ONCOLOGY

The Role of Heat Shock Protein 90 in the Regulation of Tumor Cell Apoptosis

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Programmed death of Jurkat tumor cells was studied under conditions of culturing with 17-AAG selective inhibitor of heat shock protein with a molecular weight of 90 kDa and etoposide. Apoptosis realization was evaluated by fluorescent microscopy with FITC-labeled annexin V and propidium iodide. Activity of caspase-3 was evaluated spectrophotometrically. Inhibition of heat shock protein with a molecular weight of 90 kDa activated the apoptotic program in Jurkat tumor cells and etoposide-induced apoptosis. The heat shock protein with a molecular weight of 90 kDa acted as apoptosis inhibitor in tumor cells.

Key Words: *heat shock protein 90; apoptosis; caspase-3; tumor cells*

Various mechanisms are involved in initiation and regulation of apoptosis processes. The fate of cells (death or survival) after apoptosis induction depends on the presence or activation of numerous factors and processes modulating programmed cell death. These factors include proteins constantly present in the cell, such as Bcl-2 and IAP, and stress-induced molecules: NF-κB and p53 transcription regulation factors, ceramide, JNK, p38, and ERK stress-induced kinases [1,2].

Heat shock proteins (HSP) are the most significant of the stress-induced molecules. These proteins are involved in the formation of newly synthesized polypeptides, functional activity of intracellular proteins and elimination of damaged protein forms, and transport of proteins through cell membranes, intracellular supramolecular complexes association–dissociation processes, and protein protection from aggregation. In addition, HSP are characterized by anti- and pro-apoptotic functions [3,8]. Apoptosis regulation by HSP is a defense mechanism reducing cell sensitivity to untoward factors and promoting survival of cells, including tumor cells. Heat shock protein-90 is a unique molecular chaperone. The majority of its known substrates are proteins involved in transmission of intracellular signals. This protein controls numerous stages of mitogenic signal cascade, cyclin-dependent progress through cell cycle phases G_1 and G_2, and centrosomes in mitosis. The HSP-90 binds actin and tubulin, the main components of the cytoskeleton; it is the main protein reacting with microtubules [3,4,8,12].

We evaluated the role of HSP-90 in etoposide-induced apoptosis of Jurkat tumor cells.

MATERIALS AND METHODS

The study was carried out on Jurkat tumor cells (human T-lymphoblastoid cells) obtained from Cell Culture Bank of Institute of Cytology, St. Petersburg. The cells were cultured in Petri dishes in nutrient medium

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with 90% RPMI-1640, L-glutamine (0.3 μg/ml), gentamicin (100 μg/ml), and 10% FCS (Biolot), inactivated at 56°C for 30 min. The cells were maintained in the logarithmic growth phase by repeated (every 2-3 days) passages. Cell viability was evaluated by trypan blue staining.

Etoposide in a concentration of 8 μg/ml served as inductor of tumor cell apoptosis. In order to evaluate the role of HSP-90 in regulation of programmed cell death, 17-AAG (HSP-90 selective inhibitor; Sigma Aldrich) in a concentration of 5 μmol/ml was added into Jurkat culture and incubation was carried out for 18 h at 37°C and 5% CO_2 with and without etoposide.

Apoptosis realization was evaluated by fluorescent microscopy under an Axiostar plus microscope (Carl Zeiss) with FITC-labeled annexin V according to the instruction. The method is based on specific binding of FITC-labeled annexin V to phosphatidylserine and propidium iodine intercalation with DNA molecule.

Activity of caspase-3 was evaluated spectrophotometrically according to manufacturer's instruction (Abcam). The method is based on light emission by p-nitroanilide (pNA) chromophore after its cleavage from DEVD-pNA caspase-3 labeled substrate. The results were expressed in caspase-3 activity coefficients showing the proportion of pNA optical density increment for experimental and control samples with consideration for protein content in the sample. Protein concentration in the sample was measured by Bradford's method.

The data were processed by methods of variation statistics. The significance of differences was evaluated by nonparametric Mann–Whitney (for independent samples) and Wilcoxon (for related samples) tests. Bonferroni's correction was used for nonparametric tests. The data were presented as the median (Me), upper and lower quartiles (Q_1-Q_3).

