Syed LH and Vali M: A pyruvic acid analog primarily targets GAPDH to promote cancer cell death. FASEB J *23(1_MeetingAbstracts)*: 678.2, 2009.

15 Ganapathy-Kanniappan S, Geschwind JF, Kunjithapatham R, Buijs M, Vossen JA, Tchernyshyov I, Cole RN, Syed LH, Rao PP, Ota S and Vali M: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is pyruvylated during 3-bromopyruvate-mediated cancer cell death. Anticancer Res *29(12)*: 4909-4918, 2009.

16 Tang Z, Yuan S, Hu Y, Zhang H, Wu W, Zeng Z, Yang J, Yun J, Xu R and Huang P: Overexpression of GAPDH in human colorectal carcinoma as a preferred target of 3-bromopyruvate propyl ester. J Bioenerg Biomembr *44(1)*: 117-125, 2012.

17 Ihrlund LS, Hernlund E, Khan O and Shoshan MC: 3-Bromopyruvate as inhibitor of tumour cell energy metabolism and chemopotentiator of platinum drugs. Mol Oncol *2(1)*: 94-101, 2008.

18 Ganapathy-Kanniappan S, Geschwind JF, Kunjithapatham R, Buijs M, Syed LH, Rao PP, Ota S, Kwak BK, Loffroy R and Vali M: 3-Bromopyruvate induces endoplasmic reticulum stress, overcomes autophagy and causes apoptosis in human HCC cell lines. Anticancer Res *30(3)*: 923-935, 2010.

19 Ganapathy-Kanniappan S, Kunjithapatham R, Torbenson MS, Rao PP, Carson KA, Buijs M, Vali M and Geschwind JF: Human hepatocellular carcinoma in a mouse model: Assessment of tumor response to percutaneous ablation by using glyceraldehyde-3-phosphate dehydrogenase antagonists. Radiology *262(3)*: 834-845, 2012.

20 Lemma T and Pawliszyn J: Human serum albumin interaction with oxaliplatin studied by capillary isoelectric focusing with the whole column imaging detection and spectroscopic method. J Pharm Biomed Anal *50(4)*: 570-575, 2009.

21 Groessl M, Terenghi M, Casini A, Elviri L, Lobinski R and Dyson PJ: Reactivity of anticancer metallodrugs with serum proteins: New insights from size exclusion chromatography-ICP-MS and ESI-MS. J Anal At Spectrom *25(3)*: 305-313, 2010.

22 Singal PK and Iliskovic N: Doxorubicin-induced cardiomyopathy. N Engl J Med *339(13)*: 900-905, 1998.

23 Giri SN, Al-Bayati MA, Du X, Schelegle E, Mohr FC and Margolin SB: Amelioration of doxorubicin-induced cardiac and renal toxicity by pirfenidone in rats. Cancer Chemother Pharmacol *53(2)*: 141-150, 2004.

24 Vali M, Vossen JA, Buijs M, Engles JM, Liapi E, Ventura VP, Khwaja A, Acha-Ngwodo O, Shanmugasundaram G, Syed L, Wahl RL and Geschwind JF: Targeting of VX2 rabbit liver tumor by selective delivery of 3-bromopyruvate: A biodistribution and survival study. J Pharmacol Exp Ther *327(1)*: 32-37, 2008.

25 Pinheiro C, Longatto-Filho A, Azevedo-Silva J, Casal M, Schmitt FC and Baltazar F: Role of monocarboxylate transporters in human cancers: State of the art. J Bioenerg Biomembr *44(1)*: 127-139, 2012.

26 de Oliveira AT, Pinheiro C, Longatto-Filho A, Brito MJ, Martinho O, Matos D, Carvalho AL, Vazquez VL, Silva TB, Scapulatempo C, Saad SS, Reis RM and Baltazar F: Coexpression of monocarbo-xylate transporter 1 (MCT1) and its chaperone (CD147) is associated with low survival in patients with gastrointestinal stromal tumors (GISTs). J Bioenerg Biomembr *44(1)*: 171-178, 2012.

27 Thangaraju M, Karunakaran SK, Itagaki S, Gopal E, Elangovan S, Prasad PD and Ganapathy V: Transport by SLC5A8 with subsequent inhibition of histone deacetylase 1 (HDAC1) and HDAC3 underlies the antitumor activity of 3-bromopyruvate. Cancer *115(20)*: 4655-4666, 2009.

