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Influence of Proteasome Inhibitor Bortezomib on the Expression of Multidrug Resistance Genes and Akt Kinase Activity

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Abstract—The goal of this work was to study the mechanisms of ABC family transport proteins' regulation by a new-generation antitumor drug – the proteasome inhibitor bortezomib (Velcade). ABC transporters determine the multidrug resistance of tumor cells (MDR). We confirmed our previously discovered observation that bortezomib affects the expression of genes involved in the formation of MDR (*ABCB1* gene, also known as *MDR1*, and *ABCC1-MRP1*), reducing the amount of their mRNA. This effect was found to depend on Akt kinase activity: the Akt activity inhibitor Ly 294002 increased the amount of *MRP1* mRNA in KB 8-5 cells. It was also shown that bortezomib increased the amount of Akt kinase phosphorylated form in cell lines of malignant cells KB 8-5 and K 562/i-S9 that overexpressed ABCB1 transporter (Pgp), and did not affect the amount of activated Akt in the corresponding wild-type cells. When exposed to bortezomib, selection of resistant to it cell variants was much faster for a Pgp-overexpressing cell population (compared to wild-type cells). It is shown that bortezomib affects the amount of *MRP1* gene mRNA, relocating the multifunctional protein YB-1, dependent on Akt activity, from cytoplasm to nuclei of MCF-7 breast cancer cells. The data indicate that the transcriptional activity of YB-1 might be one of the mechanisms that determine the effect of bortezomib on the amount of *MRP1* gene mRNA.

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Key words: ABC protein family, multidrug resistance of tumors, bortezomib, Akt kinase

In this work we investigated the mechanisms of regulation of ABC family transport proteins under the influence of a proteasome inhibitor, bortezomib, using a tumor cell population, and in particular the participation of the PI3K/Akt signaling pathway and YB-1 protein in this process. The new-generation antitumor drug bortezomib (Velcade) is the first and most famous selective proteasome inhibitor used in cancer treatment [1]. It is used to treat multiple myeloma, lymphomas, and other tumors. Bortezomib is a water-soluble compound – a dipeptidyl of boronic acid, which covalently binds to the active Thr1 residue on the N-terminus of catalytic subunit β5 of 20S proteasome core part and inhibits its chymotrypsin-like activity. Bortezomib, along with its main function as a proteasome inhibitor, is able, as we have previously shown, to affect the amount of mRNA of some genes from ABC family and proteins encoded by it that may determine the multidrug resistance (MDR) of tumor cells [2]. Literature data suggest that bortezomib has an effect on the activity of key signaling pathways and cell signaling molecules, including the PI3K/Akt signaling pathway [3, 4].

The PI3K/Akt cascade is the pathway of signal transmission associated with phosphatidylinositol-3-kinase (PI3K). PI3K phosphorylates phosphatidylinositol (PI), phosphatidylinositol-4-monophosphate (PI4P), and phosphatidylinositol-4,5-diphosphate (PI-4,5-P2) to PI-3-P, PI-3,4-P2, and PI-3,4,5-P3 [5, 6]. The PI3K/Akt signaling pathway regulates processes of cell growth, proliferation, angiogenesis, and intracellular metabolism. Activation of the PI3K/Akt signaling pathway in tumor cells after stress may regulate the resistance of tumor cells to chemotherapeutic drugs, as well as the degree of sensitivity of tumors to radiotherapy [7]. One of the main parts of the PI3K/Akt signaling pathway is serine/threonine kinase Akt (also known as PKB). Since its discovery as a proto-oncogene, Akt kinase has attracted the attention of many researchers because of its important regulatory role in various processes including tumor progression and the metabolism of insulin.

Abbreviations: CML, chronic myeloleukemia; MDR, multidrug resistance; Pgp, P-glycoprotein; PI3K, phosphatidylinositol-3-kinase.

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It was shown earlier in our laboratory that there is a connection between the activity of the PI3K/Akt signaling pathway and the development of MDR. We have shown that the PI3K/Akt signaling pathway and its component mTOR contribute to the development of multifactor MDR of cancer cells when exposed to cytotoxic drugs, both through direct effects on cell survival and by influencing the activity of transport proteins of the ABC family [8, 9].

The ABC (ATP binding cassette) family includes more than 100 proteins from various organisms – from bacteria to humans [10]. Most of these proteins transport various substances in cells, from ions to polypeptide chains. Some of these proteins determine multidrug resistance of cells. In the study of tumors resistant to therapy, increased expression of one of these ABC transporters is detected most often: ABCB1 (P-glycoprotein, hereinafter Pgp), ABCC1 (MRP1), and ABCG2 (BCRP) [11, 12]. Increased expression of several ABC transporters may also be observed in cases of MDR. In previous work we showed that bortezomib affects mRNA expression of some genes from the ABC family and their proteins, which may underlie the MDR of tumor cells [2].

