Life Sciences 227 (2019) 212–223

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Metastatic prostate cancer cells are highly sensitive to 3-bromopyruvic acid

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ARTICLE INFO

Keywords:
Actin cytoskeleton
ATP
3-Bromopyruvate
Cell mobility
Glutathione
Prostate cancer

ABSTRACT

Aims: 3-Bromopyruvate (3-BP), an alkylating agent and a glycolytic inhibitor, is a promising anticancer agent, which can be efficient also against multidrug-resistant cancer cells. The aim of this study was to examine how 3-BP affects the survival and mobility of rat (MAT-LyLu and AT-2) and human (DU-145 and PC-3) metastatic prostate cancer cell lines.

Main methods: Cytotoxicity was estimated with Neutral Red. Cell mobility was analyzed by time-lapse microscopic monitoring of trajectories of individual cells at 5-min intervals for 6 h. ATP was estimated with luciferin/luciferase and glutathione (GSH) with *o*-phthalaldehyde. Actin cytoskeleton was visualized with phalloidin conjugated with Atto-488.

Key findings: All metastatic prostate cell lines studied were very sensitive to 3-BP (IC_{50} of 4–26 μM). 3-Bromopyruvate drastically reduced cell movement even at concentrations of 5–10 μM after 1 h treatment. This compound depleted also cellular ATP and GSH, and disrupted actin cytoskeleton.

Significance: The data obtained suggest that 3-BP can potentially be useful for treatment of metastatic prostate cancer and, especially, be efficient in limiting metastasis.

1. Introduction

Cancer metastasis (cancer cell migration and invasion throughout the body) causes approximately 90% of all cancer-related death in spite of the advancement of cancer therapy [1]. Clinically and biologically, metastasis is intricately linked with resistance to chemotherapy [2,3]. Prostate cancer is the main cause of cancer associated mortality in men worldwide. In Europe, the mortality rate of the patients diagnosed with prostate cancer is the highest among all types of cancer [4]. Cancer patients frequently suffer serious side effects when treated with chemotherapy/radiotherapy, so novel drugs have been developed to treat prostate cancer. Prostate cancer tumor grow relatively fast and migrate to other parts of the body, including lymph nodes, bone tissues as well as soft tissues, if proper treatment is not applied in time [5,6].

Cancer cells are generally more dependent on glycolysis than normal cells ("Warburg effect"), which results in a higher consumption of glucose, due to the lower efficiency of energy production by anaerobic glycolysis and high vitality, even in the absence of sufficient levels of oxygen [7,8]. In contrast to existing chemotherapy/radiotherapy strategies, inhibiting glycolysis to limit ATP yield seems to be an efficient approach for suppressing cancer cell proliferation. Nevertheless, most current inhibitors of glycolysis cause adverse effects. D-glucose has low bioavailability, competitively inhibits glucose transporters, shows nonspecific delivery as well as a narrow therapeutic window [9,10]. An alkylating antiglycolytic compound, 3-bromopyruvic acid (3-BP) is a promising anticancer agent, able to act on multidrug-resistant cells. 3-Bromopyruvic acid as an analogue of lactic acid or pyruvic acid can "trick" cancer cells and enter via a monocarboxylate transporters (MCTs) as a Trojan horse [11–14].

Apart from inhibiting glycolysis, 3-BP causes oxidative stress and depletes cellular glutathione (GSH) [15,16]. We demonstrated that the glutathione depletion in 3-BP treated cells is mainly due to the formation of a conjugate of 3-BP with glutathione (pyruvylo-*S*-glutathione) [17]. High glutathione content of tumors decreases their sensitivity to 3-BP and requires additional means to lower the glutathione level. Not surprisingly, 3-BP demonstrates synergistic activity with other compounds that reduce intracellular levels of GSH. The examples of such compounds are buthionine sulphoximine (BSO), methionine sulfoximine (MSO) and paracetamol (acetaminophen) [16,18,19].

It should be emphasized that 3-BP, in contrast to the commonly used

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https://doi.org/10.1016/j.lfs.2019.03.066
Received 24 February 2019; Received in revised form 23 March 2019; Accepted 25 March 2019
Available online 28 March 2019

cytostatics, shows low toxicity. No hepato- and nephrotoxicity was observed in nude mice and Kunming mice given a 3-BP dose of 8 mg/kg [20]. 3-BP seems to be not toxic for human erythrocytes (normal cells heavily dependent on glycolysis) at therapeutic concentrations; only high 3-BP concentration (0.5 mM) affects erythrocytes in vitro [2,22]. In summary, 3-bromopyruvic acid is a promising anticancer compound due to high potency, stability and negligible toxicity. Here, for the first time to the best of our knowledge we have shown the effect of 3-BP on the survival, movement and ATP level of rat and human prostate carcinoma cells, finding high sensitivity of these metastatic cells and inhibition of their mobility even by low concentrations of 3-BP.

2. Materials and methods

2.1. Materials and equipment

Human prostate carcinoma cell lines [DU-145 (HTB-81) and PC-3 (CRL-1435)] were obtained from American Type Culture Collection (ATCC) and cultured according to the catalogue instructions; rat prostate carcinoma cell lines of markedly different metastatic potential were obtained from Imperial College, London (UK).

The DU-145 cell line (grade II) was derived from a brain metastatic site of a 69-year old Caucasian man, with 0Rh+ blood type. These cells have epithelial morphology and adherent culture properties. DU-145 cell line is tumorigenic and insensitive to hormones. The line is weakly positive for acid phosphatase. There are no prostate antigens expressed by this line. Ultrastructural analyses confirmed the consistency between the cell line and original tumor cells, considering microvilli, tonofilaments, desmosomes, few mitochondria, well developed Golgi and heterogenous lysosomes. The PC-3 cell line was established from bone metastasis of grade IV of prostate cancer in a 62-year-old Caucasian male. These cells are insensitive to androgens, glucocorticoids or fibroblast growth factors. The PC-3 cell line is more invasive than the DU-145 cell line. Those cells have epithelial morphology and adherent culture properties and can be adapted to suspension growth. The cells express HLAA1 and A9 antigens and exhibit low acid phosphatase and testosterone-5-alpha reductase activities [23,24].

Both rat cancer cell lines, MAT-LyLu (the Dunning rat model) and AT-2 were established from spontaneously occurring prostate tumor among Copenhagen rats. Those cell lines exhibit different invasiveness – MAT-LyLu cell line is highly invasive and metastasizing in over 90% of cases of injections into Copenhagen rats, whereas AT-2 cell line metastasizes in < 10%. MAT-LyLu cells were derived from successive in vivo passages of the AT-1 tumor cell line. The cells are anaplastic, androgen-dependent and metastatic to the lung [25–28]. All the lines exhibit no contact inhibition.

