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Up-regulation of hexokinaseII in myeloma cells: targeting myeloma cells with 3-bromopyruvate

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Abstracts Hexokinase II (HKII), a key enzyme of glycolysis, is widely over-expressed in cancer cells. However, HKII levels and its roles in ATP production and ATP-dependent cellular process have not been well studied in hematopoietic malignant cells including multiple myeloma (MM) cells. We demonstrate herein that HKII is constitutively over-expressed in MM cells. 3-bromopyruvate (3BrPA), an inhibitor of HKII, promptly and substantially suppresses ATP production and induces cell death in MM cells. Interestingly, cocultures with osteoclasts (OCs) but not bone marrow stromal cells (BMSCs) enhanced the phosphorylation of Akt along with an increase in HKII levels and lactate production in MM cells. The enhancement of HKII levels and lactate production in MM cells by OCs were mostly abrogated by the PI3K inhibitor LY294002, suggesting activation of glycolysis in MM cells by OCs via the PI3K-Akt-HKII pathway. Although BMSCs and OCs stimulate MM cell growth and survival, 3BrPA induces cell death in MM cells even in cocultures with OCs as well as BMSCs. Furthermore, 3BrPA was able to diminish ATP-dependent ABC transporter activity to restore drug retention in MM cells in the presence of OCs. These results may underpin possible clinical application of 3BrPA in patients with MM.

Keywords Multiple myeloma · Gycolysis · Hexokinase II · 3-bromopyruvate · Osteoclast

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Introduction

Multiple myeloma (MM) eventually relapses and still remains essentially incurable for the vast majority of patients, although the recent implementation of novel agents has significantly improved the response rates and survival outcome. The development of novel therapeutic modalities is therefore urgently wanted. MM cells preferentially reside and grow within the bone marrow microenvironment (Abe 2011; Mahindra et al. 2010; Roodman 2010; Yaccoby 2010), and potently induce osteoclastogenesis (Abe et al. 2002; Choi et al. 2000) and suppress osteoblastogenesis (Ehrlich et al. 2005; Giuliani et al. 2005; Oshima et al. 2005; Standal et al. 2007; Takeuchi et al. 2010; Tian et al. 2003) to cause bone destruction. We and others reported that MM cells and osteoclasts (OCs) mutually interact in MM bone lesions to confer aggressiveness and drug resistance in MM cells while progressing bone destruction (Abe et al. 2004, 2006; Ge et al. 2006; Yaccoby et al. 2008, 2004)

The emergence of drug-resistant clones during the course of treatment and the presence of cancer stem cells or cancer-initiating cells are among predominant causes of drug resistance in cancers (Cheng et al. 2011; Dean 2009). Such drug-resistant cells often up-regulate their expression of ATP-binding cassette (ABC) transporters, which function as ATP-dependent efflux pumps for a variety of chemotherapeutic drugs and become a

major target of the treatment in cancers (de Figueiredo-Pontes et al. 2008; Dean 2009; Fletcher et al. 2010; Szakacs et al. 2006). In malignant cells ATP production is managed largely through glycolysis under normal oxygen concentrations (aerobic glycolysis; the Warburg effect) in contrast to that in normal cells which utilize a tricarboxylic acid (TCA) cycle in mitochondria (Cairns et al. 2011; Lunt and Vander Heiden 2011; Mathupala et al. 2009; Pedersen 2007b). We have recently reported that inhibition of glycolysis effectively deplete ATP in MM or leukemic cells, and thereby inactivate ATP-dependent ABC transporters to restore drug sensitivity while impairing clonogenic side population (SP) cells with enhanced glycolysis (Nakano et al. 2011). Therefore, enhanced glycolysis appears to become an effective cancer-specific target.

Hexokinases (HKs) catalyze the first irreversible step of glycolysis, and play a critical role in the regulation of glycolytic activity (Lunt and Vander Heiden 2011; Mathupala et al. 2006, 2009; Pedersen 2007b). HKs have four isoforms; and among them, HKII is predominantly overexpressed in a wide variety of malignant cells (Mathupala et al. 2006, 1995; Shinohara et al. 1991; Wolf et al. 2011). HKII interacts with the voltage dependent anion channel (VDAC) in the outer membrane of mitochondria, and converts glucose to glucose-6-phosphate (Mathupala et al. 2006, 2009; Pedersen 2007b). The PI3K-Akt signaling facilitates the association of HKII with VDAC on a mitochondrial membrane, leading to an increase in stabilized HKII levels to drive glycolysis (Gottlob et al. 2001; Mathupala et al. 2006; Stiles 2009; Wolf et al. 2011). In addition, HKII occupies the binding site of pro-apoptotic proteins, Bax and Bad, on VDAC to prevent the release of cytochrome *c* from mitochondria and subsequent activation of apoptotic machinery (Mathupala et al. 2006, 2009). However, the regulation of HKII levels and their roles in ATP production and ATP-dependent cellular process have not been well studied in hematopoietic malignant cells, including MM cells. We demonstrate herein that HKII is constitutively over-expressed in MM cells, and further up-regulated by the interaction with OCs, and that 3-bromopyruvate (3BrPA), an inhibitor of HKII (Kim et al. 2007; Ko et al. 2001; Pedersen 2007a, b), promptly and substantially suppresses ATP production and induces cell death in MM cells even in cocultures with bone marrow stromal cells (BMSCs) and OCs. These results may underpin possible clinical application of 3BrPA in patients with MM.

