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The Inhibition of Hsp27 Chaperone Affects the Level of p53 Protein in Tumor Cells

E. V. Kaigorodova[1,2], L. S. Litvinova[3], E. V. Konovalova[1], M. V. Klimova[2], L. A. Tashireva[1,2], O. L. Nosareva[1] & V. V. Novitskiy[1]

[1] Siberian State Medical University, Tomsk, Russian Federation

[2] Cancer Research Institute of Siberian Branch of the Russian Academy of Medical Sciences, Tomsk, Russian Federation

[3] Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation

Correspondence: E. V. Kaigorodova, Cancer Research Institute of Siberian Branch of the Russian Academy of Medical Sciences, 5 Kooperativny Street, Tomsk 634050, Russian Federation. Tel: 7-382-241-8041. E-mail: zlobinae@mail.ru

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Abstract

The characteristics of p53 protein content in Jurkat and THP-1 tumor cell line, and mononuclear leukocytes were evaluated from *in vitro* studies with selective inhibition of the chaperone Hsp27. For the inhibition of Hsp27 KRIBB3 ((5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxasole) was used. The p53 protein concentration was determined by Western blot analysis. The apoptoticly transformed cells with selective inhibitor Hsp27 were assessed by *in vitro* fluorescence microscopy using FITC-labeled annexin V and propidium iodide. The present study showed that the *in vitro* inhibition of the chaperone Hsp27 leads to an increase in p53 concentration in tumor cells and a growth of the amount of apoptotic cells in modified Jurkat and THP-1 cultures but revealed no such effects in the mononuclear leukocytes culture. Thus, Hsp27 appeared to play an important regulatory role in the activation of p53 protein of tumor cells.

Keywords: p53 protein, molecular chaperone Hsp27, tumor cells, apoptosis

1. Introduction

It is known that a cell incorporates different mechanisms that regulate the processes of survival and death. The key transcription factor involved in the implementation of apoptosis is p53. This protein is activated in response to various forms of cellular stress and is essential for control of anti-proliferative processes. P53 can be induced by DNA damage, hypoxia, or aberrant expression of oncogenes. This protein regulates DNA reparation, cellular senescence, and apoptosis (Levine, Hu, & Feng, 2006; Lin et al., 2004). Dysfunctions of p53 are accompanied by defects of the protection, genetic instability, cell immortality, which allows the damaged cells to survive and evolve (Beroud & Soussi, 2003). Taking these facts into account, it ts evident that p53 is the most frequently inactivated suppressor gene of carcinogenesis. p53 in mutated or deactivated form is detected in more than 50% of human tumors (Kern et al., 1992; Milner, Medcalf, & Cook, 1991). A number of authors have shown *in vivo* that a mutant p53 specifically interacts with the molecular chaperone Hsp90 (Heat shock protein-Hsp) (Wang & Chen, 2003; Smith, Whitesell, & Katsanis, 1998; Blagosklonny, Toretsky, Bohen, & Neckers, 1996). However, the molecular interconnections of heat shock protein Hsp27 and p53 remain unknown. Hence, in order to investigate the influence of Hsp27 on the general concentration of the transcription factor p53 in hematological cells and mononuclear leukocytes, obtained from healthy donors, the *in vitro* specific inhibition with KRIBB-3 was used.

2. Material and Methods

2.1 Material of Research

The materials used in this study are Jurkat tumor cell lines (T-lymphoblastic human leukemia), THP-1 (monocytic leukemia), obtained from Russian Cell Cultures Collection of the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg), and mononuclear leukocytes isolated from relatively healthy donor blood (donors: 11 men and 16 women, aged 18-45).

Mononuclear leukocytes (MNL) were isolated under sterile conditions from venous heparin-treated blood by centrifugation with Ficoll-Paque density gradient (ρ=1.077 g/cm^3; «Pharmacia», Sweden) with the ratio 1:2.

The main criteria for participant selection were as follows: absence of infectious diseases, exacerbation of chronic somatic diseases and cancer, mental disorders, alcohol and drug abuse in medical background; age from 18 to 50 years, and informed consent of thee participant.