Dulbecco's Modified Eagle Medium: Nutrient mixture F-12 (Ham's) (DMEM/F12) (cat. no. 11330-032) was purchased from Thermofisher Scientific (Waltham, MA, USA). Fetal Bovine Serum (FBS) (cat. no. 04-001-1A), Trypsin-EDTA solution (10×) (cat. no. 03-051-5B), Phosphate-Buffered Saline (PBS) without Ca^{2+} and Mg^{2+} ions (cat. no. 02-023-1A) and Penicillin-Streptomycin solution (cat. no. 03-031-1B) were obtained from Biological Industries (Cromwell, CT, USA). 0.4% Trypan Blue Solution (cat. no. T8154), 0.33% Neutral Red (NR) solution (cat. no. N2889), 4′,6-diamidino-2-phenylindole (DAPI, Dihydrochloride) (cat. no. D9542), Triton X-100 (cat. no. 9002-93-1), phalloidin conjugated with Atto-488 (cat. no. 49409), 3-bromopyruvic acid (cat. no. 16490), *N*-ethylmaleimide (NEM) (cat. no. E3876), trichloroacetic acid (TCA) (cat. no. T4885), diethylenetriaminepentaacetic acid (DTPA) (cat. no. D1133), L-ascorbic acid (cat. no. A0278), sodium hydrosulfite (sodium dithionite) (cat. no. 15,795-3), 96% ethanol (cat. no. 396420113), glacial acetic acid (cat. no. 568760114) as well as methanol (cat. no. 621990110) were obtained from Avantor Performance Materials Poland. 37% Formaldehyde Solution was provided by CHEMPUR (Poland). CellTiter-Glo® Luminescent Cell Viability Assay (cat. no. G7571) was purchased from Promega (Madison, WI, USA).

Cell culture 75 cm^2 flasks were obtained from Thermofisher Scientific (cat. no. 156499) (Waltham, MA, USA). Transparent 96-well culture plates (cat. no. 92096) were provided by TPP (Trasadingen, Switzerland). Black flat bottom 96-well plates (cat. no. 655209) were obtained from Greiner (Kremsmünster, Austria). Black 96-well plates with optical bottom (cat. no. 3603) were purchased from Corning (Corning, NY, USA). Other sterile cell culture materials were provided by Nerbe (Winsen, Germany).

Stock solutions of 3-BP were freshly prepared in PBS and filtered through a 0.22 μm filter before each experiment, considering short lifetime of 3-BP in solution [29]. 3-Bromopyruvic acid (3-BP) (cat. no. 16490) (purity of ≥97%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Fluorometric and absorptiometric measurements were done in a Spark multimode microplate reader (Tecan Group Ltd., Männedorf, Switzerland). All measurements were performed in triplicate and repeated at least three times on different preparations.

2.2. Cell culture

The cells were cultured in DMEM/F12 supplemented with 10% v/v heat-inactivated fetal bovine serum (FBS) and 1% v/v penicillin and streptomycin solution. Cells were incubated at 37 °C under 5% carbon dioxide and 95% humidity. Medium was changed twice a week and cells were passaged at about 80% confluence. Cell morphology was examined under an inverted microscope with phase contrast Zeiss Primo Vert (Oberkochen, Germany), viability was estimated by Trypan Blue exclusion test, cells were counted in a Thoma hemocytometer (Superior Marienfeld, Lauda-Königshofen, Germany). The cells were monitored periodically for mycoplasma contamination.

2.3. Cytotoxicity

The cells were seeded in 96-well clear plate at amount of 1×10^4 cells/well (0.5×10^4 cells/well for MAT-LyLu cell line) in 100 μL culture medium. After 24 h incubation, medium was removed and replaced with cell culture medium supplemented with 3-BP at concentrations ranging from 5 to 75 μM. After 24 h or 48 h exposure, medium was removed and replaced with 100 μL of 2% Neutral Red solution in cell culture medium and incubated for 1 h in a CO_2 incubator. Then the cells were washed twice with PBS and fixed with 100 μL/well 50% ethanol, 49% H_2O, 1% acetic acid glacial solution and shaken for 15 min (500 rpm) at room temperature. Absorbance was measured at 540 nm against 620 nm.

2.4. Time-lapse monitoring of movement of individual cells

The cells were seeded on 12-well plates at the density of 600 cells/mm^2. After 12 h, 5 μM, 10 μM, 20 μM or 50 μM 3-BP was added and cells were incubated for the next 1 or 24 h. Then the cell movement was recorded for 6 h at 5-minute time intervals under isotropic condition or in the presence of 3-BP. At least 50 cells were analyzed for each condition.

The tracks of individual cells were determined from the series of changes in cell centroid positions, pooled and analyzed as previously described [30]. The following parameters were estimated: (i) the total length of cell trajectory [μm], (ii) the average speed of cell movement [μm/min], i.e. total length of cell trajectory/time of recording, (iii) the total length of cell displacement [μm], i.e. the distance from the starting point directly to the cell's final position, (iv) the rate of cell displacement [μm/min], i.e. the distance from the starting point directly to the cell's final position/time of recording, (v) the coefficient of movement efficiency (CME) corresponding to the ratio of cell displacement to cell trajectory length [31].