Materials and methods

Reagents

The following reagents were purchased as indicated: 3BrPA, 2-deoxyglucose (2DG), and mouse monoclonal anti-β-actin antibody from Sigma (St. Louis, MO); LY294002 from Calbiochem (Darmstadt, Germany); goat polyclonal anti-human HKII antibody and normal goat IgG from Santa Cruz Biotechnology (Santa Cruz, CA); horseradish peroxidase (HRP)-rabbit anti-goat IgG from Zymed Laboratories (San Francisco, CA); rabbit polyclonal antibodies against, STAT3, pSTAT3, Akt, Erk, pErk and apoptosis-inducing factor (AIF), rabbit monoclonal antibody against pAkt, HRP-conjugated horse anti-mouse IgG, and HRP-conjugated goat anti-rabbit IgG from Cell signaling technology (Danvers, MA).

Cells and cultures

Human MM cell lines, RPMI8226 and U266, and human leukemic cell lines, KG1, HL-60 and U937, were obtained from American Type Culture Collection (ATCC) (Rockville, MD). INA6, MM.1S and KMS-12BM MM cell lines were kindly provided by Dr. Renate Burger (University of Kiel, Kiel, Germany), Dr. Steven Rosen (Northwestern University, Chicago, IL) and Dr. Otsuki (Kawasaki Medical University, Okayama, Japan), respectively. TSPC-1 and OPC MM cell lines were established in our laboratory (Abe et al. 2002). Bone marrow mononuclear cells (BMMCs) were isolated from fresh bone marrow aspirates of patients with MM and primary $CD138^+$ MM cells were further sorted using CD138 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously (Oshima et al. 2005). Peripheral blood mononuclear cells (PBMCs) were isolated from fresh peripheral blood from healthy donors (Abe et al. 2004). The cells were cultured in RPMI1640 medium (Sigma) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin G (Sigma), and 100 μg/ml of streptomycin (Sigma). INA-6 cells were cultured in the presence of 1 ng/ml of rhIL-6 (PEPROTECH EC, London, UK). Primary bone marrow stromal cells (BMSCs) derived from fresh BM aspirates from patients with MM were isolated as previously described (Asano et al. 2011). OCs were generated from PBMCs as previously reported (Abe et al. 2004). BMSCs and OCs cells were cultured in αMEM supplemented with 10% FBS, 2 mM L-Glutamine (Sigma), 100 U/ml of penicillin G and 100 μg/ml of streptomycin. All procedures involving human specimens were performed with written informed consent according to the Declaration of Helsinki and using a protocol approved by the Institutional Review Board for human protection in University of Tokushima.

Cell viability assays

Viable cells were counted by the Cell Counting Kit-8 assay (DOJINDO, Kumamoto, Japan) according to the manufacturer's instructions. The absorbance of each well was measured at 450 nm with a microplate reader (Model 450 micro plate reader; Bio-Rad Laboratories, Hercules, CA). Dead cells were determined after staining with propidium iodide

(PI) using Coulter Epics XL-MCL (Beckman & Coulter) and analyzed by CellQuest software (BD Bioscience).

Intracellular ATP measurements

Cells were plated in 96-well culture plates. Cellular ATP levels were determined using the CellTiter-Glo luminescent Cell Viability Assay (Promega, Madison, USA) according to the manufacturer's instructions. Luminescent levels were measured by a microplate reader (Thermo Fisher Varioskan Flash; Waltham, MA).

Immunohistochemistry

Bone marrow clot sections from MM patients were fixed in neutral-buffered formalin and embedded in paraffin. Paraffin-embedded tissue sections were deparaffinized and hydrated. For antigen retrieval the sections were microwaved in 10 mM sodium citrate buffer (pH 6.6). After a 10-minute blocking process, the sections were incubated with goat polyclonal anti-human HKII antibody or control goat IgG overnight at 4 °C. After washing, immunoreactivity was detected by biotinylated secondary antibodies and horseradish peroxidase-conjugated streptavidin (DAKO LSAB + System, HRP; DAKO, Carpinteria, CA) followed by diaminobenzidine substrate (DAKO) according to the manufacturer's instructions.

Drug accumulation and efflux assays

After exposing to 100 ng/ml of mitoxantrone for 30 min in the presence or absence of OCs, cells were harvested, and their intracellular levels of fluorescence emitted from mitoxantrone were analyzed by flow cytometry (accumulation phase: AP). For determining drug efflux activity, the cells were further incubated without mitoxantrone for 120 min in the coculture with OCs, and intracellular fluorescence levels were analyzed (efflux phase: EP). Intracellular drug levels after AP and EP were represented as mean fluorescence intensity (MFI) after subtracting that of background (ΔMFI_{AP} and ΔMFI_{EP}, respectively).

Lactate measurements

Culture media were filtered to remove protein, and their a lactate levels were measured using a Lactate Assay Kit (BioVision, Mountain View, CA).

Immunoblotting

Cells were lysed in lysis buffer (Cell Signaling) supplemented with 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail solution (Sigma). Membrane fractions were extracted using a Mem-PER Eukaryotic Membrane Protein Extraction Kit (Thermo Scientific, Rockford, IL). The lysates were subjected to immunoblot analysis as described previously (Oshima et al. 2005).

