Citrate induces apoptosis of the acute monocytic leukemia U937 cell line through regulation of HIF-1α signaling

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Abstract. The present study aimed to investigate the anti-tumor effect of citrate on acute monocytic leukemia (AML) and its mechanisms. The apoptosis of the AML cell line, U937, was assessed by MTT and Hoechst staining, the expression of Bcl-2, caspases-3 and -9, hypoxia-inducible factor 1 α (HIF-1 α) and its target gene GLUT-1, were assayed by western blotting and the role of HIF-1 α was evaluated through siRNA. The results showed that citrate inhibits the expression of Bcl-2, while it induces the activation of caspases-3 and -9. In addition, citrate induces U937 apoptosis in a dose- and time-dependent manner by regulating the expression of HIF-1 α and its downstream target GLUT-1. The results suggest that citrate performs an anti-acute monocytic leukemia action by targeting HIF-1 α signaling and may be a promising clinical approach.

Introduction

Acute monocytic leukemia (AML) is a serious life threatening clinical disorder and its prognosis is usually poor due to its resistance to the majority of chemotherapy treatments. Therefore, it is important to seek novel agents to overcome this disorder (1).

In normal cells, glucose is firstly metabolized through oxidative phosphorylation in mitochondria when oxygen is

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available. However, if oxygen is deficient, pyruvate is converted to lactate in the cytoplasm and then lactate is metabolized through glycolysis for further metabolism to produce ATP. In cancer cells, even with sufficient oxygen supply, cells prefer to undertake a glycolytic process to generate ATP and lactate production. This cancer metabolic phenotype is characterized by high glucose uptake, increased aerobic glycolysis, decreased mitochondrial activity and is known as the Warburg effect (2,3). Previously, targeting glucose metabolism has been used as an anticancer strategy (4), for example, aminopterin is targeted to induce remission of leukemia by blocking the activity of the enzyme dihydrofolate reductase, an NADPH-dependent enzyme, which has a central role in nucleic acid synthesis, which is activated by the high redox state induced by aerobic glycolysis and/or glutamine breakdown. Methotrexate, is another example of a related aminopterin compound that is a clinically used anticancer drug (5-7). The use of citrate has been reported in the treatment for a number of cancers, however, the underlying mechanism remains unknown (4,8-10).

The transcription factor hypoxia-inducible factor 1α (HIF-1 α) has been implicated in regulating genes which are responsible for the metabolic differences between cancer and normal cells (11,12). HIF-1 α is closely associated with multiple oncogenes, including ras, FOXO3a, bcl-2, VEGFA and PKM2 (11,13,14). Exploiting HIF-1α to switch to glycolysis in the process of glucose metabolism to produce ATP for cancer growth is a primary function of the oncogenes. However, it remains unclear how this aerobic glycolysis is regulated to sustain the growth of tumor cells (11,15,16). New data suggest that HIF-1a exerts its role by increasing glycolysis rather than by suppressing mitochondrial activity (17,18). In addition, studies on tumor glycolysis have identified pyruvate kinase-M2 (19) as an intriguing novel interacting partner for HIF-1 α (14,20-22), revealing a potential mechanism for the Warburg effect and this characteristic may be exploited in targeting cancer (23-25).

The present study investigated the effects of citrate on the monocytic leukemia cell line U937 *in vitro* and a number of genes involved in glycolysis and cell death, including bcl-2, caspases-9 and -3, and HIF-1 α have been assessed to explore the mechanisms underlying the anticancer activity of citrate.

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Materials and methods

Cell line and culture. The human established monocytic leukemia cell line, U937, was commercially obtained from the Institute of Biochemistry and Cell Biology, SIBS, Chinese Academy of Science (Shanghai, China). The human lymphoblast cell line HYM2CIR, a de novo cell line derived from a 56 year-old patient was a gift from Yongchuan Wang (Department of Surgery, Shanghai First People's Hospital, Shanghai, China). The de novo cell line was established following written consent from the patient and this study was approved by the Ethics Committee of Shanghai First People's Hospital, Shanghai Jiaotong University, Shanghai, China (approval number: 2012K071). The cell lines were cultured in RMPI-1640 medium, 10% fetal calf serum and 1% (v/v) penicillin/streptomycin (all Gibco-BRL, Carlsbad, CA, USA). DMEM (no glucose) medium was purchased from Gibco-BRL. Cells were maintained in a 5% CO₂ humidified atmosphere at 37°C and powered tribasic sodium citrate was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Lactate measurement. Following termination of incubation, medium was separated by centrifugation at 200 x g for 5 min. Lactate was measured on an ABL700/800 series analyzer (Radiometer, Copenhagen, Denmark) with a lower detection limit of 0.1 mM/l.