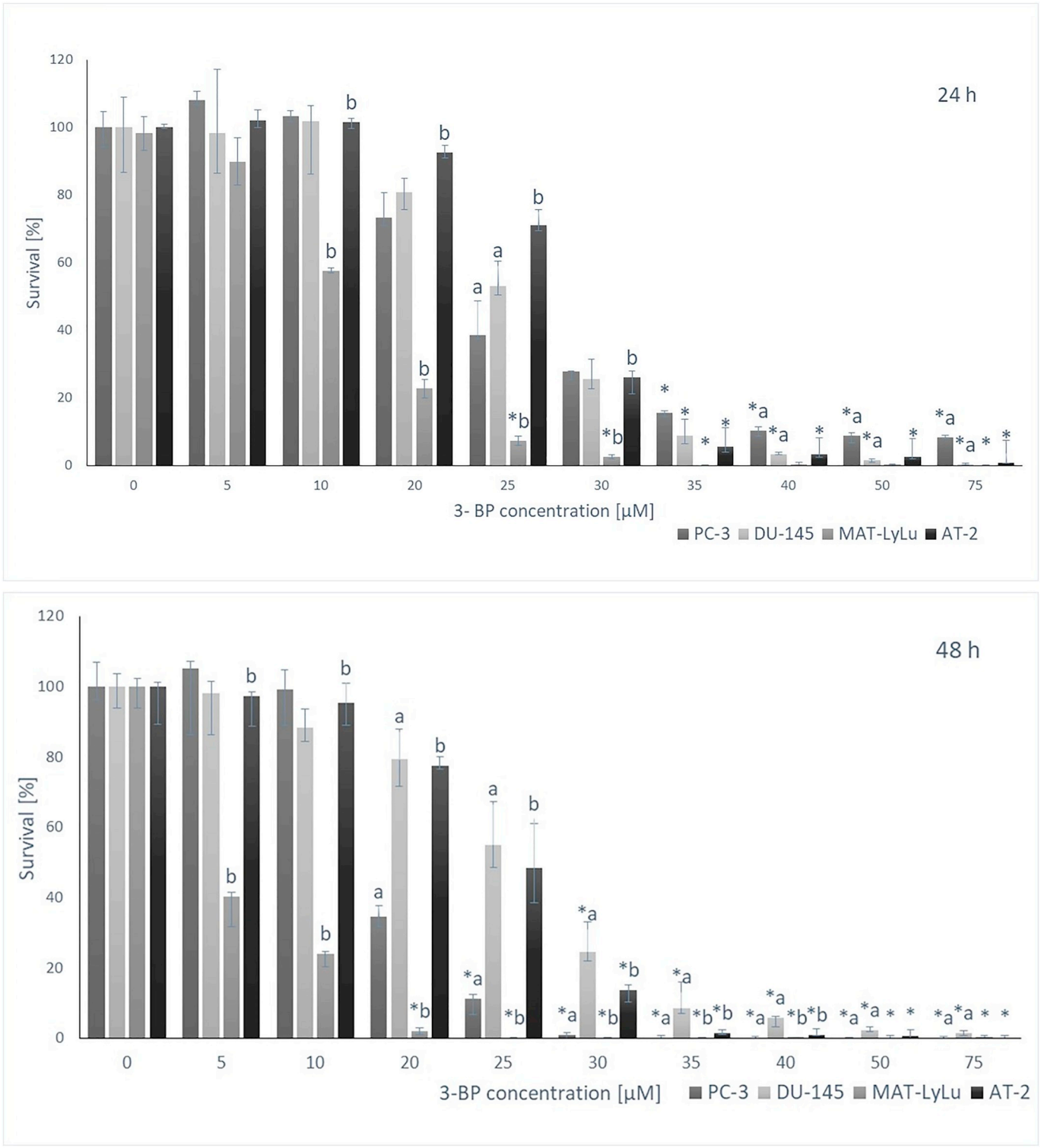

Fig. 1. Effect of 3-BP on the survival of human (DU-145 and PC-3) and rat (AT-2 and MAT-LyLu) metastatic prostate cell lines 24 h and 48 h after addition of 3-BP. Cell survival was estimated with Neutral Red. The whiskers are lower (25%) and upper (75%) quartile ranges. $^{*}P < 0.05$ with respect to control (100%), Kruskal–Wallis test; $^{a}P < 0.05$ (PC-3 vs DU-145), $^{b}P < 0.05$ (Mat-LyLu vs AT-2), Mann-Whitney *U* test.

2.5. Actin cytoskeleton staining

The cells were seeded on a 96-well black plate with optical bottom at amount of 1×10^4 cells/well (0.5×10^4 cells/well for MAT-LyLu cell line) in 100 µL culture medium and then treated as described above. The test was performed after 1 and 24 h incubation with 3-BP. Following the treatment, the medium was removed, the cells were washed with PBS (100 µL/well) and fixed with 100 µL/well 3.7% formaldehyde for 15 min. Then the cells were washed twice with PBS and permeabilized with 0.1% Triton X-100 solution at an amount of 100 µL/well for 15 min. The cells were washed with PBS two times (100 µL/well) and 100 µL of phalloidin working solution (prepared accordingly to the manufacturer's protocol) were added per well and incubated for 60 min. After that the cells were washed with PBS (100 µL/well) and

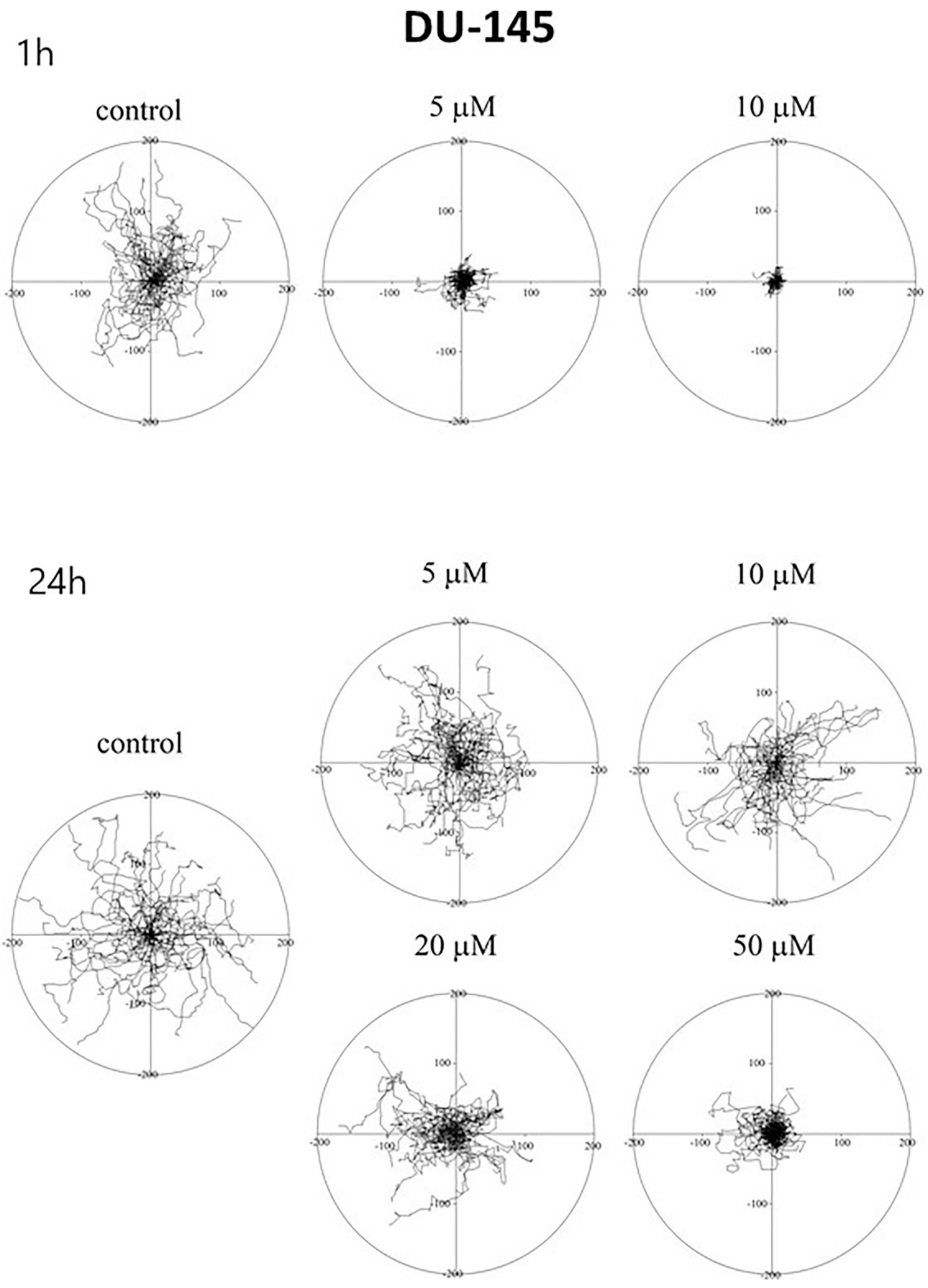

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Fig. 2. The effect of 3-BP on the migration of human (DU-145 and PC-3) and rat (AT-2 and MAT-LyLu) prostate cancer cells. Composite trajectories of the cells migrating in the absence or in the presence of 3-BP are shown as circular diagrams. In diagrams, the initial point of each trajectory was placed at the center of the circle. Each trajectory was constructed from 72 successive positions of cell centroids recorded at 5 minute time intervals. The movement of cells was recorded for 6 h, after 1 h or 24 h after changing the medium with or without 3-BP. Scale in μm.

nuclei were stained with 600 nM DAPI for 60 min (100 μL/well). Images were taken using a ZEISS LSM 710 inverted confocal microscope (Oberkochen, Germany).