2.2 Cultivation of Cells

Jurkat, THP-1 and MNL cells were cultured in suspension in complete nutrient medium with the following composition: 90% RPMI-1640 («Vector-Best», Russia), 10% FCS («Invitrogen», USA) inactivated at 56°C for 30 min, 0.3 mg/ml L-glutamine («Vector-Best», Russia), 100 µg/ml gentamicin (INS, USA) at 37°C and 5% CO_2 atmosphere. The cells were maintained in the logarithmic growth phase and were subcultured in 3 days. The Hsp27 in the cells was inhibited by adding KRIBB3 (5-(5-ethyl-2-hydroxy -4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxasole) («Sigma-Aldrich», USA) into culture medium.

In order to estimate the Hsp27 apoptotic inhibitor dose, Jurkat tumor cell line ($2x10^6$ per 1 ml) was incubated for 18 hours in culture medium with KRIBB3 final concentrations of 0.1, 1, 5, and 10 µM. Apoptotic and necrotic cells were counted by the method of fluorescence microscopy. The maximum amount of apoptotic cells with corresponding minimum of necrosis ones was registered at 0.1 µM of KRIBB3, which was chosen as inhibitor concentration for the further study.

2.3 Evaluation of Apoptic Cells

The apoptosis was evaluated by means of fluorescent microscopy on Axiostar plus microscope («Carl Zeiss», Germany) using FITC-labeled annexin V and propidium iodide («PI»; «Abcam», Great Britain). The method is based on specific ability of FITC-labeled annexin V to bind to phosphatidyl serine and PI and to intercalate with DNA molecule. The number of FITC+/PI– and FITC+/PI+-labeled lymphocytes were counted for 200-300 cells and then expressed in percentage.

2.4. Western Blotting Analysis Based Determination of p53 Concentration

The cells lysate proteins were separated by electrophoresis under the following conditions: 10 V/cm electric field, 5% and 10% polyacrylamide gel SDS-PAGE, 14.3-220.0 kDa protein molecular weight markers («Fermentas», EU). Proteins were transferred to the nitrocellulose membranes («Bio-Rad», USA). Then, membranes were incubated sequentially in 0.05% TTBS, pH 7.4 (20 mM Tris-HCl, 150 mM NaCl and 0.05% Tween-20) with 5% fat free dry milk containing primaries with monoclonal antibodies to p53 («Biosource», USA) and then with peroxidase-labeled secondary antibodies («Biosource», Belgium). The results were recorded on Kodak X-ray film (USA) with Novex chemiluminescent substrate («Invitrogen», USA). The conclusion about the content of the target antigen in cell was based on the signal ratio from the target protein label and the glyceraldehyde-3-phosphate dehydrogenase enzyme, measured with the software TotalLab (G3PDH) («Chemicon», USA). The results were expressed in conventional units.

2.5 Statistical Analysis

The data were processed by statistical methods using SPSS 13.0 software. The normality of distribution was verified by the Shapiro-Wilk test. The validity of differences was confirmed by the nonparametric Mann–Whitney test (for samples with abnormal distribution) and the Student two-sample test (for samples with normal distribution). The results are presented as a median (Me) and upper and lower quartiles (Q1-Q3). The differences are considered significant at $p < 0.05$.

3. Results

The present study of the total content of p53 transcription factor in lysates of Jurkat and THP-1 tumor cell lines has shown a significantly higher concentration of this parameter in tumor-transformed cells than in mononuclear leukocytes of relatively healthy donors (Figure 1).

This can be explained by the fact that a wild-type p53 is a labile protein with high volatility. It is quickly destroyed via proteolysis and ubiquitin-proteasome pathway, which leads to the low concentrations of p53 in cells. Alongside this, mutated p53 is less dependent on proteolysis or ubiquitination and, therefore, is accumulated in the cells (Beroud & Soussi, 2003). A higher concentration of p53 in tumor cells can also be explained by the action of heat shock proteins, which are highly expressed in ontogenesis, perform the chaperone functions, stabilize and protect the mutant form of the transcription factor p53 from degradation. Previously, it was shown that the expression level of mRNA Hsp27 is 4 times higher in Jurkat and THP-1tumor cell lines than in mononuclear leukocytes obtained from healthy donors (Kaigorodova et al., 2012; Kaigorodova, 2011).

In vitro study of the general concentration of p53 by Western blotting with inhibition of Hsp27 in hematological and normal blood cells has shown a significant increase of this parameter in Jurkat and THP-1tumor cell lines (Figure 1).