Activity of the YB-1 protein is associated both with MDR and the PI3K/Akt signaling pathway. It is a member of the DNA- and RNA-binding proteins family with an evolutionarily conserved cold shock domain. YB-1 is involved in many DNA- and mRNA-dependent processes [13, 14]. This protein is found both in the nucleus and cytoplasm of mammalian cells. Functioning as a transcription factor in the nucleus, YB-1 regulates in cells the expression of genes containing Y-boxes in their promoters, including MDR genes *MDR1* and *LRP* [13, 14]. In the cytoplasm, YB-1 is one of the major proteins that bind to mRNA and form RNP (ribonucleoprotein particle) with unique physical and chemical characteristics. The synthesis rate of various proteins depends on its quantity in RNP. Thus, the localization of YB-1 in cells is associated with different molecular mechanisms that determine its effect on tumor cell phenotype. It is known that overexpression of YB-1 is observed in tumors of different histogenesis [15]. We found that the activity of YB-1 is stimulated by Akt kinase [16, 17].

The goal of this study was to determine whether the effect of bortezomib on the expression of MDR genes and proteins is associated with its impact on the PI3K/Akt-signaling pathway and on YB-1 protein. We also tried to understand with which features of the cellular context the differences in responses of studied cells on bortezomib may be related. We found that bortezomib activates Akt kinase under Pgp overexpression and that prolonged exposure to bortezomib leads to the selection of cells with constitutively activated Akt, especially in the population of malignant cells with Pgp overexpression. The effect of bortezomib on MDR malignant cells may be associated with the relocation of YB-1 protein from the cytoplasm to cell nucleus under its influence.

MATERIALS AND METHODS

Reagents. Bortezomib (Velcade) was from BEN VENUE Laboratories, Inc. (USA). Polyclonal rabbit antibodies against YB-1 protein were supplied by the laboratory of L. P. Ovchinnikov (Institute of Protein Research, Russian Academy of Sciences). Monoclonal antibodies against actin (MAB1501R; Chemicon, USA), fluorescently labeled secondary antibody to rabbit immunoglobulin (Alexa Fluor 488 (green); Invitrogen, USA), secondary antibody to mouse immunoglobulins conjugated with peroxidase (AR160R; Chemicon), polyclonal rabbit antibodies against phosphorylated AKT1(Ser473) (AV3132; Chemicon), secondary antibodies to rabbit immunoglobulin conjugated with peroxidase (AR132R; Chemicon), antibodies against Pgp directly labeled with phycoerythrin (CD243-PE; Beckman Coulter, USA), and monoclonal antibodies CD38-PE and CD34-PE (Beckman Coulter) were used in this study.

Cell lines. Chronic myeloid leukemia cell line K 562 [18] and its sublines overexpressing Pgp (K 562/i-S9) [19] were maintained on RPMI 1640 medium with 10% fetal bovine serum. We also used cells of human epidermoid oral cavity carcinoma KB 3-1 and cells of its subline KB 8-5, which overexpress Pgp. It was selected for resistance to colchicine [20]. We used MCF-7 cells – a cell line of human breast adenocarcinoma [21]. The cells were grown in DMEM medium with 10% serum. All cells were cultured at 37°C in an atmosphere of 5% CO_2.

MTT test. The method is based on the ability of mitochondrial dehydrogenases of living cells to transform "yellow" MTT – 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide – into "blue" formazan. Details of the method were described previously [8]. At least three independent experiments were conducted.

RNA isolation and RT-PCR. The procedure was described previously [22]. Total RNA was isolated using TRI Reagent (Sigma, USA) according to the protocol of the manufacturer. Reverse transcription (RT) was performed using hexameric primers. To equalize the amount of cDNA, housekeeping gene *GAPDH* was amplified in different samples. The following specific primers were used to amplify the products of desired length: GAPDH, 513 bp (CCCCTGGCCAAGGTCATCCATGACAACTTT (direct), GGCCATGAGGTCCACCACCCTGTTGCTGTA (reverse)); MDR1, 167 bp (CCCATCATTGCAATAGCAGG (direct), GTTCAAACTTCTGCTCCTGA (reverse)); MRP1, 180 bp (ATCAAGACCGCTGTCATTGG (direct), GAGCAAGGATGACTTGCAGG (reverse)); YB1, 476 bp (ACAAGAAGGTCATCGCAACGAAG (direct), GGTTGGAATACTGTGGTCGACG (reverse)). Amplification conditions: 30 sec at 94°C, then 25 cycles: 10 sec at 94°C, 10 sec at 60°C, 10 sec at 72°C, then 1 min at 72°C. Amplification products in reaction mixture with the volume of 20 μl were separated

by electrophoresis in 2% agarose gel with ethidium bromide. The gel was photographed with a digital camcorder.