Quantitative real-time PCR

RNA isolation and quantitative real-time PCR were performed as described previously (Hiasa et al. 2009). The primers used were as follows: h*HKII* sense 5'-TGGAGGGACCAACTTCCGTGTGCT-3' and antisense 5'-TCAAACAGCTGGGTGCCACTGC-3', h*HPRT1*, used as a housekeeping gene for quantity normalization, sense 5'-TTTGCTTTCCTTGGTCAGGC-3', and antisense 5'-GCTTGCGACCTTGACCATCT-3'.

Statistical analysis

Statistical significance was determined by a one-way analysis of variance (ANOVA) with Scheffe's post hoc tests or Student's *t*-test. The minimal level of significance was $P=0.05$.

Results and discussion

3BrPA reduces HKII and ATP levels in MM cells to cause cell death

Although HKII is regarded as a cancer-specific HK isoform in various solid cancers (Mathupala et al. 2006, 2009; Pedersen 2007b; Wolf et al. 2011), its expression has not been well demonstrated in hematopoietic malignant cells, especially in MM cells. Therefore, we first looked at the levels of HKII in MM cells. Most MM and leukemic cell lines constitutively over-expressed HKII at a protein level, while normal PBMCs did not (Fig. 1a). The immunohistochemical study on bone marrow aspirates revealed hHKII expression in primary MM cells in all the eight cases (Supplementary Fig. 1). ATP production was also increased in MM cell lines and primary MM cells compared to that in normal PBMCs (Fig. 1b).

3BrPA, a lactate analog, is able to enter cells through lactate transporters overexpressed in malignant cells (Pedersen 2007a, b). When it is incorporated in malignant cells, it dissociates HKII from a mitochondrial membrane to be inactivated, and thus act as an HKII inhibitor (Chen et al. 2009; Kim et al. 2007). Indeed, HKII in membrane fractions had mostly disappeared in MM cells after the treatment with 3BrPA (Fig. 2a), suggesting the dissociation of membrane-bound HKII by 3BrPA to suppress the HK-mediated glycolytic process in MM cells. We next examined the impact of 3BrPA on ATP production and the viability on MM cells. 3BrPA promptly and substantially suppressed ATP production in

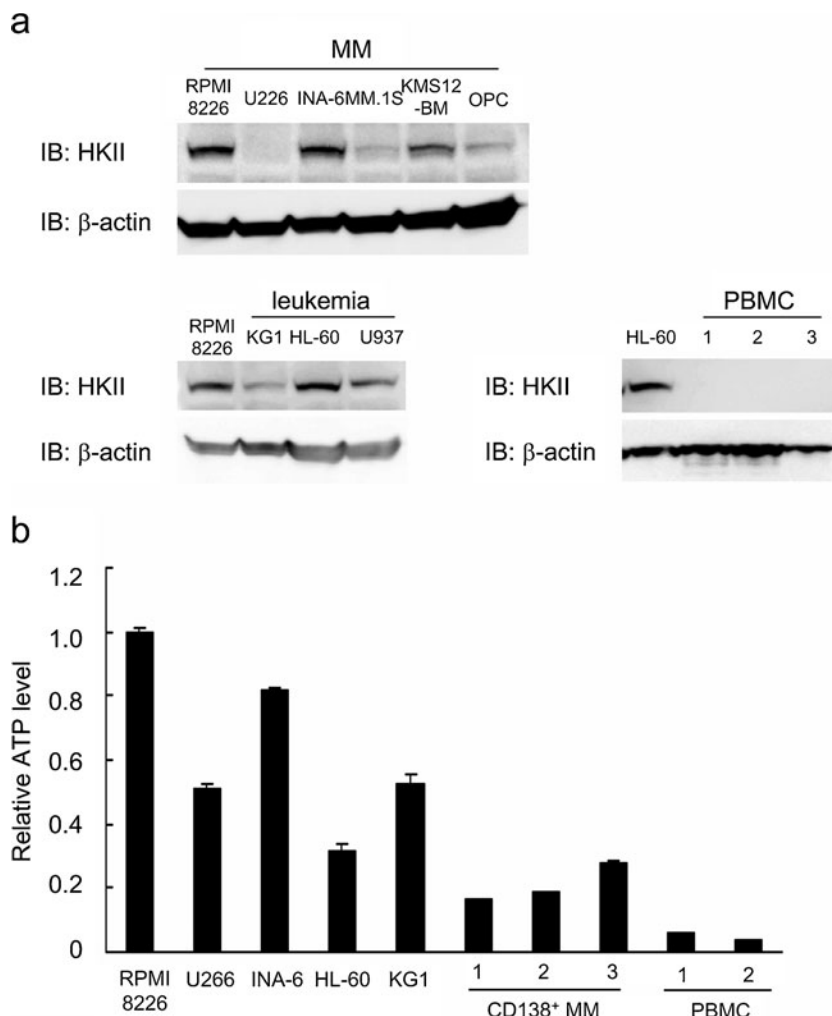

Fig. 1 HKII expression and ATP production in MM cells. **a** HKII protein expression was examined by Western blot analysis in MM and leukemia cell lines as well as normal PBMCs. β-actin was blotted as a loading control. **b** Cellular ATP levels were measured in MM and leukemia cell lines, primary CD138+ MM cells from patients with MM, and PBMCs. The results were expressed as relative levels of ATP per cell compared to ATP levels in RPMI8226 cells (mean +/− SD)