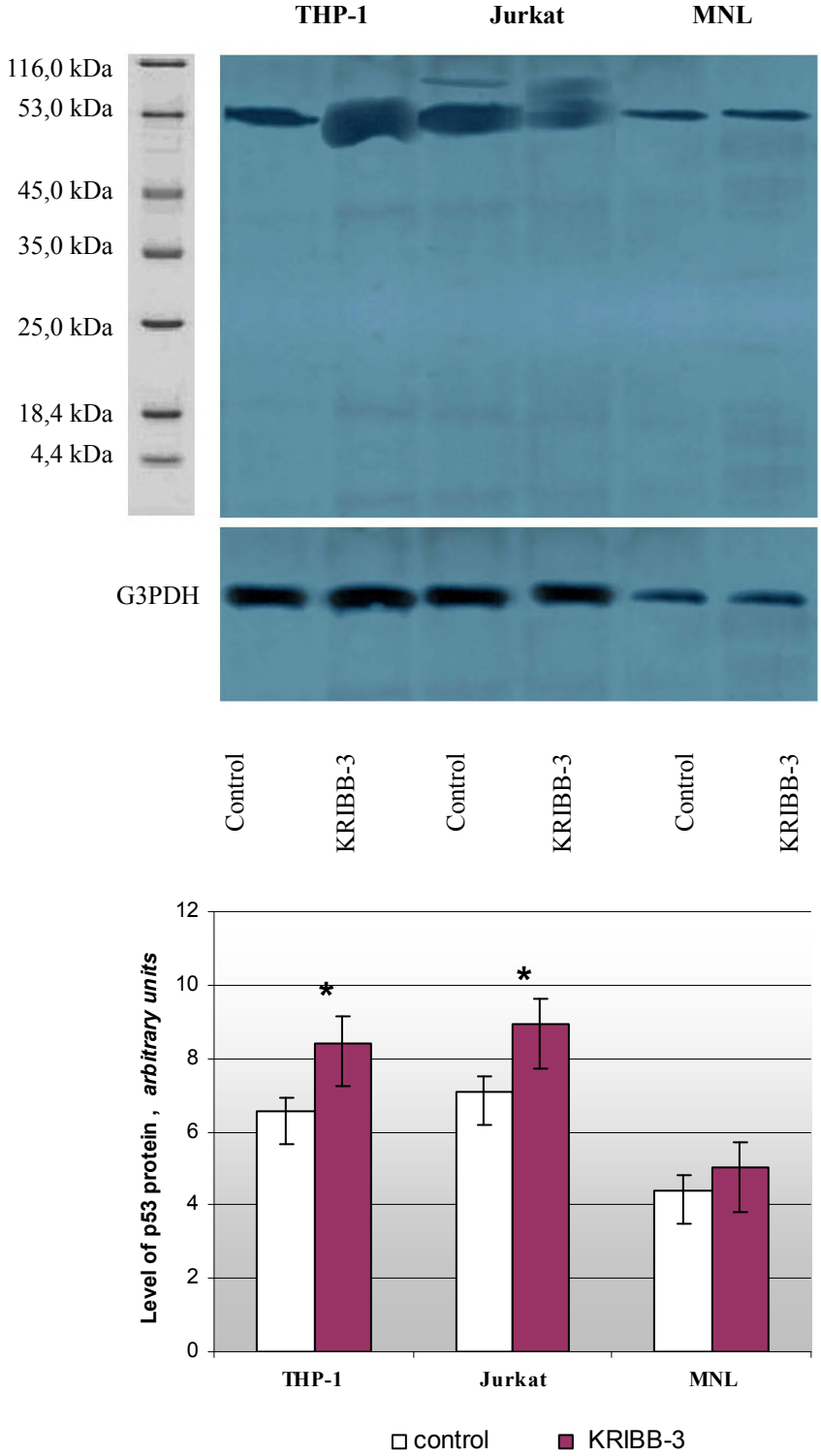

Figure 1. The concentration of p53 protein in Jurkat and THP-1 tumor cell lines and mononuclear leukocytes (MNL) under the effect of inhibitor of Hsp27 chaperone in vitro (according to Western blotting data)

Note: GAPDH – glyceraldehyde 3-phosphate dehydrogenase, KRIBB-3 - selective inhibitor Hsp27; * - certainty factor of differences in comparison with intact cells of the correspondent line with $p < 0.05$.