Immunocytochemical analysis of proteins. The method was described previously [22]. We used monoclonal antibodies to proteins Pgp, CD34, and CD38. The prepared samples were analyzed on a flow cytometer from Becton Dickinson. The analysis was performed using the Cell Quest program by two indicators: estimated percentage of positively stained cells in the sample and average fluorescence intensity for each sample. At least 10,000 cells per sample were analyzed.

Immunocytochemical detection of YB-1 protein intracellular localization. To determine the intracellular localization of YB-1, cells were grown on Petri dishes 30 mm in diameter on cleaved cover glasses, fixed with 4% solution of *para*-formaldehyde, washed with PBS buffer three times, and the integrity of cell membrane was disrupted with 0.1% Triton X-100. The cells were then incubated with polyclonal antibodies against YB-1. After incubation with the primary antibodies, the cells were washed three times with PBS. The cells were then incubated with secondary fluorescently labeled antibodies against rabbit immunoglobulin. To specify the localization of YB-1, the same preparations were stained with nuclear dye Hoechst 33258. The number of cells with cytoplasmic and nuclear localization of YB-1 was counted using a fluorescent microscope (Zeiss, Germany).

Western-blot analysis. Cells ($2 \cdot 10^6$) were suspended in 100 μl of lysis buffer, prepared from 5× (1 M Tris-HCl, pH 6.8, 10% SDS, 50% glycerol, 10% mercaptoethanol, and bromophenol blue), and left for 5 min at room temperature. The samples were then placed in a boiling water bath for 10 min. The proteins were separated in 10% polyacrylamide gel and transferred onto a nitrocellulose membrane (Amersham, UK). They were further incubated in 5% milk in 1× TBS, 0.1% Tween-20 (TBST) for 1 h or in 1% BSA at room temperature with constant shaking. Then the membrane was washed in TBST and incubated with specific polyclonal antibodies against p-Akt(Ser473) at 1 : 1000 dilution or with antibodies against actin at 1 : 2000 dilution overnight at 4°C. The membrane was then washed with TBST three times for 10 min and incubated with secondary antibodies conjugated with peroxidase (Chemicon). Then the membrane was washed in TBST and developed using ECL reagent (Amersham).

RESULTS

Changes in activity of Akt and ERK1/2 kinases under the influence of bortezomib in drug-resistant and parental cells. In previous work, we showed that cells of oral cavity carcinoma KB 8-5 (possessing MDR and overexpressing Pgp) differ from the parental KB 3-1 cells in bortezomib response [2]. It was shown that bortezomib did not affect the amount of ABC proteins in KB 3-1 cells, whereas it increases the expression of Pgp and MRP1 in resistant KB 8-5 cells. To understand what may determine the differences between KB 3-1 and KB 8-5 cells, we investigated the effects of bortezomib on Akt-kinase activity. KB 8-5 and KB 3-1 cells were incubated with bortezomib for 6, 12, and 24 h, with effective IC_{50} dose. It turned out that bortezomib increases the amount of phosphorylated form of Akt-kinase in the KB 8-5 population, whereas in the KB 3-1 cells this drug reduces the phosphorylation of Akt (Fig. 1a). Thus, the resistant and parental cells differed by the nature of changes in Akt-kinase activity in response to treatment with bortezomib. We tested whether bortezomib affects Erk1/2-kinase. We used the same cell lines (KB 8-5 and KB 3-1), the same concentration of bortezomib, and the same time of exposure of the cells with the drug. We found that bortezomib significantly reduced the amount of phosphorylated Erk1/2-kinase form in KB 8-5 cells after incubation with the drug for 12 and 24 h, while having no effect on this target in KB 3-1 cells (Fig. 1b). This shows that bortezomib affects the Erk-MAP kinases in tumor cells with overexpression of Pgp and does not affect the original tumor cells with lower levels of P-glycoprotein in this pair of cell lines.

To determine whether altering Akt activity influences the amount of ABC transporter mRNA, we investigated the effect of Ly 294002 (specific inhibitor of Akt-kinase activation) on the amount of *MDR1* and *MRP1* gene mRNA in KB 8-5 cells (Fig. 1c). Ly 294002 increased the amount of *MRP1* mRNA. Its influence on the amount of *MDR1* mRNA in these experiments was not detected (data not shown). It is possible that high levels of *MDR1* mRNA in resistant cells do not permit to detect small variations in the content of this mRNA. Combined treatment of cells with bortezomib and Ly 294002 somewhat reduced the mRNA amount of both genes, but it remained higher than in the group with bortezomib treatment only. These data indicate that active Akt-kinase has a pronounced effect on the mRNA amount of at least one ABC transporter, even against the background bortezomib influence.