2.6. Determination of ATP level

Intracellular ATP level was performed using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA). The assay is based on luminescent, enzymatic transformation of luciferin to oxyluciferin in the presence of ATP. Cells were cultured and treated as described above, but 3-BP concentration was ranging from 5 to 50 μM. CellTiter-Glo® Assay was performed after 1 h and 24 h incubation (on separate plates), by adding 100 μL CellTiter-Glo® Reagent to cell culture medium present in each well, shook and incubated, according to the manufacturer's protocol. Luminescence was recorded by TECAN Spark® multimode plate reader.

2.7. Glutathione content

The content of reduced glutathione (GSH) was assayed with *ortho*-phthalaldehyde (OPA) [32]. The cells were seeded in 96-well clear plate at an amount of 1×10^4 cells/well (0.5×10^4 cells/well for MAT-LyLu cell line) in 100 μL culture medium. After 24 h incubation, medium was removed and replaced with cell culture medium supplemented with 3-BP at concentrations ranging from 5 to 50 μM. Tests were performed after 1 and 24 h treatment with 3-BP. After incubation with the drug, medium was removed by aspiration and cells were washed with PBS 150 μL/well. Phosphate-Buffered Saline was gently removed by aspiration. Then 60 μL/well of freshly prepared cold lysis buffer (RQB buffer: 20 mM HCl, 5% TCA, 5 mM DTPA, 10 mM L-ascorbic acid) were added. Plate was shaken at 700 rpm for 5 min and centrifuged at 4000 rpm for 5 min, at room temperature. After that cell lysate was transferred into two black 96-well plates with black bottom ('+ NEM' and '− NEM') in amount of 25 μL/well. Within the first plate '+ NEM',

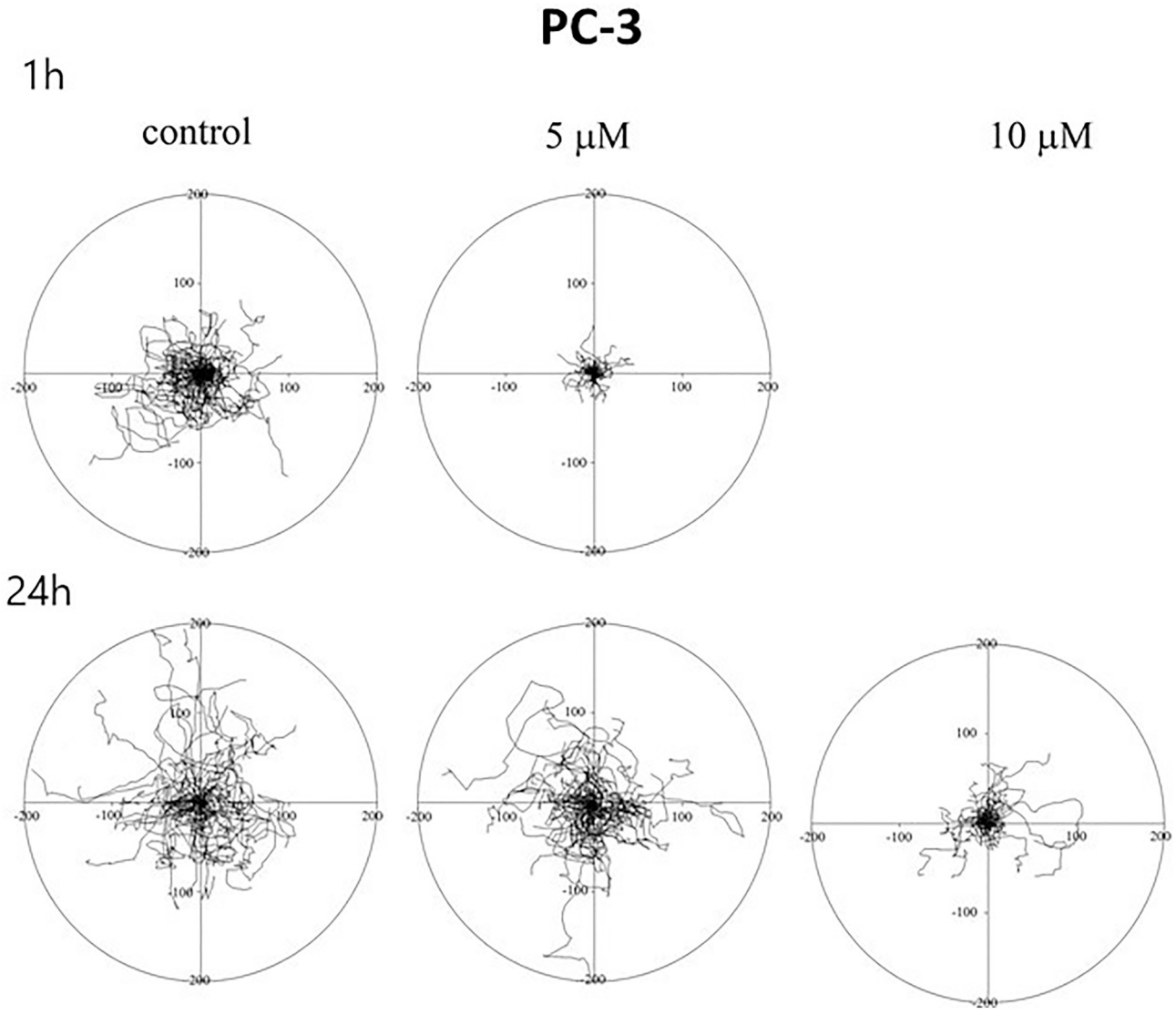

Fig. 2. (*continued*)