It was shown that blocking of HSF-1 (HSF1 - heat shock factor 1) and a decrease in the intracellular level of Hsp27 is a trigger for accumulation of transcriptionally active mutant p53 (Kanagasabai, Krishnamurthy, Druhan, & Ilangovan, 2011). The results obtained indicate that Hsp27 chaperone regulates the transcriptional activity of p53. There is evidence in favor of this fact in the relevant literature which shows that Hsp27 reduce the doxorubicin-induced apoptosis of fibroblasts and cardiomyocytes, lowering the transcriptional activity of p53 and increasing the activity of p21 (Venkatakrishnan et al., 2008).

It should be emphasised that a sharp increase in the number of apoptotically modified cells was noted under the inhibition of Hsp27 in Jurkat and THP-1tumor cell lines (Kaigorodova, Ryazantseva, Novitsky, Maroshkina, & Belkina, 2012; Kaigorodova, 2011). Therefore, adding KRIBB3 results in a significant increase of the number of apoptotically altered cells in Jurkat and THP-1tumor culture lines ($p < 0,05$) in contrast to the culture of mononuclear leukocytes obtained from healthy donors ($p < 0,05$). (Figures 2 and 3).

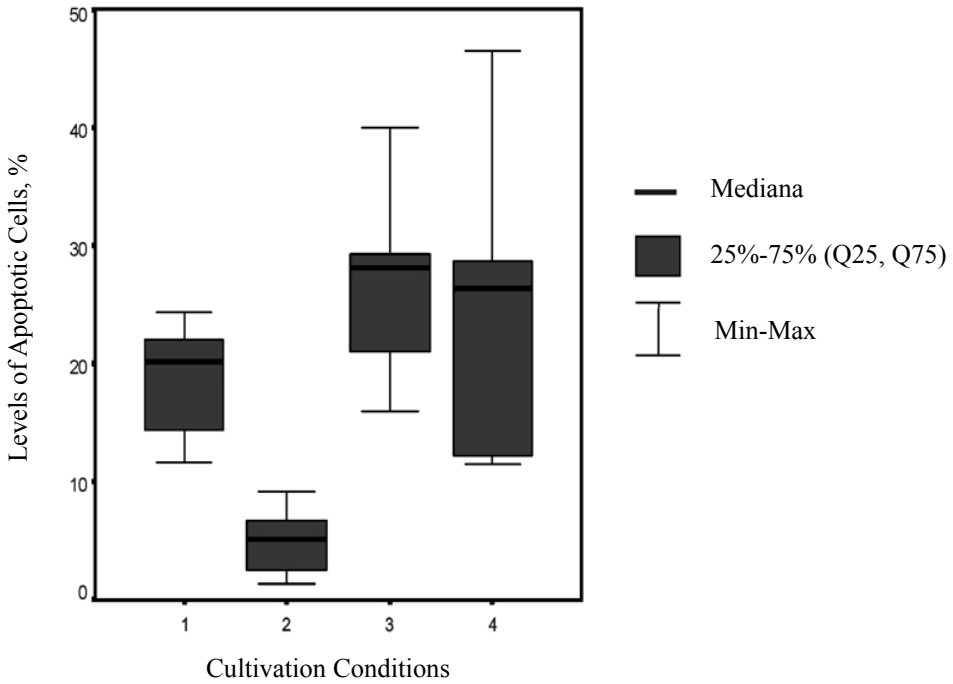

Figure 2. Levels of apoptotic cells under the effects of heat shock protein Hsp27 inhibitor (KRIBB-3) in vitro

1 - intact mononuclear leukocytes obtained from healthy donors,

2 - culture intact tumor cell line Jurkat;

3 - inhibition of Hsp27 (Jurkat cultivation of cells line with 0.1 mM KRIBB3);

4 - inhibition of Hsp27 (cultivation of mononuclear leukocytes with 0.1 mM KRIBB3).