Cell viability measured by MTT assays. Cell viability was measured using the CCK-8 assay at 48 h following citrate treatment. Briefly, U937 or HYM2CIR cells ($3x10^3$), were seeded in each well in 96-well plates and cultured overnight. The culture medium was removed and cells were incubated with fresh medium containing citrate at concentrations ranging between 0.125 and 4 mg/ml. Following 48 h, plates were incubated with 10 µl CCK-8 for 4 h and measured with a Bio-Rad 680 microtiter plate reader (Bio-Rad, Hercules, CA, USA) at 450 nm. The viability of cells treated with citrate was compared with untreated cells. All experiments were performed a minimum of three times and triplicate wells were used in each experiment.

HIF-1a silencing. Small interfering RNA (siRNA) was performed by transfection using HIF-1a directed or control (Luciferase GL2; Thermo Scientific, Waltham, MA, USA) double-stranded RNA oligonucleotides. HIF-1a was synthesized by Eurofins MWG Operon (Ebersberg, Germany). The target sequences are labeled 'siRNA Target Sequences' and the sequence is as follows: HIF-1 α forward 5'-CCACAGGACAGTACAGGATG-3' and reverse 5'-TCAAGTCGTGCTGAATAATACC-3'. Cells (1.5x10⁵) were seeded in 12.5-cm² flasks 24 h prior to treatment with siRNA. At the time of transfection, the proliferation state of the cell was in the exponential (40-50%) phase. Different concentrations and incubation times of siRNA were analyzed in pilot experiments. siRNA (75 nM) was transfected into U937 cells using Interferin (Polyplus-transfection Inc., NY, USA) following the manufacturer's instructions. Cells were pre-treated with siRNA for 4 h at 37°C under normoxic conditions and treated following incubation.

Hoechst 33258 staining. U937 cells $(1x10^5 \text{ cell/ml})$ were seeded in 24-well plates and treated with concentrations of

citrate between 0.77 and 1.55 mM/l for 12-24 h. The apoptotic morphology of U937 cells was evaluated by Hoechst 33258 (KeyGen Biotech Co., Ltd., Nanjing, Jiangsu, China) staining according the kit's instructions.

Western blot analysis. Cells were centrifuged following treatment and rinsed with ice-cold PBS, lysed in RIPA buffer (150 mM NaCl, 50 mM Tris HCl pH 8, 1% Triton[™] X-100, 4 mM PMSF, 2 mM aprotinin, 5 mM EDTA, 10 mM NaF, 10 mM NaPPi and 1 mM Na3VO4) for 30 min on ice. Lysates were clarified by centrifugation at 14,000 rpm for 15 min at 4°C and protein concentrations were determined using the BCA assay (Bio-Rad). Equal amounts of total cellular protein (40 μ g) were resolved in a Bis-tris-HCL buffered (pH 6.4) 10-12% polyacrylamide gel (Bio-Rad) for 90 min at 120 V and electrophoretically transferred onto a PVDF membrane (Bio-Rad) for 75 min at 300 MA. The membrane was blocked for 1 h at room temperature in T-TBS (132 mM NaCl, 20 mM Tris-HCl pH 7.6 and 0.05% Tween-20) supplemented with 5% non-fat dry milk. The membrane was incubated for 1 h at room temperature in T-TBS-milk with the following primary antibodies: anti-Bcl-2, anti-caspases-9 and -3 (all 1:1,000, Cell Signaling Technology, Inc., Danvers, MA, USA), anti HIF-1a (1:500, Bioword Technology, Visalia, CA, USA), anti-glut-1 and anti-\beta-actin (1:500 and 1:3,000; Sigma-Aldrich).

Statistical analysis. Statistical analysis was performed using the SPSS software version 13.0 (SPSS, Inc., Chicago, IL, USA). Student's t-test was also used. P<0.05 was considered to indicate a statistically significant difference.

