Development of Drug Resistance in the Population of Colon Cancer Cells under the Effect of Multifunctional Protein YB-1

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The effects of *YB-1* gene on the expression level of P-glycoprotein and drug resistance of tumor cells were studied in cultured HCT116 colon cancer cells. Transitory transfection of chimeric *YB-1/GFP* gene rendered HCT116 cells a selective advantage in a medium with vinblastine, which caused translocation of the chimeric protein into cell nuclei. This was paralleled by an increase in the expression of P-glycoprotein (multiple drug resistance protein).

Key Words: *YB-1 protein; tumor cells; colorectal cancer; P-glycoprotein*

Mammalian YB-1 protein belongs to the multifunctional family of DNA/RNA-binding proteins [7]. Upon binding to DNA, YB-1 acts as a transcription factor and regulates expression of genes containing the Y-box element in promotors and enhancers, including the expression of multiple drug resistance gene (*MDR1*) [10]. In addition, YB-1 is involved in reparation, recombination, and replication of DNA [7]. Reacting with mRNA, this protein participates in mRNA splicing in the nucleus [3], serves as the main packing protein for mRNA in the cytoplasm [13], and regulates the life span [5] and matrix activity of mRNA in protein synthesis [4]. Hence, YB-1 regulates various processes in the cell at different levels.

Multiple drug resistance (MDR) of tumor cells is a serious obstacle for chemotherapy of malignant tumors. MDR is a system of protection of tumor cell population from many drugs differing by chemical structure and mechanism of action [1]. Drug resistance of tumor cells is an intricate phenomenon based on a series of molecular changes; several mechanisms modulating the sensitivity of tumor cell to treatment can simultaneously function in one cell [1]. Attempts at overcoming MDR gave no clinically significant results. These failures can be explained by insufficient knowledge of the molecular mechanisms of multifactor MDR; YB-1 can be an important regulator of MDR.

Protein YB-1 is translocated from the cytoplasm into the nucleus after cell exposure to DNA-damaging agents, UV, and heat shock [9,2,15]. Nuclear location of YB-1 correlates with the expression of P-glycoprotein (Pgp) in breast cancer, osteosarcoma, lung cancer [2,8,11]; however, no relationships between YB-1 and Pgp expression was detected in colon cancer [12]. It was shown that YB-1 positively regulates the transcription of MDR genes *MDR1* and *LRP* [10,14]. However, other data indicate that activity of YB-1 is insufficient for activation of *MDR1* gene in genotoxic stress [6]. Hyperthermia causes translocation of YB-1 from the cytoplasm into the nuclei of HCT116 and HCT15 colorectal cancer cells, which is paralleled by an increase in the expression of *MDR1* and *MRP1* ge-

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nes, but no drug resistance develops in cells in this case [15]. These data suggest that the effect of YB-1 on the MDR phenotype depends on tumor histogenesis. We evaluated the effect of YB-1 on colon cancer cell MDR.

MATERIALS AND METHODS

The study was carried out on colorectal cancer cell HCT116 cultured in DMEM with 10% fetal calf serum at 37°C and 5% CO_2.

YB-1 gene cDNA was inserted in the EcoR1 restriction site of pEGFP-N3 vector (Clontech). Transfection of HCT116 cells (3×10^5) with pEGFP-N3/YB-1 or pEGFP-N3 plasmids (1 μg/ml) was carried out using Escort IV (2 μl/μg plasmid, Sigma-Aldrich) according to manufacturer's instruction. Vinblastine was added to the culture medium 24 h after transfection. Cells containing GFP (green fluorescent protein) were counted after 2-day incubation in medium with the drug.

The count of transfected cells expressing GFP was evaluated using flow cytofluorometer (Becton Dickinson) and special software (3000-5000 cells were analyzed in each experiment).

For evaluation of the of Pgp expression, the cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 for 10 min at ambient temperature. The cells were then incubated with mouse monoclonal antibodies UIC2 (for Pgp; Chemicon) for 1 h at 32°C, washed 3-fold in a buffer, incubated with FITC-labeled goat anti-mouse IgG antibodies (Chemicon) for 1 h at 32°C, and washed 3 times in PBS. The count of cells with bound antibodies and intensity of their fluorescence were evaluated on a flow cytofluorometer.