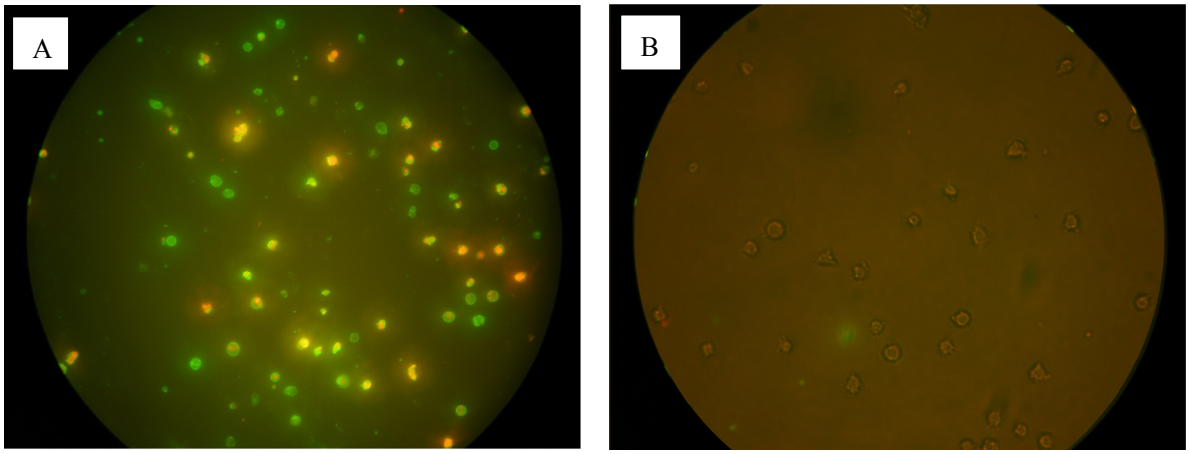

Figure 3. Fluorescent microscopy of annexin V-positive THP-1 cells, ×400

(A) THP-1 cells after addition of 0.1 μM KRIBB3; (B) intact THP-1 cells (control)

4. Discussion

The study of molecular chaperones and their regulatory mechanisms is an important task of medicine, since it could find specific ways for tumor cells avoiding apoptosis by Hsps and could be used as the basis for targeted therapy of malignant diseases.

It is known that a key transcription factor involved in the implementation of apoptosis is p53. It is also determined that in more than 50% of human cancers cases the protein is either in mutated or deactivated form. In such a case, in many cancer cells, one may observe an increased expression of the protein Hsp27 which performs a variety of chaperone functions (folding and refolding of proteins that support the functional activity of intracellular proteins and elimination of damaged protein forms, providing the transport of proteins across cell membranes, providing the association-dissociation processes of intracellular supramolecular complexes protecting proteins from aggregation) (Smith, Whitesell, & Katsanis, 1998; Nahleh, Tfayli, Najm, El Sayed, & Nahle, 2012).

The molecular mechanisms regulating cell apoptosis by transcription factor p53 are various. Thus, p53 controls the expression of proteins of the Bcl-2 family which perform the apoptotic function, transactivates the gene which encodes Apaf-1, and therefore participates in the implementation of the mitochondrial pathway apoptosis launch (Morgunkova, 2005; Joza et al., 2001). The dysregulation of a programmed cell death under the influence of a selective inhibitor of the molecular chaperone Hsp27, which was registered in vitro in the present study, can be reasonably explained in the context of p53 activation. In particular, the reduction of transmembrane mitochondrial potential can occur as the result of translocation of p53 towards mitochondria, where it binds antiapoptotic proteins (Bcl-2, Bcl-XL), thus inducing the outgo of the cytochrome c (Prives & Hall, 1999). We showed earlier that the effect of a selective inhibitor Hsp27 in vitro on Jurkat and THP-1 tumor cell lines involve changing of proapoptotic (Bax and Bad) and anti-apoptotic (Bcl-2) proteins ratio in favor of the former ones, and also lowering the level of mitochondrial transmembrane potential (Kaigorodova, Ryazantseva, Novitsky, Maroshkina, & Belkina, 2012; Ryazantseva, Kaigorodova, Maroshkina, Belkina, & Novitsky, 2012; Kaigorodova, 2011). We assume that the chaperone Hsp27 is a molecular target for the targeted impact on the programmed death of tumor cells of T-lymphoblastic leukemia and monocytic leukemia.

5. Conclusion

Thus, the results obtained in the study evidentiate the fact of an increase in the intracellular level of p53 in blocking of Hsp27 in vitro, which correspond to the previously obtained and literature data, and indicate an important regulatory role of Hsp27 chaperones in activation of p53 tumor cells.

In our view, the molecular chaperone Hsp27 is essential for stability and regulation of transcriptional activity of p53 and represent an additional control level of this transcription factor.

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