28 Matsumoto S, Saito K, Yasui H, Morris HD, Munasinghe JP, Lizak M, Merkle H, Ardenkjaer-Larsen JH, Choudhuri R, Devasahayam N, Subramanian S, Koretsky AP, Mitchell JB and Krishna MC: EPR oxygen imaging and hyperpolarized (13) C MRI of pyruvate metabolism as noninvasive biomarkers of tumor treatment response to a glycolysis inhibitor 3-bromopyruvate. Magn Reson Med. 2012 Jun 12. doi: 10.1002/mrm.24355. [Epub ahead of print].

29 Cao X, Bloomston M, Zhang T, Frankel WL, Jia G, Wang B, Hall NC, Koch RM, Cheng H, Knopp MV and Sun D: Synergistic antipancreatic tumor effect by simultaneously targeting hypoxic cancer cells with HSP90 inhibitor and glycolysis inhibitor. Clin Cancer Res *14(6)*: 1831-1839, 2008.

30 Cao X, Jia G, Zhang T, Yang M, Wang B, Wassenaar PA, Cheng H, Knopp MV and Sun D: Non-invasive MRI tumor imaging and synergistic anticancer effect of HSP90 inhibitor and glycolysis inhibitor in RIP1-Tag2 transgenic pancreatic tumor model. Cancer Chemother Pharmacol *62(6)*: 985-994, 2008.

31 Filomeni G, Cardaci S, Da Costa Ferreira AM, Rotilio G and Ciriolo MR: Metabolic oxidative stress elicited by the copper (II) complex [Cu(isaepy)2] triggers apoptosis in SH-SY5Y cells through the induction of the AMP-activated protein kinase/p38MAPK/p53 signaling axis: Evidence for a combined use with 3-bromopyruvate in neuroblastoma treatment. Biochem J *437(3)*: 443-453, 2011.

32 Yu SJ, Yoon JH, Lee JH, Myung SJ, Jang ES, Kwak MS, Cho EJ, Jang JJ, Kim YJ and Lee HS: Inhibition of hypoxia-inducible carbonic anhydrase-IX enhances hexokinase II inhibitor-induced hepatocellular carcinoma cell apoptosis. Acta Pharmacol Sin *32(7)*: 912-920, 2011.

33 El Sayed SM, Abou El-Magd RM, Shishido Y, Chung SP, Sakai T, Watanabe H, Kagami S and Fukui K: D-Amino acid oxidase gene therapy sensitizes glioma cells to the antiglycolytic effect of 3-bromopyruvate. Cancer Gene Ther *19(1)*: 1-18, 2012.

34 Yu SJ, Yoon JH, Yang JI, Cho EJ, Kwak MS, Jang ES, Lee JH, Kim YJ, Lee HS and Kim CY: Enhancement of hexokinase II inhibitor-induced apoptosis in hepatocellular carcinoma cells *via* augmenting ER stress and antiangiogenesis by protein disulfide isomerase inhibition. J Bioenerg Biomembr *44(1)*: 101-115, 2012.

35 Wintzell M, Lofstedt L, Johansson J, Pedersen AB, Fuxe J and Shoshan M: Repeated cisplatin treatment can lead to a multiresistant tumor cell population with stem cell features and sensitivity to 3-bromopyruvate. Cancer Biol Ther *13(14)*: 2012.

36 Nakano A, Tsuji D, Miki H, Cui Q, El Sayed SM, Ikegame A, Oda A, Amou H, Nakamura S, Harada T, Fujii S, Kagawa K, Takeuchi K, Sakai A, Ozaki S, Okano K, Nakamura T, Itoh K, Matsumoto T and Abe M: Glycolysis inhibition inactivates ABC transporters to restore drug sensitivity in malignant cells. PLoS One *6(11)*: e27222, 2011.

37 Zhou Y, Tozzi F, Chen J, Fan F, Xia L, Wang J, Gao G, Zhang A, Xia X, Brasher H, Widger W, Ellis LM and Weihua Z: Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells. Cancer Res *72(1)*: 304-314, 2012.

Received October 16, 2012
Revised November 6, 2012
Accepted November 7, 2012