BIOPHYSICS AND BIOCHEMISTRY

Expression of Genes for Redox-Dependent Glutathione S-Transferase Isoforms GSTP1-1 and GSTA4-4 in Tumor Cell during the Development Doxorubicin Resistance

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 3, pp. 298-301, March, 2007
Original article submitted July 11, 2006

Expression of genes for redox-dependent glutathione S-transferase isoforms GSTP1-1 and GSTA4-4 in tumor cells K562, MCF-7, and SKOV-3 was studied during the development of resistance to doxorubicin. It was found that the development of resistance was accompanied by predominant increase in the expression of hGSTP1 gene in MCF-7 cells, and hGSTA4 gene in resistant K562/DOX and SKVLB cells.

Key Words: *drug resistance; glutathione S-transferase isoforms GSTP1-1 and GSTA4-4; doxorubicin*

The development of tumor cell resistance to doxorubicin (DOX) is mainly associated with changes in cellular redox status under conditions of oxidative stress induced by this antitumor drug [1,8]. Apart from antioxidant enzymes, isoforms of the multienzyme family of glutathione S-transferases (GST, EC 2.5.1.18) play an important role in the processes blocking the development of oxidative stress [10]. At the same time GST participate in the formation of drug resistance by contributing to enhanced detoxification of antitumor drugs and/or high-reactivity metabolites via glutathione-dependent conjugation with the formation of nonactive products. These products in the form of mercapturates are easily eliminated from the organism [12]. Seven classes of cytosolic GST isoforms (α, μ, π, σ, θ, ω, and ζ) were identified in humans and animals using the primary amino acid sequence. Among them, isoforms of α and π classes play an important role in detoxification of oxidative stress products [10].

Here we studied the expression of genes for redox-dependent isoforms GSTP1-1 and GSTA4-4 during the development of tumor cell resistance to DOX. The study was performed with tumor cells of different genesis, including erythroleukemia K562 cells, breast carcinoma MCF-7 cells, and ovarian carcinoma SKOV-3 cells.

MATERIALS AND METHODS

Experiments were performed on human erythroleukemia K562 cells, human breast carcinoma MCF-7 cells, and human ovarian carcinoma SKOV-3 cells. They were sensitive (K562/S, IC_{50} 0.005 μg/ml; MCF-7/S, IC_{50} 0.003 μg/ml; and SKOV-3, IC_{50} 0.2 μg/ml) or resistant to DOX (K562/DOX, IC_{50} 4.0 μg/ml; MCF-7/DOX, IC_{50} 4.1 μg/ml; and SKVLB, IC_{50} 4.5 μg/ml). The suspension of K562 cells was

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cultured in RPMI-1640 medium (Sigma). The monolayer of MCF-7 and SKOV-3 cells was cultured in DMEM (Sigma). The media contained 10% heat-inactivated fetal bovine serum (Gibco BRL), 2 mM L-glutamine, 100 U/ml penicillin, and 50 μg/ml streptomycin. Culturing was performed in a humid atmosphere at 5% CO_2 and 37°C. Cell resistance was induced by culturing in the presence of increasing cytostatic concentrations.

The level of mRNA for GST isoforms was measured by reverse transcription polymerase chain reaction (RT-PCR). RNA was isolated using RNAwiz kit (Ambion) according to manufacturer's recommendations. Total RNA (5 μg) from each sample served as the matrix for the synthesis of the first cDNA strand with reverse transcriptase Superscript II (Invitrogen). PCR was conducted under the following conditions: 3 min at 94°C; 35 cycles: 30 sec at 94°C, 20 sec at 55-64°C, 30 sec at 72°C; 5 min at 72°C. We used primers for GST P1-1 (direct, 5'-ACCCCAGGGCTCTATGGGAA-5'; reverse, 5'-TG AGGGCACAAGAAGCCCCT-3'), GST A4-4 (direct, 5'-ATGGCAGCAAGGCCCAAGCTCCACT TC-3'; reverse, 5'-TTATGGCCTAAAGA TGTTG TAGACGGTTCT-3'), and β-actin (direct, 5'-CCAC GAAACTACCTTCAACTCC-3'; reverse, 5'-TCG TCATACTCCTGCTTGCTGATCC-3'). PCR products were separated by electrophoresis in 1.5-2.0% agarose gel followed by densitometry. mRNA content was standardized by β-actin mRNA level. GST activity was measured spectrophotometrically by the formation of products from 1-chloro-2,4-dinitrobenzene (1 mM), ethacrynic acid (0.2 mM), or 4-hydroxynonenal (0.1 mM), respectively [2,9].