RPMI8226 cells (Fig. 2b). Furthermore, 3BrPA dose-dependently induced cell death in MM and leukemic cell lines (Fig. 2c). All MM cell lines tested except U266 cells underwent almost complete cell death in the presence of 3BrPA at 30 μM. Similarly, 2-deoxyglucose (2DG), an analog of glucose (Zhang et al. 2006), suppressed ATP production, and induced cell death in MM cells (Fig. 2b and c). 3BrPA also induced cell death in leukemic cell lines, KG1, HL60 and U937 (Fig. 2c) although these leukemic cell lines appeared to be less susceptible to 3BrPA than the MM cell lines. However, 3BrPA appeared to more potently and effectively deplete ATP, and induce cell death in these malignant cells than 2DG. These results collectively suggest that HKII plays a critical role in ATP production and survival in MM cells, and that 3BrPA is able to effectively induce cell death in MM cells.

OCs activate the PI3K-Akt-HKII pathway in MM cells

MM cells reside in the bone marrow microenvironment, and interact with bone marrow stromal cells and OCs to enhance MM cell growth and survival (Abe 2011; Abe et al. 2004; Mahindra et al. 2010; Roodman 2010; Yaccoby 2010; Yaccoby et al. 2004). When INA-6 and TSPC-1 MM cells, were cocultured with BMSCs or OCs, HKII levels in MM cells was increased by OCs but not by BMSCs (Fig. 3a). The increase in HKII levels was also observed in RPMI8226, MM.1S, U266 and OPC MM cells when cocultured with OCs (Fig. 3b).

Although BMSCs enhance MM cell growth and survival largely through their elaborating IL-6 (Abe 2011; Mahindra et al. 2010; Roodman 2010; Yaccoby 2010), we and others previously demonstrated that OCs are able to support the growth and survival of IL-6-dependent MM cell lines, which is mostly inhibited by the prevention of cell-cell contact (Abe et al. 2004; Yaccoby et al. 2004). Consistent with these observations, the cocultures with BMSCs markedly enhanced the phosphorylation of STAT3 and Erk in INA-6 and TSPC-1 cells while OCs only slightly enhanced the phosphorylation of STAT3 but not that of Erk (Fig. 3c). In contrast, the cocultures with OCs but not with BMSCs enhanced the phosphorylation of Akt along with an increase in HKII levels in MM cells (Fig. 3c). The PI3K inhibitor