Results

Citrate inhibits cellular proliferation and induces apoptosis of U937. Cell viability was assessed using a CCK-8 assay. Briefly, cells were treated with citrate at concentrations ranging between 0.77 and 1.55 mM/l for 48 h with or without glucose in the medium. The proliferation of cells was significantly inhibited in U937 compared with control (P<0.05; Fig. 1). The IC₅₀ values of citrate for U937 were 0.86 mM/l. However, it did not significantly affect the viability of HYM2CIR. This suggested that the proliferation inhibition of U937 by citrate is associated with glucose metabolism with little effect on normal cells.

To probe the mechanisms by which citrate inhibits cell proliferation, the morphological changes in citrate-treated cells were examined. Apoptosis was evaluated by the appearance of condensed and fragmented nuclei by Hoechst 33258 staining. Compared with controls, more nuclei of U937 cells showing condensed and apoptotic bodies were observed, due to apoptosis (Fig. 2B and C) in a dose- and time-dependent manner.

To determine the apoptotic mechanisms, western blotting was performed to assess the expression of Bcl-2 and activation of caspases-3 and -9. The results showed that citrate significantly inhibits Bcl-2 protein expression in U937 in a dose- and time-dependent manner accompanied by the cleavage activation of caspase-3 or -9 (Fig. 2E).

Lactate production of U937. Cells were incubated with citrate at a dose of 1.16 mM/l for 24, 36 or 72 h. Lactate production was compared between treatment and control groups. The



Figure 1. Citrate significantly inhibits U937 cell growth under the glucose deprived condition, compared with the results of the normal cell HYM2CIR, which is not sensitive to citrate treatment under the same incubation condition. (A) Effects of citrate on U937 cell proliferation. (B) Effects of citrate on HYM2CIR. The values represent the mean \pm SD (n=6) of triplicate experiments. HYM2CIR, human lymphoblast cell.



Figure 2. Citrate induces apoptosis of U937 cells by inducing cytochrome c release and activating caspases 9 and 3, and was observed to inhibit the expression of Bcl-2. Morphological features for apoptosis in controls and citrate treated cells were revealed by Hoechst 33258 staining. (A) untreated U937; (B and C) treated with citrate 1.55 mM/l 12 and 24 h respectively and (D) HYM2CIR cell treated with citrate 1.55 mM/l for 24 hours. (E) Western blot analysis of a number of key proteins involved in apoptosis following citrate treatment. β -actin is used as a load control. HYM2CIR, human lymphoblast cell.

results showed that citrate markedly reduced lactate production and increased the pH value (Fig. 3), suggesting that U937 cell death treatment by citrate is induced by intervening in the glycolysis process. Citrate induces the expression of HIF-1 α and GLUT-1. To determine the role which citrate plays in glycolysis inhibition, its effect on the expression of HIF-1 α , an important signaling molecule which is closely associated with glucose metabolism,



Figure 3. Citrate treatment significantly decreases lactic acid production and leads to an increase of pH value as the treatment time is prolonged for U937 cells compared with the control, HYM2CIR. The two cell lines were treated with citrate at 1.55 mM/l. Lactic acid production and pH value were measured at 24, 48 and 72 h. $^{\circ}P<0.05$, vs. control. Mean \pm SD; n=3.



Figure 4. Effects of HIF-1 α expression on the U937 cell viability. (A) MTT test of U937 cell line when treated with different concentrations of citrate. (B) The expression of HIF-1 α and Glut-1 in U937 cell line revealed by western blot analysis. (C) The results of MTT test of U937 cell line following silencing HIF-1 α and (D) the expression of HIF-1 α and Glut-1 in U937 cell following HIF-1 α silencing.

was examined. Following treatment with citrate for 24 h, western blot analysis revealed that citrate up-regulated the expression of HIF-1 α and another important protein, glucose transporter-1, which is a target of HIF-1 α , the primary transporter involved in glucose influx in U937 cells. By contrast, U937 cell viability is also assayed following HIF-1 α silencing (Fig. 4).