Changes in amount of P-glycoprotein (Pgp, ABCB1) and Akt kinase activity after prolonged exposure of parental and drug-resistant cells to bortezomib. The next task was to investigate the effect of bortezomib on MDR mechanisms in long-term treatment of a tumor cell population with this drug, since bortezomib therapy is a lengthy process. We used cell lines K 562 and K 562/i-S9, which were cultured in the presence of bortezomib at a dose equal to IC_{50} for each cell line. The cells were passaged for nine weeks. The amount of the drug was increasing through selection. After nine weeks, MTT test was used to determine the sensitivity level of cells that were cultured with bortezomib (indicated as K 562_{vlc} and K $562/i\text{-}S9_{vlc}$). It was compared with drug sensitivity of the original lines K 562/i-S9 and K 562 (Fig. 2a). We

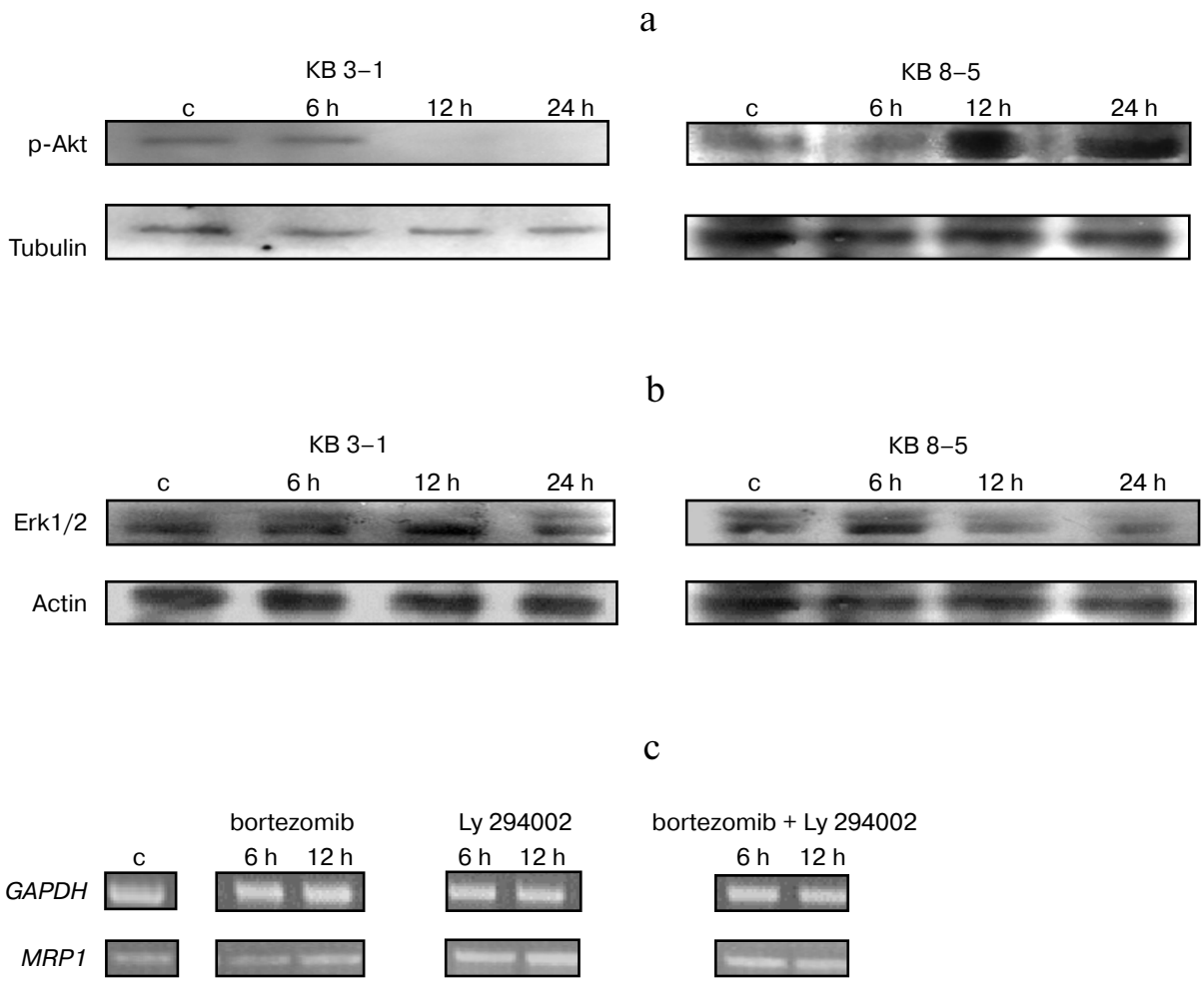

Fig. 1. Effect of bortezomib (IC_{50}) on Akt and Erk1/2 kinase phosphorylation in cells of KB 3-1 and KB 8-5 cell lines (Western blot) (a, b). Effect of Akt kinase activity suppression on mRNA expression of *MRP1* (*ABCC1*) gene in KB 8-5 cells (c). a, b) IC_{50} of bortezomib is $2.5 \cdot 10^{-8}$ and $5 \cdot 10^{-8}$ M for KB 3-1 and KB 8-5 cells, respectively. c) Expression of *MRP1* gene in KB 8-5 cells exposed to bortezomib (IC50 of bortezomib is $5 \cdot 10^{-8}$ M) and Ly 294002 (a specific inhibitor of Akt-kinase phosphorylated form, dose of $1 \cdot 10^{-2}$ M). RT-PCR. c, control.

