Expression of Genes for Thioredoxin 1 and Thioredoxin 2 in Multidrug Resistance Ovarian Carcinoma Cells SKVLB

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The expression of genes for thioredoxin isoforms Trx1 and Trx2 was studied in sensitive SKOV-3 and resistant SKVLB human ovarian carcinoma cells. The development of doxorubicin resistance was accompanied by a significant increase in the expression of *TRX1* gene and less pronounced increase in *TRX2* gene expression.

Key Words: *drug resistance; thioredoxin; doxorubicin*

Thioredoxin (Trx) is a multifunctional protein found in the majority of eukaryotic and prokaryotic cells characterized by the presence of two thiol groups in its active site. Trx acts as a protein disulfide reductase and participates in redox-dependent processes, including protein folding, regulation of apoptosis, cell proliferation, and antioxidant protection from oxidative stress [12]. The protein modulates activity of transcription factors (*e.g.*, AP-1, NF-κB, and p53).

Thioredoxin isoforms evolved as chaperone-like proteins responsible for redox-dependent regulation of the thiol/disulfide ratio in proteins providing their functional activity [11]. Cytosolic and nuclear Trx1 and mitochondrial Trx2 are the main isoforms of Trx. Disulfides in active sites of Trx1 and Trx2 is reduced by NADPH-dependent thioredoxin reductases 1 and 2, respectively [12].

High expression of Trx1 in some tumors is related to its function of growth factor [4]. At the same time, the development of drug resistance (*e.g.*, resistance to cis-diamminedichloroplatinum) is accompanied by increased expression of Trx1 [14]. However, the role of the Trx-dependent system in drug resistance of tumor cells is poorly understood.

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Here we studied the expression of genes for Trx1 and Trx2 in multidrug resistant ovarian carcinoma cells SKVLB.

MATERIALS AND METHODS

Experiments were carried out on human ovarian carcinoma cells SKOV-3 and SKVLB. SKOV-3 cells are sensitive to doxorubicin (DOX, 50% inhibitory concentration, IC_{50}=0.2 μg/ml) and SKVLB cells are DOX-resistant (IC_{50}=4.5 μg/ml). These cells were obtained from the Laboratory of Biochemistry (All-Russian Research Center for Molecular Diagnostics and Therapy). The monolayer of cells was cultured in DMEM (Sigma) containing 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. SKVLB cells were generated from parent SKOV-3 cells [1] as a result of the development of vincristine resistance [1]. SKVLB cells with cross-resistance to DOX were obtained by selection in the presence of 0.4 μg/ml DOX in the culture medium. The level of mRNA was measured in reverse transcription polymerase chain reaction (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 first

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strand cDNA synthesis with reverse transcriptase (Superscript II, Invitrogen). PCR was conducted under the following conditions: 3 min at 94°C; 30 cycles: 30 sec at 94°C, 20 sec at 55-64°C, and 30 sec at 72°C; and then 5 min at 72°C. We used primers for Trx-1 (direct, 5'-CATATGGTGAAGCAGA TCGAGAG-3'; reverse, 5'-TGTCACGCAGATGGCA ACTG-3'), Trx2 (direct, 5'-TTGGCTGACAAGCAG GGATGAG-3'; reverse, 5'-AAAGGCGTATGGG AGGGAAGAC-3'), and β-actin (direct, 5'-CCAC-GAAACTACCTTCAACTCC-3'; reverse, 5'-TCGT-CATACTCCTGCTTGCTGATCC-3'). PCR products were separated by electrophoresis in 1.5-2.0% agarose gel and analyzed by densitometry. mRNA level was calculated as a percent of β-actin mRNA level. Trx activity was measured spectrophotometrically from the decrease in NADPH concentration in the presence of thioredoxin reductase (Sigma). Insulin (160 μM) served as the substrate [7]. The results were analyzed by Student's *t* test. The data are expressed as *M*±*SD*.

RESULTS

Differences in the expression of genes for Trx1 and Trx2 were related to the development of DOX cross-resistance in SKVLB cells. The level of Trx1 mRNA in resistant SKVLB cells was 2.1-fold higher than in sensitive SKOV-3 cells. The level of Trx2 mRNA in SKVLB cells increased insignificantly (by 1.4 times; Fig. 1, *a*, *b*). Total activity of Trx increased by 7 times (Fig. 1, *c*).

The ability of DOX to induce oxidative stress due to quinone redox cycle with the formation of $O_2^{\bullet-}$, H_2O_2, and highly reactive $^{\bullet}OH$ radicals promote the development of tumor cell resistance to DOX [3]. Trx1 and Trx2 play an important role in cell antioxidant defense. *TRX1* gene expression is induced by various factors that cause oxidative stress, including H_2O_2, UV and γ-radiation, and postischemic reperfusion [8]. Increased expression of *TRX1* gene in resistant SKVLB cells is probably associated with its antioxidant properties. For example, *TRX1* gene-mutant yeast cells are highly sensitive to the cytotoxic effect of H_2O_2 [9]. Apart from the role of Trx1 as a cofactor for peroxiredoxins (proteins reducing H_2O_2 and organic hydroperoxides using Trx1 as electron donors), Trx1 acts as an $^{\bullet}OH$ radical scavenger [2,10]. Trx1 regulates activity of redox-dependent transcription factors NF-κB and AP-1 and provides their translocation