Discussion

Otto Warburg initially observed that, under aerobic conditions, normal cells metabolize glucose to pyruvate through glycolysis in the cytosol and then the pyruvate enters the mitochondria for oxidative phosphorylation to produce ATP, while, under anaerobic conditions, glycolysis is the primary mechanism to obtain energy. By contrast, in cancer cells, even under conditions of abundant oxygen, cancer cells consume glucose for their energy production through glycolysis, this is known as the Warburg effect (26,27). The Warburg effect is regarded as one of the most significant hallmarks in cancer cells and targeting glucose metabolism as an anticancer strategy has been attracting attention over the past decade (26,28,29). HIF-1 α has been shown to be important in the altered metabolism of cancer cells as it causes increased glycolysis and decreased mitochondrial function in tumors by regulating a number of genes involved in these processes (11,30,31). HIF-1 α plays its role by dimerizing with HIF-1 β to activate a number of enzymes involved in glycolysis and suppresses genes involved in mitochondrial biogenesis (30,32). For example, previous studies revealed that fibroblasts harboring activated HIF-1α showed a marked reduction in Cav-1 levels and a shift towards aerobic glycolysis and hence, a loss of mitochondrial activity and an increase in

lactate production (18,33). It has been reported that fibroblasts expressing activated HIF-1 α have an increased tumor mass by ~2-fold and tumor volume by ~3-fold and HIF-1 α induces an increase in the lymph node metastasis of cancer cells (34), suggesting a complex role played by HIF-1 α . Previous studies have observed that HIF-1 α overexpression is an indicator of poor prognosis in T1 and T2 squamous cell carcinoma of the oral floor (11,12). In the present study, the induction of HIF-1 α was observed to be associated with the expression of Glut1 and affects cell viability. The difference between the current observation and the reports are hypothesized to be attributed to cell type specificity. However, the present results suggest that HIF-1 α may be a promising target in the treatment of monocytic leukemia.

Citrate is an intermediate in the tricarboxylic acid cycle and glycolysis in glucose metabolism (2,23), it is capable of inducing apoptosis for malignant pleural mesothelioma cells by depletion of ATP as well as leading to the diminution of the expression of the anti-apoptotic proteins and inhibition of hexokinase due to the inhibition of phosphofructokinase (10,35). Thus, it is logical that citrate induces apoptosis by intervening in the glucose metabolic process. It is well established that all cells survive of ATP supply and the fast growing cells require more energy supply to meet the biomass requirement of growth and proliferation, thus, it is possible that cancer cells are more sensitive to lack of energy, specifically lack of glucose. It is understandable that malignant cells are prone to death once energy supply is insufficient (8,36). In the current experiments, the AML U937, treated, with citrate was observed to exhibit an elevation in lactate production and an increase in pH value and thus, citrate exhibited a potent apoptotic inducing activity towards AML U937 cells while sparing the normal control cell. This suggests that citrate may kill tumor cells while having little effect on healthy cells, possibly by regulating HIF-1 α and other associated signaling. The suppression of the monocytic cell line U937 by citrate is hypothesized to be closely associated with HIF-1 α signaling, which may explain, at least partially, the relatively specific targeting effects of citrate on HIF-1 α signaling in monocytic leukemia.

The intrinsic pathway of apoptosis is a major pathway and it is triggered by signals of intrinsic cell damage or multiple stresses. These signals lead to the activation of the pro-death Bcl2 family proteins, including Bax and Bak, resulting in oligomerization of these proteins and the formation of Bak or Bax based pores in the outer mitochondrial membrane, followed by cytochrome c release from the mitochondria into the cytosol through these pores. Once translocated into the cytosol, cytochrome c assembles with Apaf-1 and caspase-9 to form an apoptosome, which leads to the activation of caspase-9. Caspase-9 subsequently activates the executioner caspases-3 and -7, ultimately resulting in apoptosis (9,37). From the observations that citrate is a calcium chelate used clinically as an anticoagulant, a number of experiments have been performed to determine whether citrate is significant at a concentration that kills tumor cells, but spares the normal cell. Notably, by supplementing calcium in vitro and in vivo, studies demonstrated that citrate induces tumor cell death by activating apical apoptotic molecules of the type one apoptotic pathway, including caspases-8 and -3, and investigators attributed the activation of apoptotic effector proteins, including bax and cytochrome c to the its kosmotropic properties (4,9,10). Multiple genes in the apoptotic process were investigated. The results showed that the expression of Bcl-2 is down regulated and that caspases-9 and -3 were activated. According to the observations, an intrinsic apoptotic pathway is hypothesized to be important during the process of AML cell apoptosis induced by citrate.