For evaluation of intracellular location of YB-1, the cells were cultured on coverslips in 30-mm Petri dishes, fixed in 4% paraformaldehyde, washed 3 times in PBS, and permeabilized with 0.1% Triton X-100. The cells were then incubated with rat polyclonal anti-YB-1 antibodies (antibodies to YB-1 were obtained from rats immunized with a synthetic peptide corresponding to 15 C-terminal amino acids of YB-1, AENSSAPEAEQGGAE). After incubation with the first antibodies, the cells were washed 3 times in PBS and incubated with the second antirat tetramethylrhodamine isothiocyanate-labeled antibodies. Cells with cytoplasmic and nuclear location of YB-1 were counted using a fluorescent microscope (Zeiss).

RESULTS

The efficiency of cell transfection varied from 15 to 30%. In our experiments transitory transfection of experimental and control plasmid did not affect HCT116 cell proliferation, the number of cells in the control was the same.

On day 2 of incubation with the drug, the number of cells with GFP (carrying the transgene) decreased in the culture transfected with *GFP* in comparison with untreated population (Fig. 1, *a*). The count of cells containing GFP increased significantly in the culture of cells transfected with *YB-1/GFP* and treated with vinblastine in comparison with untreated population (Fig. 1, *a*), which attests to selection of cells carrying the *YB-1/GFP* gene (but not cells transfected with *GFP* gene) during incubation with vinblastine. This effect on HCT116 cells transfected with *YB-1/GFP* depended on drug concentration in the medium (Fig. 1, *b*).

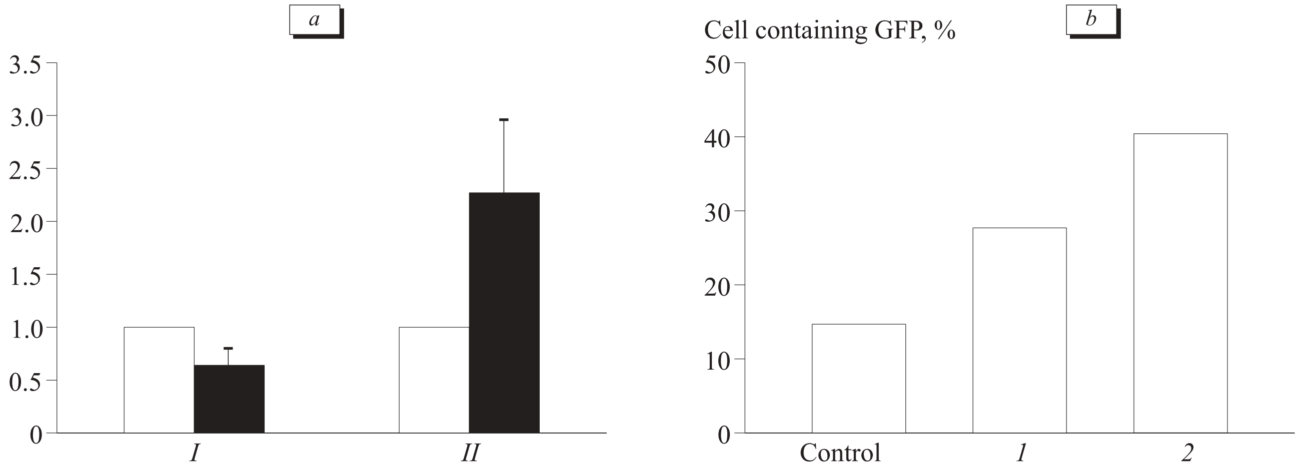

Fig. 1. Survival of HCT116 cells transitory transfected with different constructs. *a*) transfection with constructs containing *GFP* (*I*) or *YB-1/GFP* (*II*) genes followed by vinblastine treatment (10^{-9} M). Ordinate: changes in the number of cells with GFP (dark bars) in medium with vinblastine compared to the control (light bars); *b*) transfection with a construct containing *YB-1/GFP* gene followed by vinblastine treatment in a dose of 10^{-10} M (*1*) and 10^{-9} M (*2*).