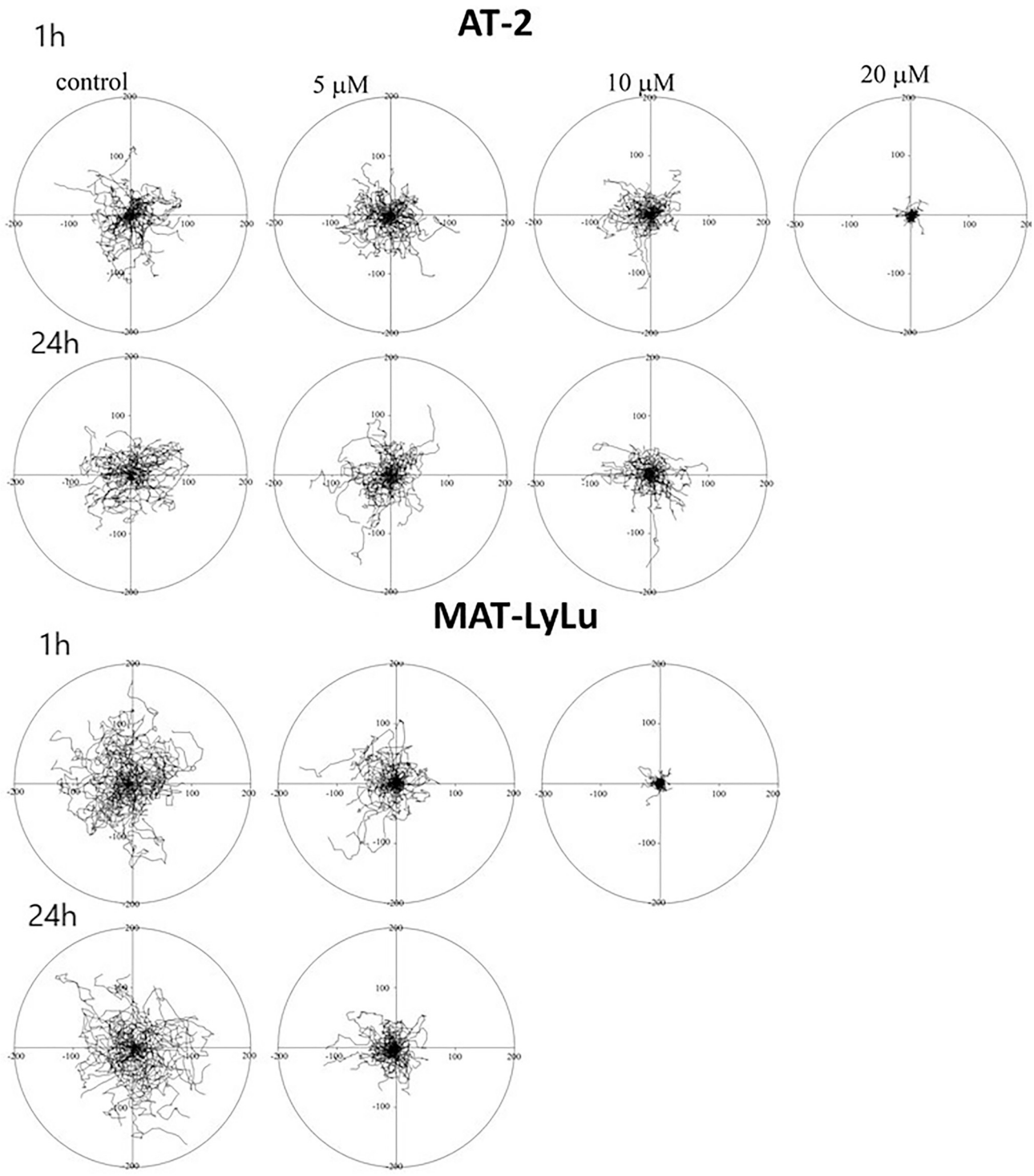

Fig. 2. (*continued*)

4 μL/well of freshly prepared 7.5 mM NEM in cold RQB buffer were added. Then, 40 μL/well of 1 M phosphate buffer (pH 7.0) were added into both plates, which then were shaken for 5 min at 700 rpm. Following 160 μL/well of cold 0.1 M phosphate buffer (pH 6.8) and 25 μL/well of freshly prepared 0.5% OPA in methanol were added into both plates. Then plates were shaken at 700 rpm for 30 min. Fluorescence was measured with TECAN Spark® multimode plate reader at 355/430 nm. The concentration of reduced glutathione was determined by subtracting the fluorescence of the ('− NEM') plate from the fluorescence of the ('+ NEM') plate and calculated with respect to the protein content.

2.8. Protein assay

Protein content was determined according to Lowry et al. [33].

2.9. Statistical analysis

Kruskal-Wallis test was performed to estimate differences between 3-BP treated and non-treated cells. The difference of survival between human and rat cell lines and theirs invasiveness was estimated using the Mann-Whitney U non-parametric test. Cell trajectories from no less than three independent experiments (number of cells = 50) were taken for

Table 1
Effect of 3-BP on the mobility parameters of human and rat metastatic prostate cells.

Time: 1 h

Parameter (± SEM)	Cell line	3-Bromopyruvic acid			
		0	5 μM	10 μM	20 μM
Total length of cell trajectory [μm]	DU-145	217.6 ± 6.5	120.1 ± 6.7*	46.3 ± 3.9*	41.8 ± 2.3*
	PC-3	238.7 ± 13.5	44.4 ± 3.9*		
	AT-2	196.5 ± 6.1	204.6 ± 6.9	156.5 ± 6.1*	
	Mat-LyLu	329.2 ± 7.2	197.8 ± 7.9*	48.6 ± 2.7*	
Average speed of cell movement [μm/min]	DU-145	0.60 ± 0.02	0.33 ± 0.02*	0.13 ± 0.01*	0.12 ± 0.01*
	PC-3	0.66 ± 0.04	0.12 ± 0.01*		
	AT-2	0.55 ± 0.02	0.57 ± 0.02	0.43 ± 0.02*	
	Mat-LyLu	0.91 ± 0.02	0.55 ± 0.02*	0.13 ± 0.003*	
Total length of cell displacement [μm]	DU-145	78.8 ± 6.4	23.2 ± 1.9*	8.7 ± 0.9*	11.5 ± 1.2*
	PC-3	45.6 ± 4.9	14.5 ± 1.8*		
	AT-2	56.6 ± 4.5	53.3 ± 3.8	43.7 ± 3.5*	
	Mat-LyLu	76.8 ± 5.1	49.6 ± 5.1*	11.8 ± 1.2*	
The rate of cell displacement [μm/min]	DU-145	0.22 ± 0.02	0.06 ± 0.01*	0.02 ± 0.003*	0.03 ± 0.01*
	PC-3	0.13 ± 0.01	0.04 ± 0.01*		
	AT-2	0.16 ± 0.01	0.15 ± 0.01	0.12 ± 0.01*	
	Mat-LyLu	0.21 ± 0.01	0.14 ± 0.01*	0.03 ± 0.003*	
Coefficient of movement efficiency CME	DU-145	0.36 ± 0.03	0.22 ± 0.02*	0.22 ± 0,02*	0.28 ± 0.03*
	PC-3	0.19 ± 0.02	0.33 ± 0.03*		
	AT-2	0.23 ± 0.02	0.26 ± 0.02*	0.28 ± 0.02*	
	Mat-LyLu	0.23 ± 0.02	0.24 ± 0.02	0.23 ± 0.02	

Time: 24 h

Parameters (± SEM)	Cell line	3-Bromopyruvic acid				
		0	5 μM	10 μM	20 μM	50 μM
Total length of cell trajectory [μm]	DU-145	248.3 ± 9.3	265.8 ± 6.2	219.5 ± 7.7	226.9 ± 9.9	192.5 ± 11.7*
	PC-3	258.3 ± 13.4	252.4 ± 10.6	90.9 ± 6.3*		
	AT-2	204.9 ± 4.4	193.5 ± 7.1	161.2 ± 5.4*		
	Mat-LyLu	291.1 ± 9.9	179.4 ± 7.9*			
Average speed of cell movement [μm/min]	DU-145	0.69 ± 0.03	0.74 ± 0.02	0.61 ± 0.02	0.63 ± 0.03	0.53 ± 0.03*
	PC-3	0.71 ± 0.04	0.71 ± 0.03	0.25 ± 0.02*		
	AT-2	0.57 ± 0.01	0.54 ± 0.02	0.45 ± 0.01*		
	Mat-LyLu	0.81 ± 0.03	0.49 ± 0.02*			
Total length of cell displacement [μm]	DU-145	100.9 ± 7.3	84.7 ± 5.2	78.6 ± 6.7*	61.9 ± 5.8*	24.5 ± 2.4*
	PC-3	76.9 ± 7.5	62.9 ± 6.1	28.2 ± 2.2*		
	AT-2	58.1 ± 3.6	51.2 ± 4.7	45.2 ± 4.2*		
	Mat-LyLu	77.1 ± 5.6	36.9 ± 4.1*			
The rate of cell displacement [μm/min]	DU-145	0.28 ± 0.02	0.28 ± 0.02	0.22 ± 0.02*	0.17 ± 0.02*	0.17 ± 0.02*
	PC-3	0.21 ± 0.02	0.17 ± 0.01	0.08 ± 0.01*		
	AT-2	0.16 ± 0.01	0.14 ± 0.01	0.13 ± 0.01*		
	Mat-LyLu	0.21 ± 0.02	0.10 ± 0.01*			
Coefficient of movement efficiency CME	DU-145	0.41 ± 0.03	0.32 ± 0.02*	0.34 ± 0.03*	0.28 ± 0.02*	0.15 ± 0.01*
	PC-3	0.31 ± 0.03	0.25 ± 0.02*	0.3 ± 0.02		
	AT-2	0.23 ± 0.01	0.26 ± 0.02*	0.28 ± 0.02*		
	Mat-LyLu	0.27 ± 0.02	0.30 ± 0.02*			