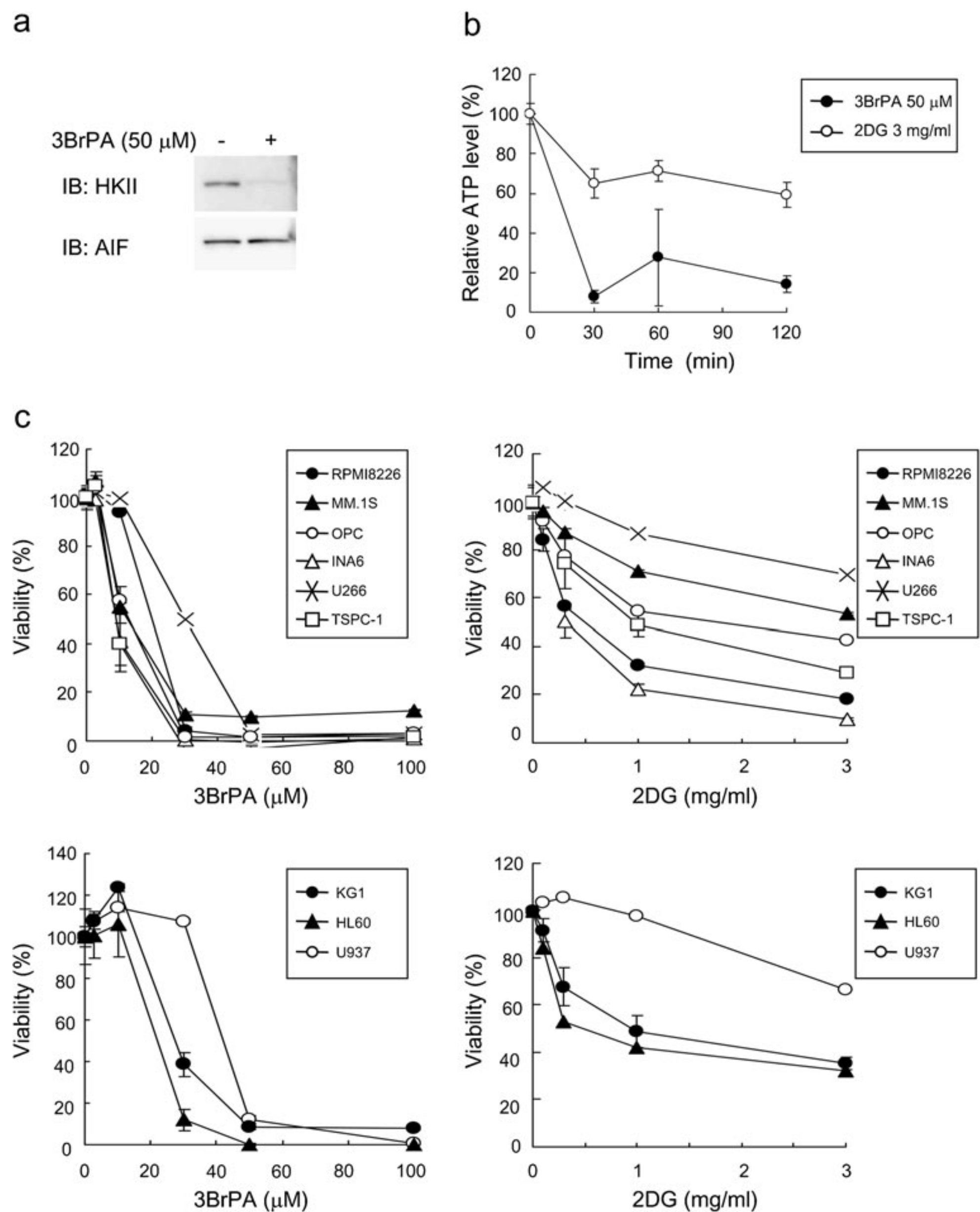

Fig. 2 Effects of 3BrPA on HKII levels, ATP production and viability in MM cells. **a** A membrane fraction was harvested after incubating with or without 3BrPA at 50 μM for 4 h in RPMI8226 cells. A membrane-bound HKII level was examined by Western blot analysis. AIF was used as internal control of the membrane fraction. **b** Time course of ATP reduction by 3BrPA and 2DG. RPMI8226 cells were treated with 3BrPA or 2DG, and cellular ATP levels were measured over time. The results were expressed as percentage of ATP levels per cell relative to non-treatment controls (mean +/− SD). **c** Cytotoxic effect of 3BrPA and 2DG. MM and leukemia cell lines were incubated for 24 h with 3BrPA or 2DG at the concentrations as indicated, and their viability was determined by a WST-8 assay (mean +/− SD)

LY294002 mostly abrogated the up-regulation of HKII levels in MM cells upon coculturing with OCs (Fig. 3d). Furthermore, lactate production by these MM cells was enhanced in the cocultures with OCs, which was diminished by the treatment with LY294002 (Fig. 3e). The PI3K-Akt signaling has been demonstrated to phosphorylate HKII to facilitate its association with a mitochondrial membrane, and thereby stabilize HKII to post-translationally increase intracellular HKII levels (Gottlob et al. 2001; Mathupala et al. 2006; Stiles 2009; Wolf et al. 2011). Consistently, *HKII* mRNA levels were not significantly changed in these MM cells by LY294002 in the presence or absence of OCs (Fig. 3f). Thus, the activation of PI3K-Akt signaling by OCs enhances HKII protein levels in MM cells without significantly affecting *HKII* mRNA expression.

3BrPA induces MM cell death and inactivates ABC transporters in MM cells in the presence of OCs

BMSCs or OCs stimulate MM cell growth and survival in the bone marrow (Abe 2011; Abe et al. 2004; Mahindra et al. 2010; Roodman 2010; Yaccoby 2010; Yaccoby et al. 2004). We next examined the effects of 3BrPA on the viability of MM cells in cocultures with BMSCs or OCs. As shown in Fig. 4a, the cocultures with BMSCs or OCs reduced spontaneous cell death in INA-6 and TSPC-1 cells. However, the treatment with 3BrPA at 30 μM showed potent induction of their death in the presence of OCs as well as BMSCs to the levels similar to those in these MM cells cultured alone.

We recently reported that inhibition of glycolysis by 3BrPA is able to effectively deplete intracellular ATP levels,