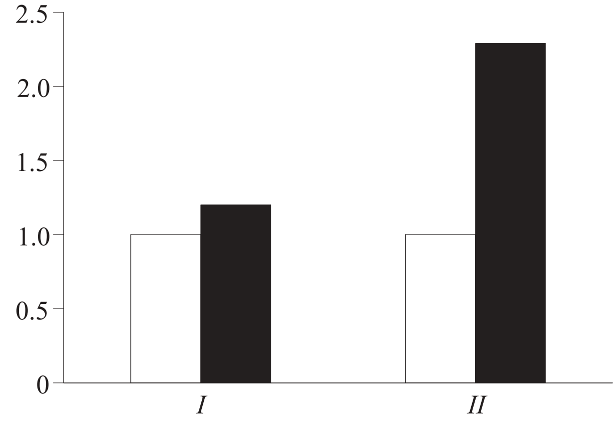

Fig. 2. Binding of antibodies to Pgp (UIC2) by HCT116 cells after treatment with vinblastine. Ordinate: changes in the number of cells binding UIC2 in a medium with vinblastine compared to the control. Light bars: control; dark bars: vinblastine. *I*: *GFP*; *II*: *YB-1/GFP*.

These results showed that incubation with the drug predominantly selected cells carrying the *YB-1/GFP* transgene. YB-1 incorporated into cells significantly increased the number of vinblastine-resistant cells in HCT116 cell population. The drug did not select cells transfected with control *GFP* gene. The selective advantage of cells transfected with *YB-1/GFP* in the medium with vinblastine was obviously due to the development of the MDR phenotype in these cells.

Staining with UIC2 monoclonal antibodies showed that on day 2 of culturing in medium with vinblastine the count of cells binding UIC2 significantly increased in the population transfected with *YB-1/GFP*. This increase was highly pronounced after drug treatment and was observed only in cell population transfected with *YB-1/GFP* construct. No changes of this kind were observed in *GFP*-transfected population (Fig. 2).

Staining with antibodies to YB-1 showed that incubation of HCT116 cells with vinblastine led to translocation of YB-1 from the cytoplasm into the nucleus (Fig. 3). YB-1 functions both in cell cytoplasm and nucleus. Translocation of YB-1 from the cell cytoplasm into the nucleus was observed after stress exposure of cell, including treatment with