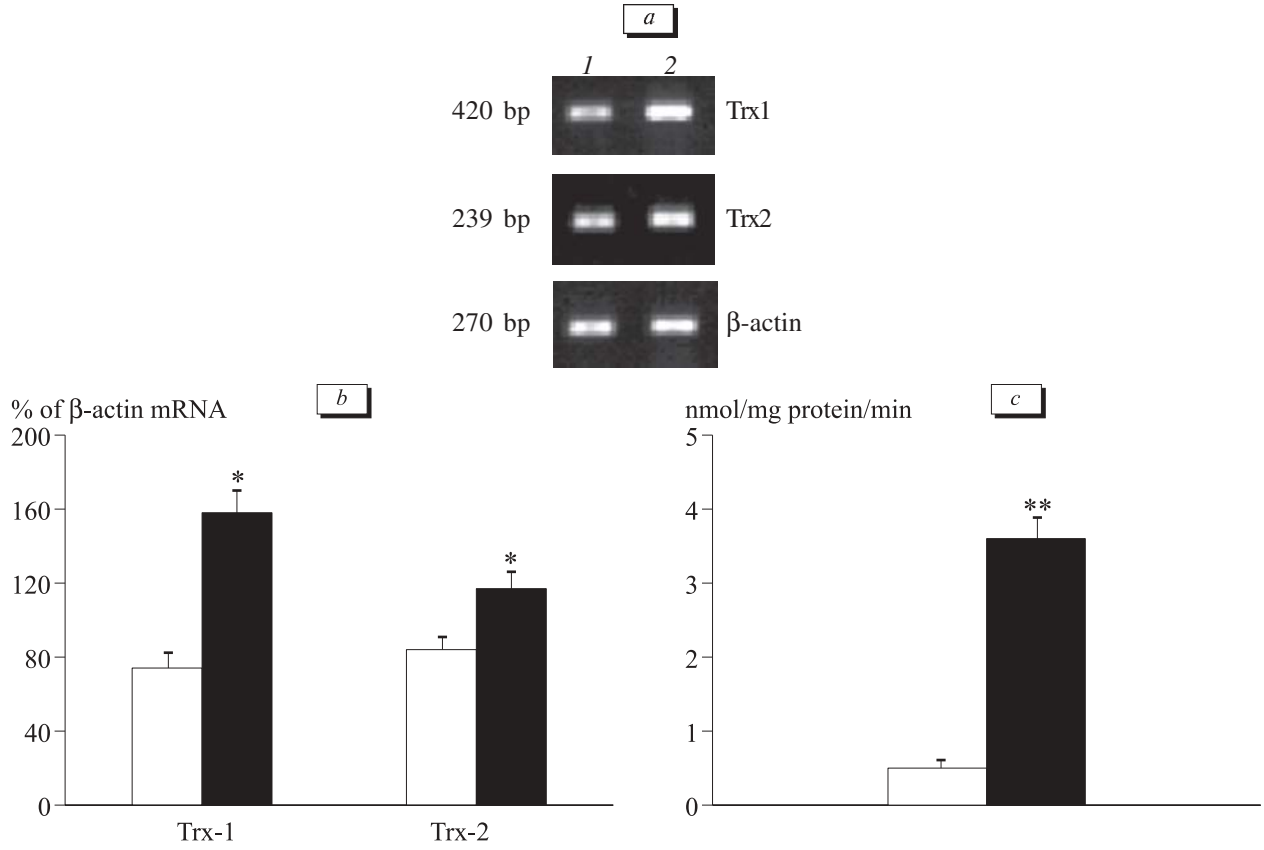

Fig. 1. Expression of *Trx1* and *Trx2* genes in sensitive SKOV-3 and resistant SKVLB cells. *a*: electrophoretogram of RT-PCR products, β-actin mRNA served as a positive control. SKOV-3 (*1*) and SKVLB (*2*). *b*: Trx1 and Trx2 mRNA content according to the results of densitometry (4 measurements). *c*: total Trx activity. Light bars: SKOV-3; dark bars: SKVLB. $p<0.05$ and $^{**}p<0.001$ compared to sensitive cells.

from the cytoplasm into the nucleus under conditions of oxidative stress [6], thus modulating transcription of genes of antioxidant enzymes. Trx1 directly binds and has the inhibitory effect on protein kinase ASK1 and p53 MAP kinase, which prevents activation of apoptosis [5]. Similarly to Trx1, Trx2 can act as a free radical scavenger. Trx2 and peroxiredoxin 3 are involved in H_2O_2 catabolism, which significantly increases antioxidant protection of mitochondria. Moreover, Trx2 counteracts the release of cytochrome C. These specific features prevent apoptosis under conditions of oxidative stress [12].

Less pronounced increase in the expression of the *TRX2* gene (compared to the *TRX1* gene) in resistant SKVLB cells is probably related to specific regulation of gene expression during adaptation to oxidative stress. This assumption is supported by the data that Trx1 mRNA level increases 5-fold in mouse lens cells 3 weeks after photochemical oxidative stress, while Trx2 mRNA level remains unchanged at this term, but progressively increased (by 2 times) in the follow-up period [13].

Our results indicate that increased expression of *TRX1* and *TRX* genes is an important component of the adaptive antioxidant response during the development of multidrug resistance in SKVLB cells.

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