* $P < 0.05$ against non-treated control, Kruskal–Wallis test.

the estimation of statistical significance by the Mann-Whitney *U* test. $P \leq 0.05$ was considered as statistically significant in both cases. Every test was performed in triplicate. Statistical analysis of the data was performed using STATISTICA software package (version 12 and 13.1, StatSoft Inc. 2016, Tulsa, OK, USA, www.statsoft.com).

3. Results

Both rat and human metastatic prostate cells proved to be sensitive to the action of 3-BP. The cell survival after 48 h and after 24 h in the range of lower 3-BP concentrations was lower for the more invasive cell lines when rat and human cell lines were compared in pairs (Fig. 1), which was reflected in the mean IC_{50} values (24, 26, 20 and 26 μM 3-BP after 24 h and 16, 25, 4 and 24 μM 3-BP after 48 h for PC-3, DU-145, Mat-LyLu and AT-2 cell lines, respectively). An unexpected finding was the dramatic effect of 3-BP on the movement of the cells. As low concentrations of 3-BP such as 5 μM and 10 μM drastically inhibited cell movement while concentrations higher than 10 completely blocked the movement after 1 h treatment. Later on, after 24 h, the surviving cells recovered their mobility to some extent, but still, the total length of cell displacement, the rate of cell displacement and the coefficient of movement efficiency were decreased in cells treated with 3-BP at concentrations ≥10 μM while the total length of cell trajectory and total length of cell displacement were decreased in cells treated with 50 μM 3-BP (Fig. 2, Table 1).

Decreased mobility was associated with changes in the organization of cytoskeleton and, at higher doses, decreased staining of cytoskeleton filaments with Atto-488-phalloidin, especially after 24 h (Fig. 3).

The restriction of movement of metastatic prostate carcinoma cells was accompanied by decrease in the ATP content of the cells (Fig. 4) and decrease in the content of GSH, statistically significant after treatment with 30 μM and 50 μM 3-BP (Fig. 5). Differences in the effect of 3-BP on ATP and GSH levels between the more and less invasive human and rat cell lines only in some cases were in the same direction

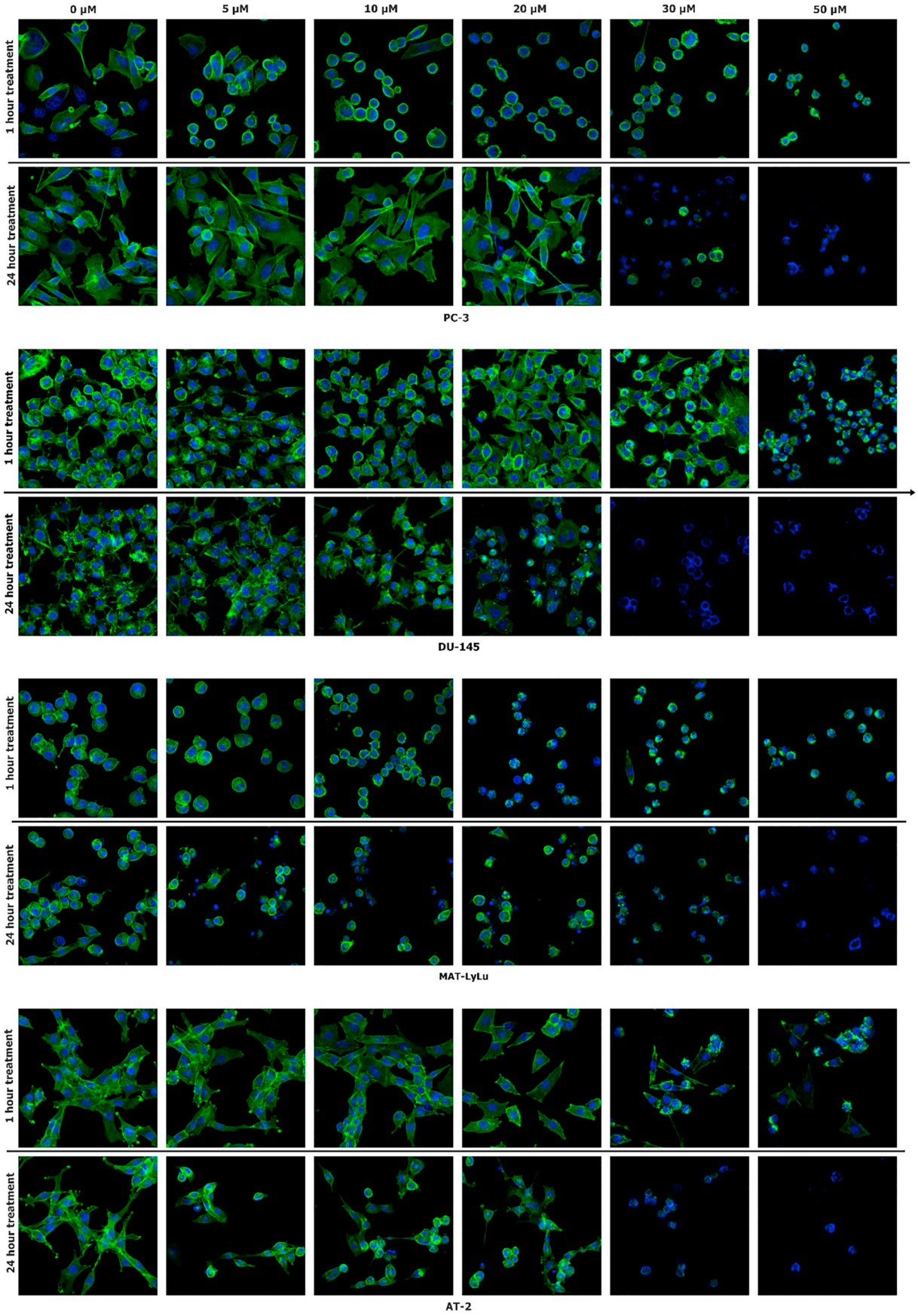

Fig. 3. Changes in cytoskeleton of human and rat prostate cancer cells after 1- and 24 h treatment with 3-BP. Fluorescent staining with Atto-488-phalloidin and DAPI.