The results were analyzed by Student's *t* test. The data are expressed as $M \pm SD$.

RESULTS

Evaluation of the expression of GST isoforms in tumor cell lines showed that the development of resistance to DOX was associated with intensive accumulation of GSTP1-1 mRNA (Fig. 1, *a*, *b*). The increase in mRNA level was maximum in resistant MCF-7/DOX cells (by 17 times compared to sensitive cells) and less pronounced in SKVLB and K562/DOX cells (by 4 times). Simultaneously, GST activity in MCF-7/DOX cells increased more significantly than in K562/DOX and SKVLB cells: for the universal substrate for all GST isoforms 1-chloro-2,4-dinitrobenzene by 17.2, 2, and 1.7 times, re-

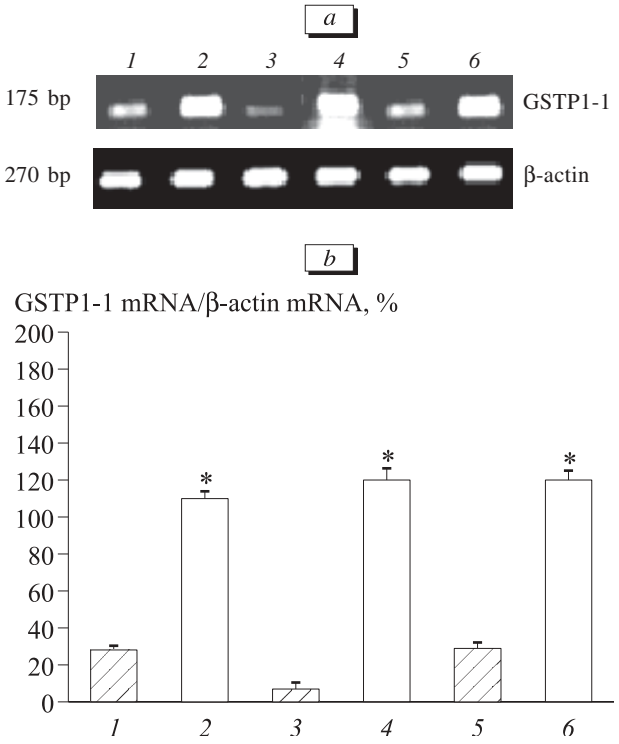

Fig. 1. RT-PCR for GSTP1-1 mRNA level in DOX-sensitive (K562/S, *1*; MCF-7/S, *3*; and SKOV-3, *5*) and resistant cells (K562/DOX, *2*; MCF-7/DOX, *4*; and SKVLB, *6*). Here and in Fig. 2: electrophoretogram of RT-PCR products, β-actin mRNA was used as the positive control (*a*); GSTP1-1 mRNA level relative to β-actin mRNA level (%) according to the results of densitometry (n=4, *b*). *p<0.05 compared to sensitive cells of each line (shaded bars).

TABLE 1. GST Activity in Tumor Cells K562, MCF-7, and SKOV-3 during the Development of DOX Resistance ($M \pm m$)

Cell line	GST activity, nmol/mg protein/min		
	1-chloro-2,4-dinitrobenzene (n=6)	ethacrynic acid (n=5)	4-hydroxy-2,3-nonenal (n=6)
K562/S	13.6±0.6	3.51±0.42	1.62±0.54
K562/DOX	27.2±0.9***	5.22±0.53*	4.70±0.48*
MCF-7/S	6.9±0.6	2.14±0.92	2.73±0.27
MCF-7/DOX	123±12***	7.43±0.61**	3.82±0.25*
SKOV-3	16.3±1.7	4.12±0.26	0.99±0.18
SKVLB	28.0±1.1**	6.64±0.45**	3.25±0.49**

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to sensitive cells of the corresponding line.