found that bortezomib resistance of cell subline K 562/i-$S9_{vlc}$ as compared with original cell line K 562/i-S9 increased tenfold. At the same time, bortezomib sensitivity of K 562_{vlc} cells remained almost unchanged as compared with K 562 cells (Fig. 2a).

Thus, it is evident that resistant sublines could not be obtained from a population of cells with low levels of Pgp after the same period of selection that was enough to create a bortezomib-resistant population from the cell line with Pgp overexpression.

Comparison of K 562/i-S9 subline and K 562/i-$S9_{vlc}$ showed that K 562/i-$S9_{vlc}$ cells (after cultivation with bortezomib) contained more phosphorylated Akt-kinase form (Fig. 2b). Investigation of Pgp protein expression and stem cells markers CD34 and CD38 in K 562/i-S9 and K 562/i-$S9_{vlc}$ populations showed that Pgp content increased twofold in K 562/i-$S9_{vlc}$ cell populations as compared with K 562/i -S9 cells (table). These data indicate that it was not the number of cells expressing Pgp increasing, but the amount of Pgp protein in the cells. The number of cells expressing CD34 also increased in the K 562/i-$S9_{vlc}$ cell population. CD38 expression remained at the same low level (data not shown), which is characteristic of blood stem cells [23]. These data indicate that the selection of K 562/i-S9 cells with bortezomib induced selection of variants with constitutively activated Akt kinase and overexpression of Pgp and CD34 marker of hemopoietic stem cells.

When comparing K 562 line and its subline K 562_{vlc}, there were no differences observed in the expression of Pgp, CD34, and CD38, the expression of these proteins remaining low in the cells of both populations, which is characteristic of K 562 cells.

Changes in YB-1 protein status under bortezomib treatment of MCF-7 cells. To further explore the molecular mechanisms that determine the effect of bortezomib