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References

- Gupta K, Chakrabarti A, Rana S, Ramdeo R, Roth BL, Agarwal ML, Tse W, Agarwal MK and Wald DN: Securinine, a myeloid differentiation agent with therapeutic potential for AML. PloS One 6: e21203, 2011.
- 2. Cairns RA, Harris IS and Mak TW: Regulation of cancer cell metabolism. Nat Rev Cancer 11: 85-95, 2011.
- Garber K: Energy deregulation: licensing tumors to grow. Science 312: 1158-1159, 2006.
- 4. Lu Y, Zhang X, Zhang H, Lan J, Huang G, Varin E, Lincet H, Poulain L and Icard P: Citrate induces apoptotic cell death: a promising way to treat gastric carcinoma? Anticancer Res 31: 797-805, 2011.
- Agarwal NK, Mueller GA, Mueller C, Streich JH, Asif AR and Dihazi H: Expression proteomics of acute promyelocytic leukaemia cells treated with methotrexate. Biochim Biophys Acta 1804: 918-928, 2010.
- Morshed SR, Tokunaga T, Otsuki S, Takayama F, Satoh T, Hashimoto K, Yasui T, Okamura M, Shimada J, Kashimata M and Sakagami H: Effect of antitumor agents on cytotoxicity induction by sodium fluoride. Anticancer Res 23: 4729-4736, 2003.
- 7. Verburg M, Renes IB, Einerhand AW, Büller HA and Dekker J: Isolation-stress increases small intestinal sensitivity to chemotherapy in rats. Gastroenterology 124: 660-671, 2003.
- 8. Bayley JP and Devilee P: Warburg tumours and the mechanisms of mitochondrial tumour suppressor genes. Barking up the right tree? Curr Opin Genet Dev 20: 324-329, 2010.
- Kruspig B, Nilchian A, Orrenius S, Zhivotovsky B and Gogvadze V: Citrate kills tumor cells through activation of apical caspases. Cell Mol Life Sci 69: 4229-4237, 2012.
- Zhang X, Varin E, Allouche S, Lu Y, Poulain L and Icard P: Effect of citrate on malignant pleural mesothelioma cells: a synergistic effect with cisplatin. Anticancer Res 29: 1249-1254, 2009.
- Cairns RA, Papandreou I, Sutphin PD and Denko NC: Metabolic targeting of hypoxia and HIF1 in solid tumors can enhance cytotoxic chemotherapy. Proc Natl Acad Sci USA 104: 9445-9450, 2007.
- 12. Fillies T, Werkmeister R, van Diest PJ, Brandt B, Joos U and Buerger H: HIF1-alpha overexpression indicates a good prognosis in early stage squamous cell carcinomas of the oral floor. BMC Cancer 5: 84, 2005.
- Denko NC: Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer 8: 705-713, 2008.