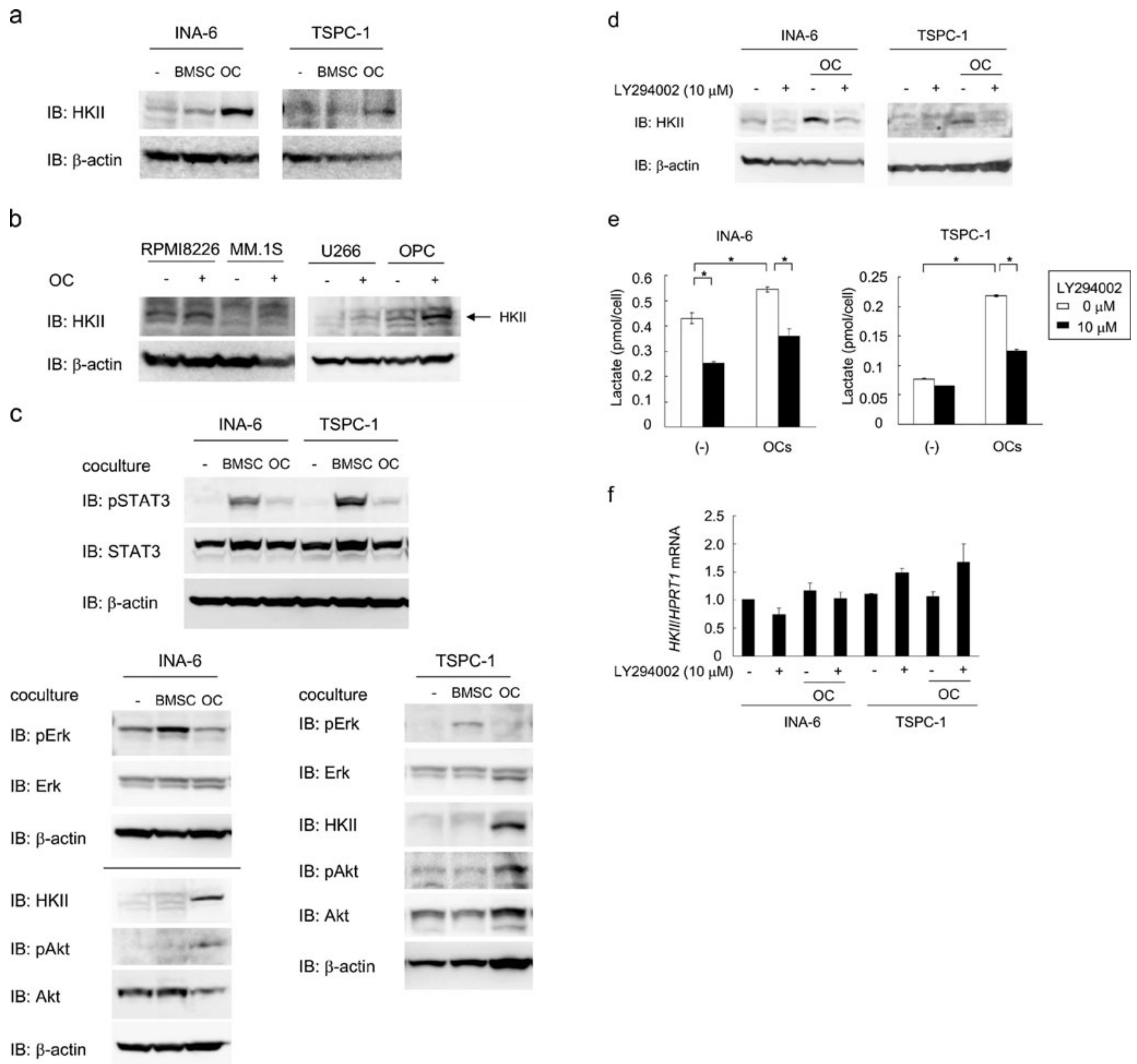

Fig. 3 HKII and lactate levels in MM cells in cocultures with BMSCs and OCs. **a** HKII protein expression was examined by Western blot analysis in INA-6 and TSPC-1 cells after coculturing with BMSCs or OCs for 2 days. **b** HKII protein expression was examined by Western blot analysis in RPMI8226, MM.1S, U266 and OPC cells after coculturing with OCs for 2 days. **c** The phospohrylation of STAT3, Erk and Akt and the expression of HKII were examined by Western blot analysis in INA-6 and TSPC-1 cells after coculturing with BMSCs or OCs for 2 days. **d** INA-6 and TSPC-1 cells were pretreated with 10 μM of LY294002 for 30 min, followed by culturing alone or coculturing with OCs for 24 h as indicated. HKII protein expression was examined by Western blot analysis. -actin was blotted as a loading control. **e** INA-6 and TSPC-1 cells were pretreated with 10 μM of LY294002 for 30 min, followed by culturing alone or coculturing with OCs for 24 h. Then, INA-6 and TSPC-1 cells were washed, and cultured alone for 40 and 120 min, respectively, for the measurement of lactate production. Lactate levels of their culture media were measured (mean +/− SD). *, $p<0.05$. **f** INA-6 and TSPC-1 cells were pretreated with 10 μM of LY294002 for 30 min, followed by culturing alone or coculturing with OCs for 24 h as indicated. h*HKII* mRNA level was determined by real time PCR. *hHPRT1* was used as internal control

and thereby suppress ABC transporter activity and restore drug sensitivity in malignant cells in vitro and in vivo (Nakano et al. 2011). Because OCs up-regulate HKII levels and stimulate glycolytic activity in MM cells, we next examined whether 3BrPA is able to inactivate ABC transporters in MM cells in cocultures with OCs. RPMI8226 cells constitutively over-express breast cancer resistant proteins (BCRP; ABCG2) as previously demonstrated (Nakano et al. 2011) and the activity of BCRP was determined by measuring the intracellular retention of