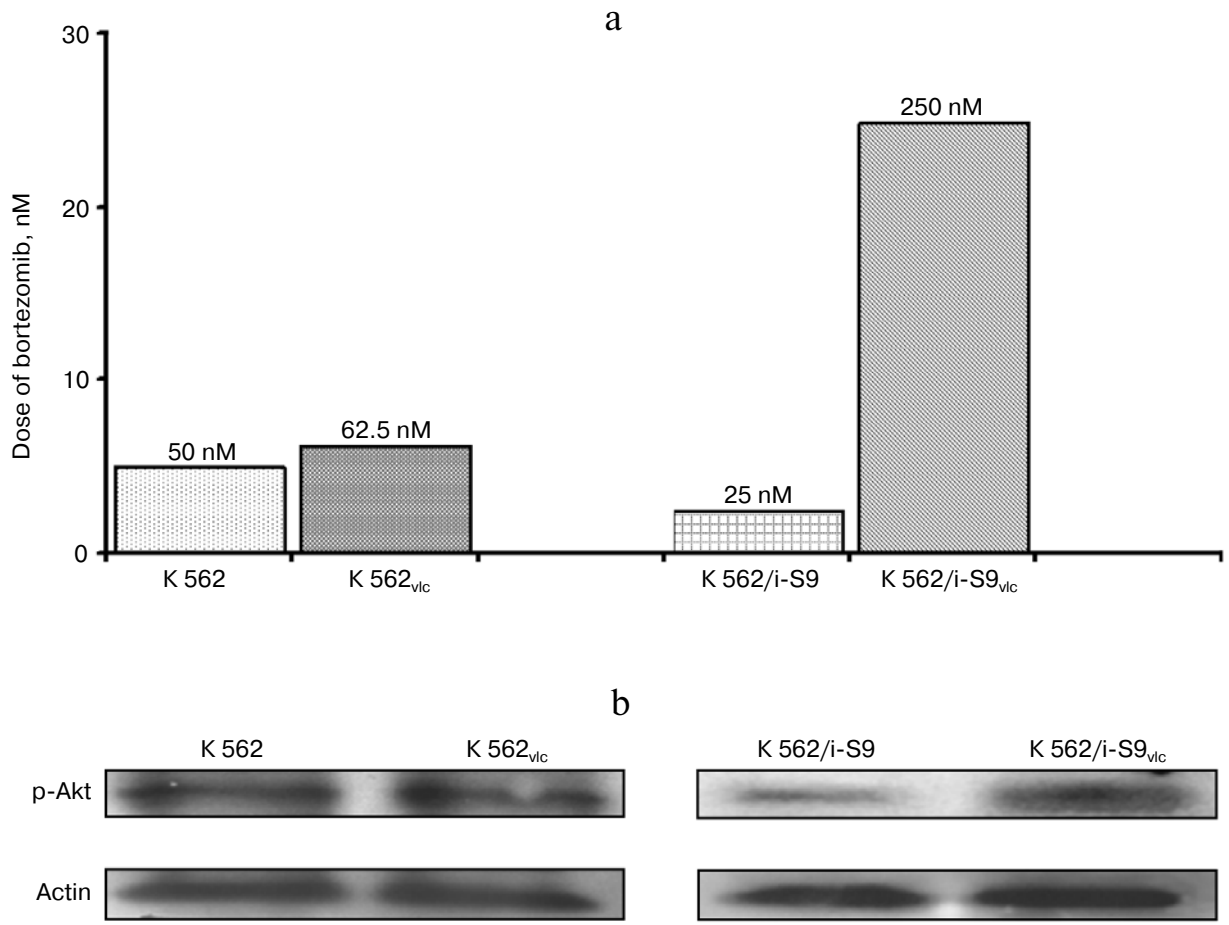

Fig. 2. a) Comparison of IC_{50} (concentration causing death of 50% of cells) for K 562 cells and their derivatives K 562/i-S9 with overexpression of P-glycoprotein (Pgp, ABCB1) before and after culturing in the presence of bortezomib for nine weeks. The cells cultured in the presence of bortezomib are labeled as $K\ 562_{vlc}$ and $K\ 562/i\text{-}S9_{vlc}$. MTT test. b) Constitutive expression of Akt kinase phosphorylated form (Western blot) in cells of K 562 and K 562/i-S9 lines before and after culturing in the presence of bortezomib for nine weeks.

on MDR genes and proteins, we decided to determine whether bortezomib affects the amount of mRNA and localization of YB-1 protein in tumor cells. Breast adenocarcinoma MCF-7 cell line was used as a model; the cells were well spread on the substrate, which allowed observing changes in intracellular localization of YB-1.

Bortezomib reduced the content of *MRP1* mRNA in the MCF-7 population (Fig. 3a). In these cells, the *MDR1* gene is not expressed at all or is expressed very weakly. We may only say that the bortezomib treatment did not lead to an increase in the amount of mRNA of this gene. The investigation of the influence of bortezomib on

Expression of P-glycoprotein (Pgp, ABCB1) and stem cell protein CD34 by K 562/i-S9 cells with P-glycoprotein (Pgp, ABCB1) hyperexpression before and after cultivation with bortezomib for nine weeks

Protein antibodies	K 562/i-S9		$K\ 562/i\text{-}S9_{vlc}$*	
	% of cells that bound the antibodies	mean fluorescence intensity	% of cells that bound the antibodies	mean fluorescence intensity
Pgp (ABCB1)	98	325	98.4	736
CD34	10.2	58.3	17.4	54

* K 562/i-S9 cells cultivated with bortezomib for nine weeks.