- Doe MR, Ascano JM, Kaur M and Cole MD: Myc posttranscriptionally induces HIF1 protein and target gene expression in normal and cancer cells. Cancer Res 72: 949-957, 2012.
- Bohuslavová R, Kolář F, Kuthanová L, Neckář J, Tichopád A and Pavlinkova G: Gene expression profiling of sex differences in HIF1-dependent adaptive cardiac responses to chronic hypoxia. J Appl Physiol 109: 1195-1202, 2010.
 Sitkovsky M and Lukashev D: Regulation of immune cells by
- Sitkovsky M and Lukashev D: Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. Nat Rev Immunol 5: 712-721, 2005.
- 17. Kumar A, Kant S and Singh SM: Novel molecular mechanisms of antitumor action of dichloroacetate against T cell lymphoma: Implication of altered glucose metabolism, pH homeostasis and cell survival regulation. Chem Biol Interact 199: 29-37, 2012.
- 18. Priebe A, Tan L, Wahl H, Kueck A, He G, Kwok R, Opipari A and Liu JR: Glucose deprivation activates AMPK and induces cell death through modulation of Akt in ovarian cancer cells. Gynecol Oncol 122: 389-395, 2011.
- Sklar P, Ripke S, Scott LJ, Andreassen OA, Cichon S, Craddock N, Edenberg HJ, Nurnberger JI Jr, Rietschel M, Blackwood D, *et al*: Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet 43: 977-983, 2011.
- 20. Simon F, Bockhorn M, Praha C, Baba HA, Broelsch CE, Frilling A and Weber F: Deregulation of HIF1-alpha and hypoxia-regulated pathways in hepatocellular carcinoma and corresponding non-malignant liver tissue - influence of a modulated host stroma on the prognosis of HCC. Langenbecks Arch Surg 395: 395-405, 2010.
- Yang W, Xia Y, Ji H, Zheng Y, Liang J, Huang W, Gao X, Aldape K and Lu Z: Nuclear PKM2 regulates β-catenin transactivation upon EGFR activation. Nature 480: 118-122, 2011.
- Luo W and Semenza GL: Pyruvate kinase M2 regulates glucose metabolism by functioning as a coactivator for hypoxia-inducible factor 1 in cancer cells. Oncotarget 2: 551-556, 2011.
 Dang CV, Hamaker M, Sun P, Le A and Gao P: Therapeutic
- Dang CV, Hamaker M, Sun P, Le A and Gao P: Therapeutic targeting of cancer cell metabolism. J Mol Med (Berl) 89: 205-212, 2011.
- 24. Mazurek S: Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. Int J Biochem Cell Biol 43: 969-980, 2011.
- 25. Pan JG and Mak TW: Metabolic targeting as an anticancer strategy: dawn of a new era? Sci STKE 2007: pe14, 2007.

- 26. Hsu PP and Sabatini DM: Cancer cell metabolism: Warburg and beyond. Cell 134: 703-707, 2008.
- 27. Vander Heiden MG, Cantley LC and Thompson CB: Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324: 1029-1033, 2009.
- Koppenol WH, Bounds PL and Dang CV: Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer 11: 325-337, 2011.
- Maxwell RE: Calibration of Warburg Manometers. Science 110: 403-404, 1949.
- 30. Tennant DA: PK-M2 Makes Cells Sweeter on HIF1. Cell 145: 647-649, 2011.
- 31. Wittwer JA, Robbins D, Wang F, Codarin S, Shen X, Kevil CG, Huang TT, Van Remmen H, Richardson A and Zhao Y: Enhancing mitochondrial respiration suppresses tumor promoter TPA-induced PKM2 expression and cell transformation in skin epidermal JB6 cells. Cancer Prev Res (Phila) 4: 1476-1484, 2011.
- 32. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, Fleming MD, Schreiber SL and Cantley LC: The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 452: 230-233, 2008.
- 33. Koukourakis MI, Giatromanolaki A, Chong W, Simopoulos C, Polychronidis A, Sivridis E and Harris AL: Amifostine induces anaerobic metabolism and hypoxia-inducible factor 1 alpha. Cancer Chemother Pharmacol 53: 8-14, 2004.
- 34. Chiavarina B, Whitaker-Menezes D, Migneco G, Martinez-Outschoorn UE, Pavlides S, Howell A, Tanowitz HB, Casimiro MC, Wang C, Pestell RG, et al: HIF1-alpha functions as a tumor promoter in cancer associated fibroblasts, and as a tumor suppressor in breast cancer cells: Autophagy drives compartment-specific oncogenesis. Cell Cycle 9: 3534-3551, 2010.
- 35. Zhang X, Varin E, Briand M, Allouche S, Heutte N, Schwartz L, Poulain L and Icard P: Novel therapy for malignant pleural mesothelioma based on anti-energetic effect: an experimental study using 3-Bromopyruvate on nude mice. Anticancer Res 29: 1443-1448, 2009.
- 36. Bayet-Robert M, Lim S, Barthomeuf C and Morvan D: Biochemical disorders induced by cytotoxic marine natural products in breast cancer cells as revealed by proton NMR spectroscopy-based metabolomics. Biochem Pharmacol 80: 1170-1179, 2010.
- 37. Boros LG, Lee WN and Go VL: A metabolic hypothesis of cell growth and death in pancreatic cancer. Pancreas 24: 26-33, 2002.