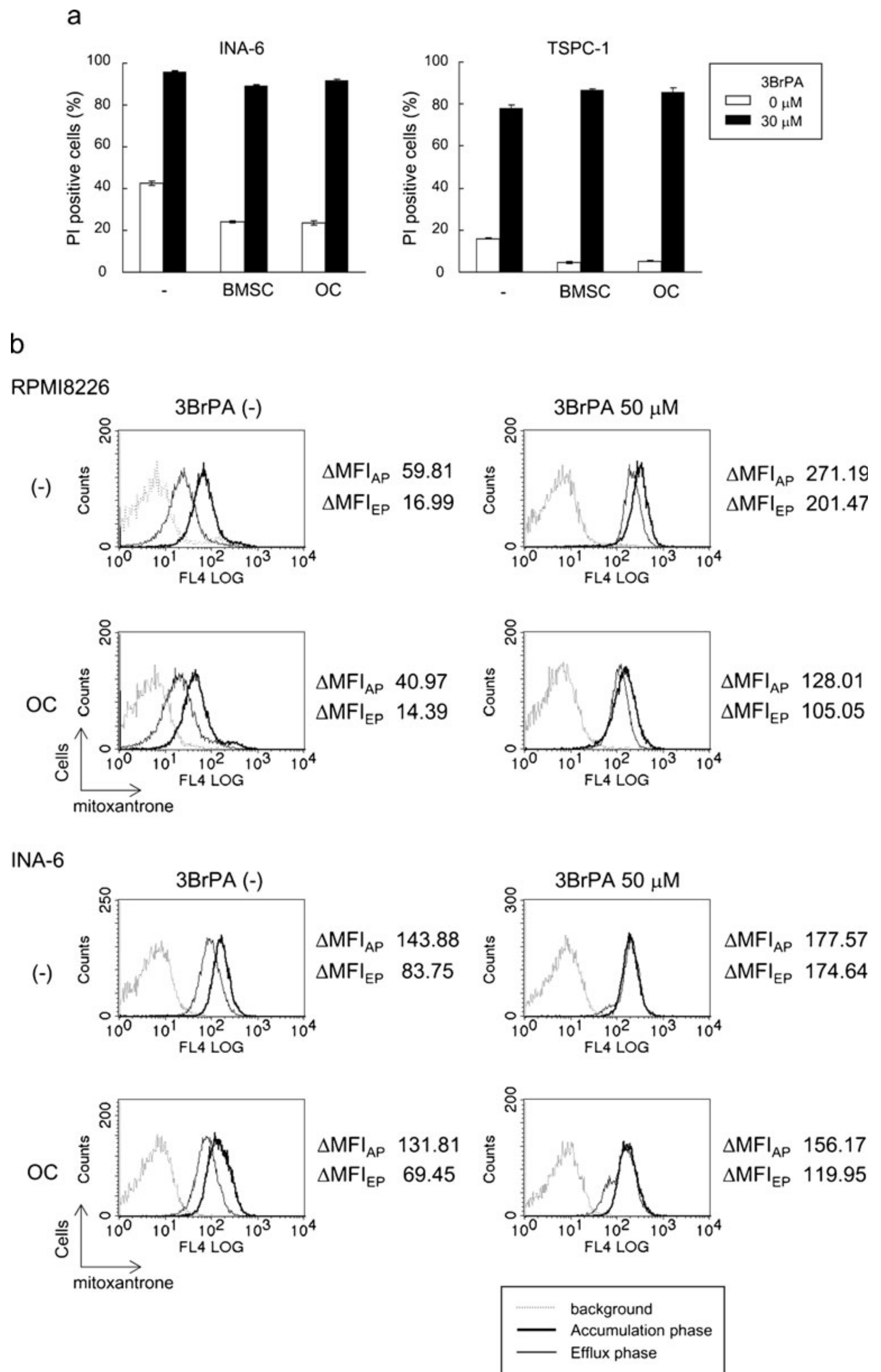

Fig. 4 Effects of 3BrPA on viability and drug retention in MM cells. **a** INA-6 and TSPC-1 cells were cultured alone or cocultured with BMSCs or OCs for 24 h in the presence or absence of 3BrPA. Dead cells were stained with 1 μg/ml of PI and analyzed by flow cytometry. The results were expressed as a mean +/− SD. **b** BCRP-expressing RPMI8226 and INA-6 cells were cultured alone or cocultured with OCs for 24 h, followed by incubation with mitoxantrone at 100 ng/ml for 30 min (accumulation phase: AP) in the presence or absence of 3BrPA at 50 μM. After AP, intracellular mitoxantrone levels in RPMI8226 and INA-6 cells were analyzed by flow cytometry (bold line). To determine their activity of expelling the drug, the cells were then washed, and were further cultured alone or cocultured with OCs in media without mitoxantrone for 120 min (efflux phase: EP) in the presence or absence of 3BrPA at 50 μM. After EP, their intracellular mitoxantrone levels were analyzed by flow cytometry (fine line). Background staining is shown in a broken line

mitoxantrone, a substrate of BCRP. The treatment with 3BrPA markedly restored the drug retention in RPMI8226 and INA-6 cells cultured alone after incubation for expelling the drug (Fig. 4b). When they were cocultured with OCs, their intracellular drug levels were slightly reduced after its passive incorporation and after the incubation for expelling the drug, as shown as bold and fine lines, respectively (Fig. 4b). Because OCs did not increase the surface levels of BCRP on RPMI8226 cells (data not shown), OCs are suggested to enhance the efflux function of BCRP in MM

cells. Treatment with 3BrPA restored the drug retention in RPMI8226 and INA-6 cells cocultured with OCs, although the recovery of their intracellular drug levels was somewhat blunted in the cocultures with OCs. These results suggest that inhibition of HKII by 3BrPA is able to induce MM cell death, and resume drug sensitivity in MM bone lesions with enhanced osteoclastogenesis.

HKII is incomparably up-regulated in malignant cells, including MM and leukemic cells, to cause robust production of ATP and a biomass in malignant cells through glycolysis for their survival and rapid proliferation (Lunt and Vander Heiden 2011; Mathupala et al. 2006, 2009; Pedersen 2007b). Malignant cells over-express lactate transporters such as monocarboxylic acid transporters while normal cells do not (Draoui and Feron 2011; Kennedy and Dewhirst 2010). Because 3BrPA preferentially gets into malignant cells through the lactate transporters, and inhibits HKII aberrantly over-expressed in malignant cells (Pedersen 2007a, b), 3BrPA can selectively target malignant cells without significantly affecting normal quiescent cells. Indeed, we reported that 3BrPA induced cell death in CD138-positive MM cells but not in normal hematopoietic cells in bone marrow samples from patients with MM (Nakano et al. 2011). In addition, the treatment with 3BrPA is suggested to incapacitate ABC transporters widely over-expressed in drug-resistant clones, including "side population" cells. Taken together, the present study suggests that the anti-MM effect of 3BrPA can be exerted even in the bone marrow microenvironment, although the interaction with OCs stimulates HKII activity and glycolysis in MM cells. Therefore, the treatment with 3BrPA seems attractive in patients with drug-resistant malignant tumors residing in the bone marrow microenvironment, including MM. The present study warrants clinical trials with 3BrPA for patients with MM to further validate the therapeutic impact of this agent with a unique mode of action.