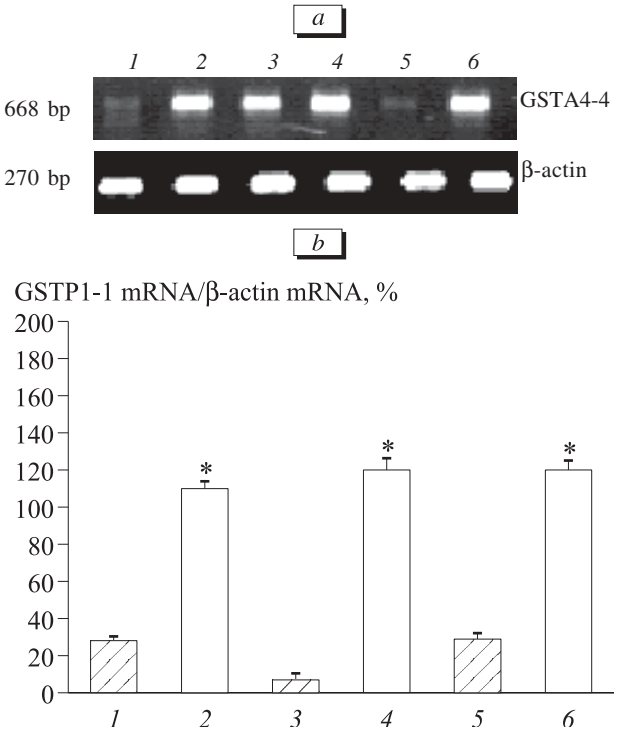

Fig. 2. RT-PCR for GSTA4-4 mRNA level in DOX-sensitive (K562/S, *1*; MCF-7/S, *3*; and SKOV-3, *5*) and resistant cells (K562/DOX, *2*; MCF-7/DOX, *4*; and SKVLB, *6*).

spectively, and for GSTP1-1-specific substrate ethacrynic acid by 3.5, 1.5, and 1.6 times, respectively.

GSTA4-4 gene expression significantly increased in resistant SKVLB and K562/DOX cells (Fig. 2, *a*, *b*). As compared to sensitive cells the level of GSTA4-4 mRNA increased most significantly in resistant SKVLB cells (by 18 times), and to a lesser extent in K562/DOX cells (by 16 times). mRNA level increased insignificantly in MCF-7/DOX cells (by 1.7 times). mRNA level was maximum in sensitive MCF-7/S cells, but 11-fold lower in K562/S and SKOV-3 cells. GST activity to specific GSTA4-4 substrate 4-hydroxy-2,3-nonenal increased most significantly in SKVLB cells (by 3.3 times), intermediately in K562/DOX cells (by 2.9 times), and minimally in MCF-7/DOX cells (by 1.4 times, Table 1).

Free radical processes also contribute to the development of DOX resistance of tumor cell, because DOX activates oxidative stress, including lipid peroxidation (LPO) in mitochondrial membranes, endoplasmic reticulum, and nucleus. Generation of $O_2^{\bullet-}$ and H_2O_2 under the influence of DOX is related to the presence of a quinone redox cycle in its structure. In the presence of mixed-valence metal ions (Fe^{2+} and Cu^{2+}), DOX contributes to the formation of high-reactivity $^{\bullet}OH$ radicals attacking nucleic acids, proteins, and lipids [6].

The induction of GSTP1-1 and GSTA4-4 isoforms is mainly associated with activation of free radical processes. GSTP1-1 prevents the destructive effect of oxidative stress. GSTP1-1 is highly reactive to peroxidation products of DNA and lipids, including propenals of nitrogen compounds and acrolein [4]. Published data show that GSTP1-1 expression reaches a high level during the development of multidrug resistance [3,12]. Transfection of tumor cells with the hGSTP1 genome is followed by a 2-fold increase in resistance to DOX [11]. At the same time, the GSTA4-4 isoform is responsible for cell protection during activation of LPO. This isoform is highly specific to 4-hydroxy-2-alkenals formed after β-cleavage of hydroperoxides in polyunsaturated fatty acids [5]. The increase in 4-hydroxy-2,3-nonenal concentration has a toxic effect and results in apoptosis [7].

The development of MCF-7 cell resistance to DOX is accompanied by predominant expression of the GSTP1-1 gene. However, the development of resistance of K562 and SKOV-3 cells mainly results in induction of GSTA4-4 gene expression.

Our results indicate that the increase in the expression of genes for GSTP1-1 and GSTA4-4 isoforms in resistant tumor cells is a part of the adaptive antioxidant response to oxidative stress induced by increasing concentrations of DOX during the formation of drugs resistance. It should be emphasized that the expression of these genes depends on the genesis of tumor cells.

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