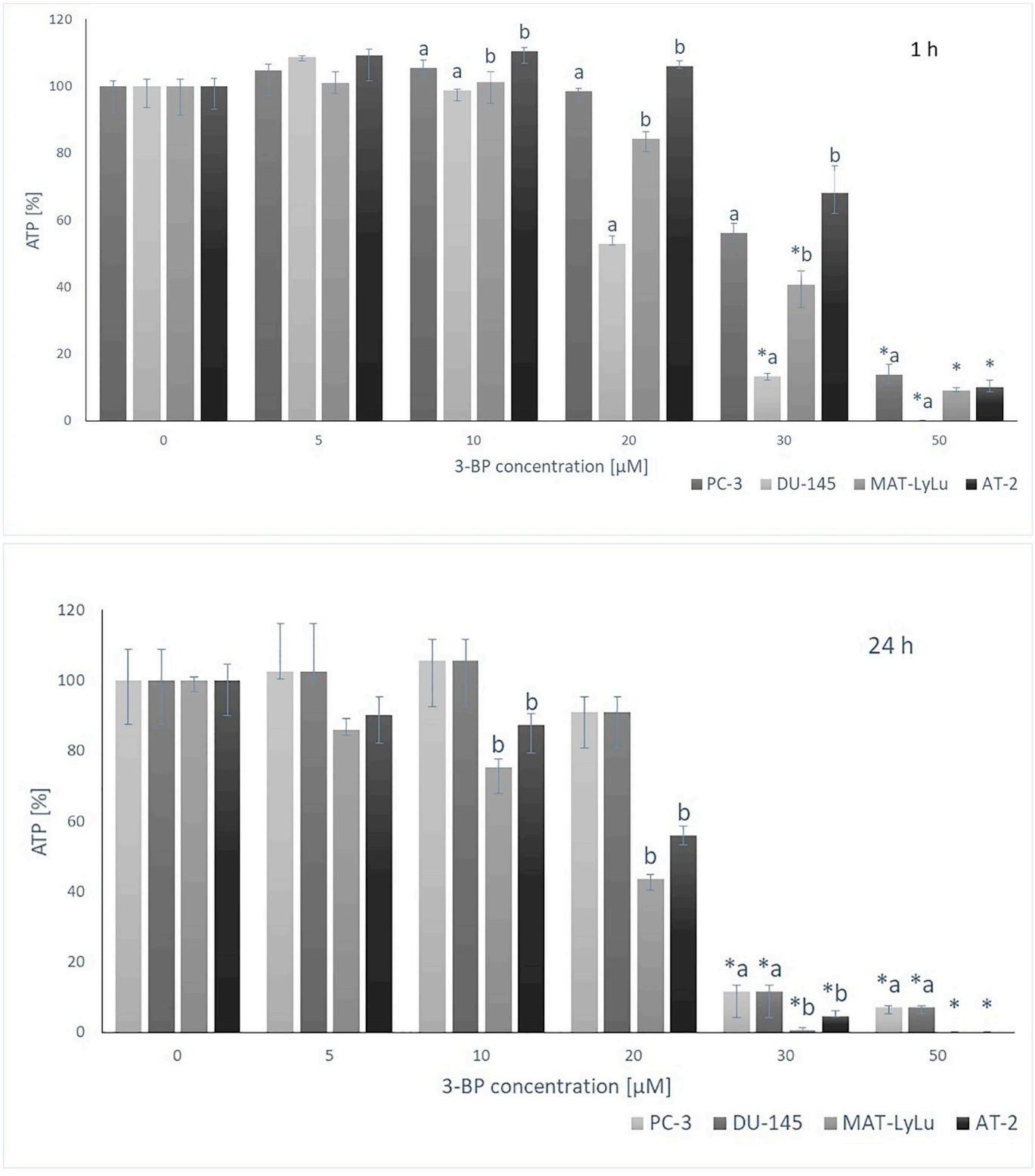

Fig. 4. The intracellular level of ATP of human and rat prostate cancer cells after 1- and 24 h treatment with 3-BP. The whiskers are lower (25%) and upper (75%) quartile ranges. $^{*}P < 0.05$ with respect to control (100%), Kruskal–Wallis test; $^{a}P < 0.05$ (PC-3 vs DU-145), $^{b}P < 0.05$ (Mat-LyLu vs AT-2), Mann-Whitney *U* test.

as the differences in survival, in other cases the direction being opposite.

4. Discussion

Unlike many conventional anticancer agents, 3-BP can kill tumor cells under both hypoxic and normoxic conditions. The broad spectrum of malignancies targeted by 3-BP includes, but is not limited to, cancers of breast, prostate, pancreas, cervix, renal, ovarian, colorectal, hepatic, melanoma, mesothelioma as well as lung origins [34]. Prostate cancer is one of the main causes of mortality in men, due mainly to metastases, the final phase of prostate cancer. On the basis of 2011–2013 data, 12.9% male population has a prostate cancer morbidity risk at any stage of life. Therefore, 90% of advanced-stage prostate cancer patients