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Conflicts of interest disclosures The authors declare no competing financial interests related to this work.

References

Abe M (2011) Int J Hematol 94(4):334–343

Abe M, Hiura K, Wilde J, Moriyama K, Hashimoto T, Ozaki S et al (2002) Blood 100(6):2195–2202

Abe M, Hiura K, Wilde J, Shioyasono A, Moriyama K, Hashimoto T et al (2004) Blood 104(8):2484–2491

Abe M, Kido S, Hiasa M, Nakano A, Oda A, Amou H et al (2006) Leukemia 20(7):1313–1315

Asano J, Nakano A, Oda A, Amou H, Hiasa M, Takeuchi K et al (2011) Leukemia 25(7):1182–1188

Cairns RA, Harris IS, Mak TW (2011) Nat Rev Cancer 11(2):85–95

Chen Z, Zhang H, Lu W, Huang P (2009) Biochim Biophys Acta 1787 (5):553–560

Cheng L, Alexander R, Zhang S, Pan CX, MacLennan GT, Lopez-Beltran A et al (2011) Expert Rev Anticancer Ther 11(7):1131–1143

Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD et al (2000) Blood 96(2):671–675

de Figueiredo-Pontes LL, Pintao MC, Oliveira LC, Dalmazzo LF, Jacomo RH, Garcia AB et al (2008) Cytometry B Clin Cytom 74(3):163–168

Dean M (2009) J Mammary Gland Biol Neoplasia 14(1):3–9

Draoui N, Feron O (2011) Dis Model Mech 4(6):727–732

Ehrlich LA, Chung HY, Ghobrial I, Choi SJ, Morandi F, Colla S et al (2005) Blood 106(4):1407–1414

Fletcher JI, Haber M, Henderson MJ, Norris MD (2010) Nat Rev Cancer 10(2):147–156

Ge Y, Zhan F, Barlogie B, Epstein J, Shaughnessy J Jr, Yaccoby S (2006) Br J Haematol 133(1):83–92

Giuliani N, Colla S, Morandi F, Lazzaretti M, Sala R, Bonomini S et al (2005) Blood 106(7):2472–2483

Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N (2001) Genes Dev 15(11):1406–1418

Hiasa M, Abe M, Nakano A, Oda A, Amou H, Kido S et al (2009) Blood 114(20):4517–4526

Kennedy KM, Dewhirst MW (2010) Future Oncol 6(1):127–148

Kim W, Yoon JH, Jeong JM, Cheon GJ, Lee TS, Yang JI et al (2007) Mol Cancer Ther 6(9):2554–2562

Ko YH, Pedersen PL, Geschwind JF (2001) Cancer Lett 173(1):83–91

Lunt SY, Vander Heiden MG (2011) Annu Rev Cell Dev Biol 27:441–464

Mahindra A, Hideshima T, Anderson KC (2010) Blood Rev 24(Suppl 1):S5–S11

Mathupala SP, Rempel A, Pedersen PL (1995) J Biol Chem 270 (28):16918–16925

Mathupala SP, Ko YH, Pedersen PL (2006) Oncogene 25(34):4777–4786

Mathupala SP, Ko YH, Pedersen PL (2009) Semin Canc Biol 19 (1):17–24

Nakano A, Tsuji D, Miki H, Cui Q, Sayed SM, Ikegame A et al (2011) PLoS One 6(11):e27222

Oshima T, Abe M, Asano J, Hara T, Kitazoe K, Sekimoto E et al (2005) Blood 106(9):3160–3165

Pedersen PL (2007a) J Bioenerg Biomembr 39(1):1–12

Pedersen PL (2007b) J Bioenerg Biomembr 39(3):211–222

Roodman GD (2010) J Bone Miner Metab 28(3):244–250

Shinohara Y, Ichihara J, Terada H (1991) FEBS Lett 291(1):55–57

Standal T, Abildgaard N, Fagerli UM, Stordal B, Hjertner O, Borset M et al (2007) Blood 109(7):3024–3030

Stiles BL (2009) Adv Drug Deliv Rev 61(14):1276–1282

Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM (2006) Nat Rev Drug Discov 5(3):219–234

Takeuchi K, Abe M, Hiasa M, Oda A, Amou H, Kido S et al (2010) PLoS One 5(3):e9870

Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B et al (2003) N Engl J Med 349(26):2483–2494

Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, Cairns R et al (2011) J Exp Med 208(2):313–326

Yaccoby S (2010) Br J Haematol 149(3):311–321

Yaccoby S, Wezeman MJ, Henderson A, Cottler-Fox M, Yi Q, Barlogie B et al (2004) Cancer Res 64(6):2016–2023

Yaccoby S, Pennisi A, Li X, Dillon SR, Zhan F, Barlogie B et al (2008) Leukemia 22(2):406–413

Zhang XD, Deslandes E, Villedieu M, Poulain L, Duval M, Gauduchon P et al (2006) Anticancer Res 26(5A):3561–3566