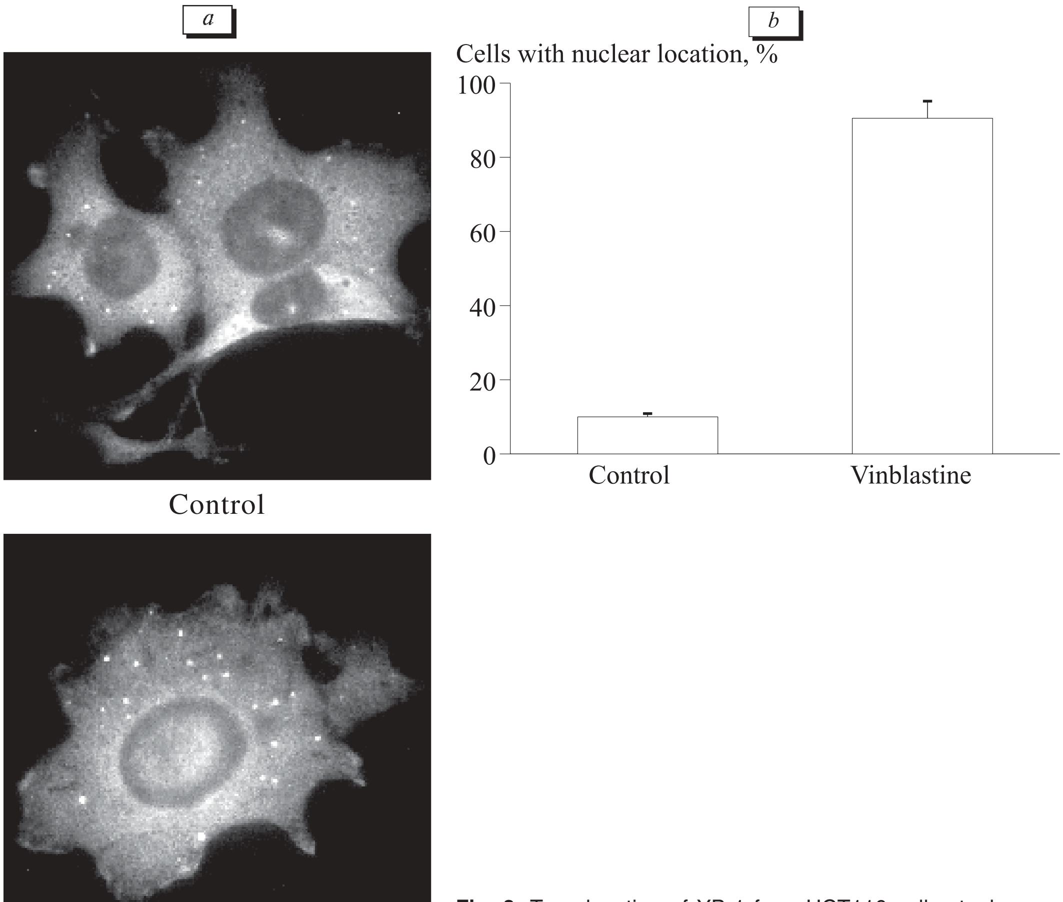

Fig. 3. Translocation of YB-1 from HCT116 cell cytoplasm into nucleus after vinblastine treatment: staining of cells with antibodies to YB-1 (*a*) and analysis of YB-1 location under a fluorescent microscope (*b*).

antitumor drugs, DNA-damaging agents, UV radiation, and hyperthermia [8,9,15]. The role of YB-1 in the regulation of MDR is usually attributed to its functioning as a transcription factor. It cannot be excluded that MDR1 is regulated at the transcription level (at least in HCT116 cells).

Translocation of YB-1 and induction of Pgp expression under the effect of the cytostatic can be explained by the results of our experiments, which showed selective advantage of cells transitory transfected with *YB-1/GFP* gene in a medium with vinblastine. This can be caused by a combination of YB-1 translocation into the nucleus under the effect of the drug and increase of its intracellular concentration as a result of transfection.

Our data indicate that YB-1 renders drug resistance to colon carcinoma cells due to activation of at least one of the ABC family transport proteins. These data indicate that activity of YB-1 can determine the initial stages of MDR formation in tumor cells population.

Hence, transitory insertion of a construct containing *YB-1/GFP* gene into HCT116 colorectal carcinoma cell renders these cells a selective advantage in a vinblastine-containing medium. Vinblastine caused translocation of YB-1/GFP chimeric protein from the cytoplasm into the nucleus of HCT116 cells. In parallel, cells transfected with chimeric *YB-1/GFP* gene exhibited high expression of Pgp protein compared to cells transfected with control *GFP* transgene.

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REFERENCES

1. A. A. Stavrovskaya, *Biokhimiya*, **65**, 112-126 (2000).
2. R. C. Bargou, K. Jurchott, C. Wagener, *et al.*, *Nat. Med.*, **3**, No. 4, 447-450 (1997).
3. H. A. Chansky, M. Hu, D. D. Hickstein, and L. Yang, *Cancer Res.*, **61**, No. 9, 3586-3590 (2001).
4. E. K. Davydova, V. M. Evdokimova, L. P. Ovchinnikov, and J. W. Hershey, *Nucleic Acids Res.*, **25**, No. 14, 2911-2916 (1997).
5. V. Evdokimova, P. Ruzanov, H. Imataka, *et al.*, *EMBO J.*, **20**, No. 19, 5491-5502 (2001).
6. Z. Hu, S. Jin, and K. W. Scotto, *J. Biol. Chem.*, **275**, No. 4, 2979-2985 (2000).
7. K. Kohno, H. Izumi, T. Uchiumi, *et al.*, *Bioessays*, **25**, No. 7, 691-698 (2003).
8. Y. Oda, A. Sakamoto, N. Shinohara, *et al.*, *Clin. Cancer Res.*, **4**, No. 9, 2273-2277 (1998).
9. T. Ohga, K. Koike, M. Ono, *et al.*, *Cancer Res.*, **56**, No. 18, 4224-4228 (1996).
10. T. Ohga, T. Uchiumi, Y. Makino, *et al.*, *J. Biol. Chem.*, **273**, No. 11, 5997-6000 (1998).
11. K. Shibahara, K. Sugio, T. Osaki, *et al.*, *Clin. Cancer Res.*, **7**, No. 10, 3151-3155 (2001).
12. K. Shibao, H. Takano, Y. Nakayama, *et al.*, *Int. J. Cancer*, **83**, No. 6, 732-737 (1999).
13. M. A. Skabkin, O. I. Kiselyova, K. G. Chernov, *et al.*, *Nucleic Acids Res.*, **32**, No. 18, 5621-5635 (2004).
14. U. Stein, S. Bergmann, G. L. Scheffer, *et al.*, *Oncogene*, **24**, No. 22, 3606-3618 (2005).
15. U. Stein, K. Jurchott, W. Walther, *et al.*, *J. Biol. Chem.*, **276**, No. 30, 28,562-28,569 (2001).