RESULTS

Tumor cells produce their own defense proteins in the course of carcinogenesis; as a result of this, they differ significantly from normal body cells, including their capacity to enter apoptosis. One of the most important antagonists of proapoptotic molecules during programmed cell death is HSP-90 [4,12]. In order to clear out the role of this protein in tumor cell apoptosis dysregulation, we used 17-AAG selective inhibitor with and without apoptosis inductor.

Etoposide (cytostatic) in therapeutic concentration of 8 μg/ml served as apoptosis inductor. Etoposide addition into Jurkat culture resulted in a significant increase in the count of annexin-positive cells in comparison with intact culture (Table 1). This cytostatic is a topoisomerase II inhibitor; it initiates disturbances in reparation of damaged DNA sites, arrest of mitosis at G_2 stage, which, in turn, triggers apoptosis. In addition, etoposide directly stimulates caspase-3, an important participant of the apoptotic cascade [5,6,10], which was confirmed by our results (Table 1). Caspase-3 stimulates caspase-6, cleaving the nuclear mitotic apparatus (NuMA) protein, and mediates shrinkage and fragmentation of the nucleus [7]. In addition, caspase-3 modulates DNA by cleaving the DNA fragmentation factor (DFF), thus inducing the formation of the "ladder" identified in the DNA electrophoregram [6].

Addition of HSP-90 selective inhibitor (17-AAG) to Jurkat cell culture significantly increased the content of apoptotic cells in comparison with intact culture (from 1.81 (0.97-2.77)% to 11.22 (9.54-15.38)%; Table 1). Combined use of etoposide and 17-AAG increased the level of annexin-positive cells to 57.21 (43.44-68.81)% (Table 1).

Heat shock proteins modulate the process of programmed cell death at different stages. They prevent activities of proapoptotic proteins promoting the release of mitochondrial inter-membrane space proteins and caspase stimulation. In addition, these proteins directly bind cytochrome *C* in the cytoplasm [11] and disorder apoptosome assembly by directly binding Apaf-1, thus preventing activation of procaspase-9 [4].

However, there is also evidence of the proapoptotic role of HSP. For example, HSP-27 promotes TNF-dependent apoptosis by inhibiting IkB degradation, while HSP-90 reacting with proapoptotic protein

TABLE 1. Levels of Apoptotic Cells and Changes in Caspase-3 Activity in Jurkat Culture under the Effects of Etoposide and 17-AAG Inhibitor (Me(Q_1-Q_3))

Incubation conditions	Percentage of apoptotic cells	Caspase-3 activity coefficient *vs.* control
Intact Jurkat culture	1.81 (0.97-2.77)	1.00
Etoposide, 8 μg/ml	28.44 (20.21-36.53)**	1.66(1.54-2.19)*
17-AAG, 5 μM	11.22 (9.54-15.38)**,++	4.07(3.12-4.77)*+
Etoposide+17-AAG	57.2 (43.44-68.81)**++xx	7.72(7.11-8.21)*+x

Note. *p<0.05, **p<0.01 compared to intact culture; +p<0.05, ++p<0.01 compared to etoposide; xp<0.05, xx$p$<0.01 compared to 17-AAG.

induces the mitochondrial pathway of programmed cell death [4].

Our study showed that HSP-90 inhibition leads to stimulation of spontaneous and etoposide-induced tumor cell apoptosis (Table 1). The data suggest an antiapoptotic effect of HSP-90 in Jurkat tumor cells.

Molecular mechanisms of HSP-90 antiapoptotic activity are still to be studied in detail; presumably, they depend on the cell type. Accordong to some data [9], HSP-90 prevents the formation of apoptosome in U-937 cells by reacting with RIP-1 kinase, this determining the cell survival with participation of NF-κB.

Analysis of changes in caspase-3 activity in Jurkat cells under the effect of etoposide and HSP-90 inhibitor showed a significant increase in this parameter in comparison with the control and a drastic increase of caspase activity coefficient in cultures treated with a combination of 17-AAG and etoposide (Table 1).

Hence, HSP-90 plays an antiapoptotic role in Jurkat cells. Inhibition of this protein leads to activation of caspase-3 and apoptotic program of human T-lymphoblastoid leukemia cells and enhances etoposide-induced apoptosis. The use of 17-AAG (HSP-90 selective inhibitor) as a tumor cell apoptosis inductor opens new vistas among the known approaches to drug therapy of malignant tumors.

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