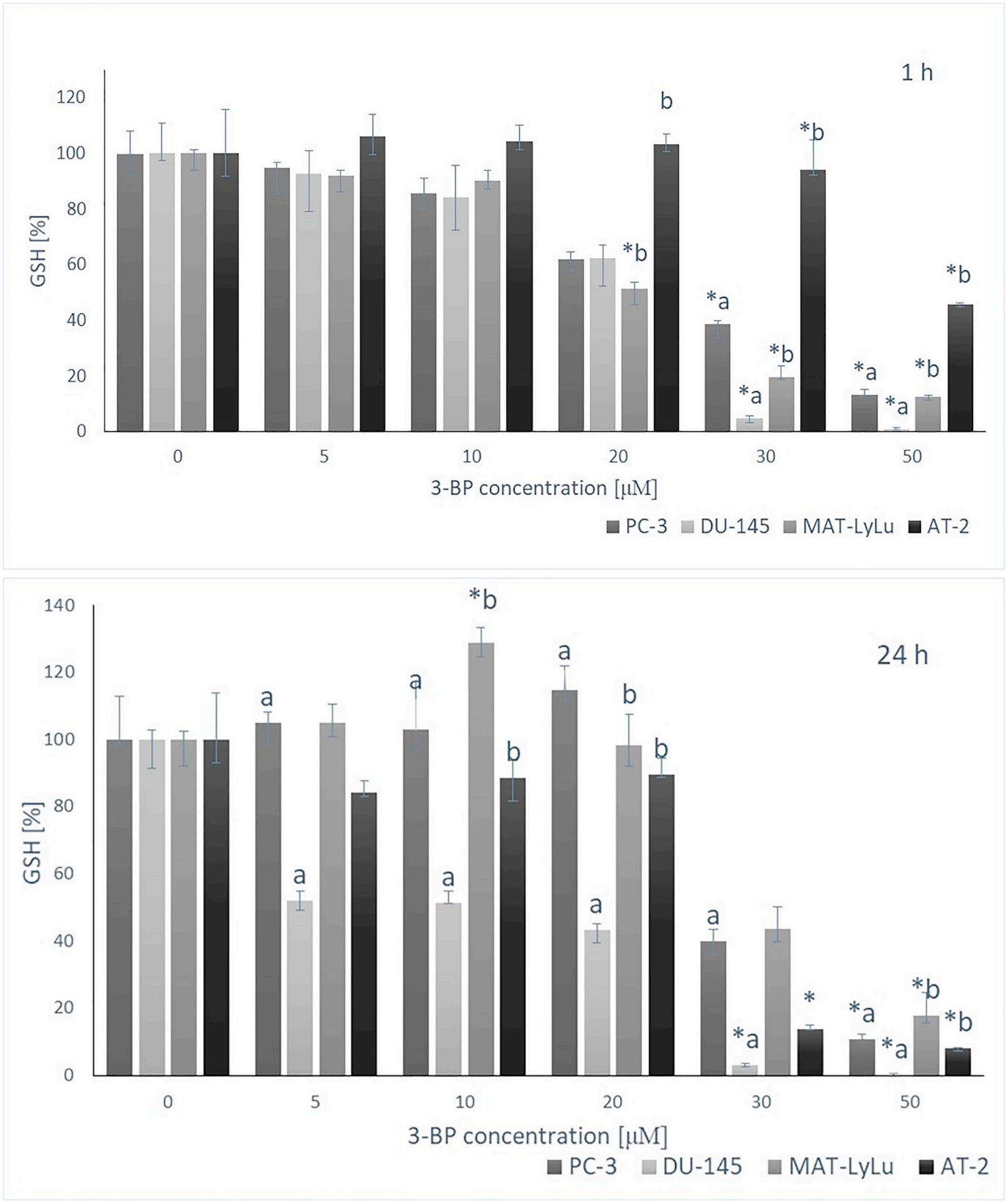

Fig. 5. Content of reduced glutathione (GSH) in human and rat prostate cancer cells after 1- and 24 h treatment with 3-BP. The whiskers are lower (25%) and upper (75%) quartile ranges $^{*}P < 0.05$ with respect to control (100%), Kruskal–Wallis test; $^{a}P < 0.05$ (PC-3 vs DU-145), $^{b}P < 0.05$ (Mat-LyLu vs AT-2), Mann-Whitney *U* test.

have very low survival rate and they are more prone to complications such as skeletal fractures, intolerable bone pain, together with spinal cord compression [35].

Cancer metastasis is critically dependent on cell mobility. The high sensitivity of the metastatic prostate cancer lines to mobility inhibition by low concentrations of 3-BP may suggest that this compound may be useful not only for eradication of prostate cancer, but even more as an inhibitor of their metastasis. The lowest concentration of 3-BP applied (5 μM) caused significant inhibition of cell mobility, and concentrations higher than 10 μM made the cells immobile. Restriction of mobility was released with time in surviving cells, nonetheless was still visible after 24 h. Restriction in mobility correlated with disassembly of actin cytoskeleton of the cells. Among the factors contributing to this effect, ATP and GSH depletion may be suggested. 3-bromopyruvic acid is a glycolytic inhibitor, affecting also mitochondrial function so it is an efficient ATP-depleting agent [13,15,36,37].

It has been established that the primary mechanism of 3-BP anti-glycolytic action is via preferential alkylation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). It has been also demonstrated that covalent modification of GAPDH by the addition of the pyruvyl moiety of 3-BP brought about the anti-glycolytic and anticancer effects. This pyruvylation of GAPDH correlated with the loss of enzymatic function. In addition, enzymes other than GAPDH which were not pyruvylated (based on the lack of ^{14}C incorporation) remained active. Further, this 3BP-GAPDH interaction causes also ATP depletion in a dose-dependent manner leading to apoptotic cell death [38]. What's more, the mitochondrial phosphate transporter, that is essential for ATP synthesis is also well known to be inhibited by compounds reacting with sulfhydryl groups (-SH) such as 3-BP [39].

3-bromopyruvic acid is also a GSH-depleting compound [15–17,21,37,40]. ATP depletion must impair cytoskeletal dynamics; also redox equilibrium is important for the proper function of the cytoskeleton [41–43]. However, in our experiments restriction of mobility occurred at lower 3-BP concentrations than those inducing ATP and GSH depletion and changes in cytoskeletal organization. Moreover, the differences in the effects of 3-BP on the ATP and GSH levels generally did not follow those observed in cell survival. Seemingly, direct interaction of 3-BP with cytoskeletal protein is mainly responsible for the observed effect, while ATP and GSH depletion may contribute at higher doses of 3-BP. Indeed, thiol alkylating reagents such as iodoacetamide or *N*-ethylmaleimide are known to alter the actin and microtubule organization even at concentrations of 10^{-6}–10^{-4} M [41] so analogous effects of 3-BP can be expected. The binding of 3-BP to cytoskeletal proteins can be expected to be higher in mobile cells as stretched protein fibrils exhibit increased availability of cysteine residues for thiol reagents [44] and periodical stretching of cytoskeletal elements occurs during cell movement.

Metastatic human and rat prostate cancer cell lines studied were highly sensitive to 3-BP. Their IC_{50} values were lower than that found for other cancer cell lines, such as primary renal carcinoma cells (49–89 μM), normal kidney cells (143–187 μM) and several renal cell lines (91–126 μM) [45], melanoma cell lines SK-mel-147 and UACC3093 (> 100 μM) [46], breast cancer cell lines MCF-7 and MDA-MB-231 (101 and 67 μM, respectively) [40], MDCK-II cells (Madin-Darby Canine Kidney) (> 100) [16] and CAOV3 ovarian cancer cell line (84 μM) [47], although some breast cancer and ovarian cell lines were characterized by IC_{50} values of 10–15 and 16–20 μM, respectively [48]. Various factors have been pointed out to determine the 3-BP sensitivity of cancer cells, including the proliferation rate [47], expression level of monocarboxylate transporters limiting 3-BP entry into the cell, GSH content [16,47] and energy requirements [48]. It can be expected that metastatic cells, like multidrug-resistant cells, have higher energy demand due to their motility and can therefore be more vulnerable to the energy depletion by 3-BP. We observed such a situation in the case of non-invasive MCF-7 and invasive MDA-MB-231 breast cancer cell lines [40]. All this supports the idea of potential suitability of 3-BP for treatment and inhibition of metastasis of prostate cancer tumors.

5. Conclusions

3-Bromopyruvate induced necrotic cell death in sensitive melanoma cell lines, even at low concentrations, inhibited the mobility of rat and human metastatic prostate cancer cell lines and was toxic to these cells at higher doses. It suggests that this compound can potentially be useful for treatment of metastatic prostate cancer and, especially, be efficient in limiting metastasis.

Author contribution statement

Conception and design: IS-B; development of methodology: IS-B, JS, ZM and MP; acquisition of data: IS-B, MP, NP and KP; analysis and interpretation of data: IS-B, MP and JS; writing and editing the manuscript: IS-B, GB and MP.

Acknowledgments

This study was performed within the research fund of the University of Rzeszów (Grant BR/ZBA/PB/1/2017). We are grateful to Dr. Sławomir Bartoszewski (University of Rzeszów) for his excellent technical help with confocal microscopy.

Conflicts of interest

The authors declare no “Conflict of interest”.

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