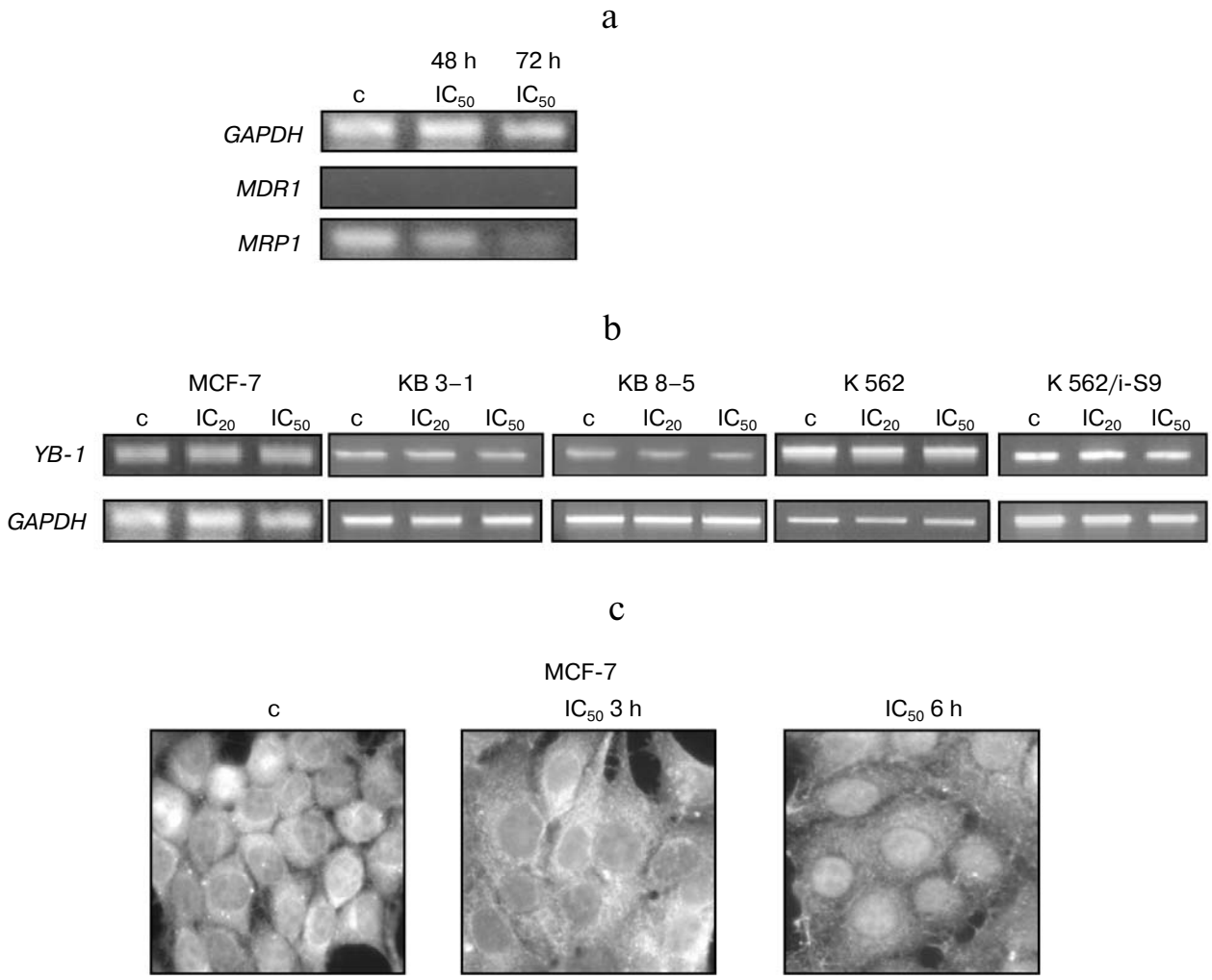

Fig. 3. Effects of bortezomib (IC50): a) on expression of *MDR1* (*ABCB1*) and *MRP1* (*ABCC1*) genes in MCF-7 cells (RT PCR); b) on expression of *YB-1* gene in cells from MCF-7, KB 3-1, KB 8-5, K 562, and K 562/i-S9 cell lines (RT PCR). $IC_{20} = 1.25 \cdot 10^{-8}$ M (KB 3-1 and K 562/i-S9), $2.5 \cdot 10^{-8}$ M (KB 8-5 and K 562) and $3.125 \cdot 10^{-9}$ M (MCF-7). $IC_{50} = 2.5 \cdot 10^{-8}$ M (KB 3-1 and K 562/i-S9) and $5 \cdot 10^{-8}$ M (KB 8-5 and K 562); c) on the intracellular localization of YB-1 in MCF-7 cells (immunohistochemical staining of cells with antibodies to YB-1).

the amount of *YB-1* mRNA in the population of MCF-7 cells and other cell lines showed no change in mRNA amount of this gene (Fig. 3b).

Using immunocytochemical staining, we determined the intracellular localization of YB-1 protein. YB-1 protein is mainly located in the cytoplasm in cells of MCF-7 line (Fig. 3c). After bortezomib treatment with a dose of IC_{50} for 6 h, YB-1 moved into the nucleus (Fig. 3c). These data indicate that the activity of YB-1 as a transcription factor can be increased under bortezomib treatment in the tumor cell population.

DISCUSSION

We previously showed that treatment of tumor cells with bortezomib led to increased amounts of these two proteins (Pgp or MRP1) in three of the four studied cell populations [2]. In the previous and present work, we showed that bortezomib not only affects the amount of proteins, but it also reduces mRNA amount of the studied proteins in some cell populations (KB 8-5 and MCF-7 lines). This decline can be attributed to various causes, especially to the connection between the accumulation of the proteins in the cell and gene activity. It may be that bortezomib inhibits the transcription of several ABC transporters genes. This kind of activity of bortezomib has recently been revealed: it was shown that bortezomib directly inhibits transcription of the estrogen receptor encoding gene in MCF-7 cells [24]. Since the effect of bortezomib on the amount of ABC transporter mRNA was detected only in some cell lines, one might think that this effect is dependent on some elements of the cellular context.

To identify the elements of the cellular context that may determine cell response to bortezomib, we compared cell line KB 8-5, selected by Pgp overexpression, and parental cells KB 3-1. We found that bortezomib increased the amount of phosphorylated form of Akt-kinase in the KB 8-5 population, whereas in KB 3-1 cells the drug reduced it. Apparently, KB 8-5 cells differ from KB 3-1 cells by increased inducibility of Akt-kinase with bortezomib. Bortezomib also reduces the amount of Erk1/2-kinase phosphorylated form in KB 8-5 cells, and it has no effect on this kinase in KB 3-1 cell populations (Fig. 1b). Our data indicate that cell selection for Pgp overexpression in selected cells was also the selection of the cells in which bortezomib is able to activate Akt-kinase. In this case, obviously, a suppression of MAP-kinase cascade occurs with an increase in Akt kinase activity in KB 8-5 cells. To our knowledge, such switching of signal transduction pathway activity has not been previously described for bortezomib treatment of tumor cells. We have found that the inhibitor of Akt-kinase activity Ly 294002 increases the number of *MRP1* mRNA in KB 8-5 cells (Fig. 1c). These results indicate that the reduction of *MRP1* mRNA amount under the influence of bortezomib in KB 8-5 cells is due to the activation of Akt-kinase by bortezomib.

Changes in activity of the PI3K/Akt signaling pathway may lead to changes in expression of genes and proteins of the ABC family. Previously, we found that the suppression of Akt-kinase activity by phosphatase PTEN is associated with multidrug resistance of prostate cell carcinomas and MRP1 transporter expression and activity [8]. Obviously, *MRP1* gene expression may be regulated through the PI3K/Akt signaling pathway, but the nature of the changes in the mRNA of the gene depends on cell type: activation of Akt in prostate carcinoma cells resulted in increase in *MRP1* mRNA amount, and in KB 8-5 cells it reduced the content of this mRNA. It is possible that these differences are caused by the fact that KB 8-5 cells, in contrast to PC3, constitutively express genes of several ABC transporters. Our data indicate that overexpression of at least one of the ABC transporters is an element of the cellular context that affects the nature of regulation of these transport proteins with bortezomib.

Results obtained in this study show that exposure of malignant cell cultures overexpressing Pgp (K 562/i-S9 cells) to bortezomib for nine weeks quickly formed a population highly resistant to bortezomib: bortezomib resistance increased tenfold. Wild type cells (K 562) did not become resistant to bortezomib after the same period of culturing with this drug. Our data indicate that in the K 562/i-S9 population under bortezomib treatment cell variants with increased Pgp expression and constitutively activated Akt kinase obtained selective advantage. According to our data, each K 562/i-$S9_{vlc}$ cell resistant to bortezomib was expressing higher amounts of Pgp, which suggests that Pgp expression may be regulated by activated Akt kinase. It is not clear whether this regulation occurs in K 562/i-S9 cells at the transcriptional level or the translational level.

Our data indicate that bortezomib may affect the transcription of ABC transporters, inducing relocation of transcription factors into the nucleus of tumor cells. We found that bortezomib treatment of breast cancer cells leads to the relocation of YB-1 protein from cytoplasm to cell nucleus. Perhaps the effect of bortezomib on ABC transporter gene transcription with the participation of Akt-kinase may be achieved by different mechanisms, one of which could be transcriptional activity of YB-1 protein, which is known to be activated by Akt-kinase. We have previously shown that drug-resistant cell populations have increased number of cells with nuclear localization of YB-1 [22]. Such an increase may also increase the number of cells in which YB-1 protein can function as a transcription factor.

The influence of bortezomib on ABC transporter protein expression may be related to tumor progression. When studying the prolonged effects of bortezomib on K 562/i-S9 cells, we obtained evidence that this exposure leads to accumulation of bortezomib-resistant cells with constitutively activated Akt kinase, increasing expression of Pgp and CD34 proteins in the population. Pgp and CD34 expression is characteristic of hematopoietic stem cells and stem cells of CML (chronic myeloleukemia), or, as they are called, tumor-initiating cells. CSC (cancer stem cells) may accumulate during the treatment of various tumors with different drugs. For example, accumulation of CSC of this neoplasm was also observed during CML treatment with imatinib (Gleevec) [25], and data obtained in the study of multiple myeloma suggest that CSC, causing the progression of multiple myeloma, are resistant to bortezomib [26]. Cells expressing CD44 protein, a marker of breast tissue stem cells, accumulated under Herceptin treatment of cultured breast cancer cell population BT474 [27]. This effect was associated with transcriptional activity of YB-1. The results we obtained in this study suggest that CSC multiply during tumor drug therapy not only because they express ABC transporters and some other protective cell systems, but also because some drugs such as bortezomib induce additional activity of cellular defense systems, including transport proteins of the ABC family and Akt kinase.

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