Original research article

ROLE OF HEAT SHOCK PROTEINS 27 AND 70, AND UBIQUITIN IN OXIDATIVE MODIFICATION OF PROTEINS, AND THE IMPLEMENTATION OF DEXAMETHASONE-INDUCED APOPTOSIS OF JURKAT TUMOR CELLS THE CONDITIONS OF INHIBITED HEAT SHOCK PROTEINS 27

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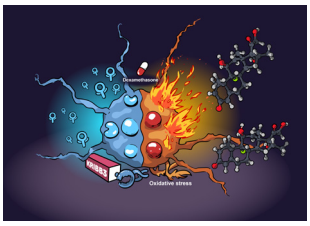

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Abstract: Examining the pathogenic aspects of tumor transformation is particularly relevant to understanding the molecular regulation of oxidative modifications in regulatory proteins and effector enzymes involved in apoptosis through their interactions with ubiquitin and heat shock proteins.
This study aims to investigate the molecular mechanisms underlying the involvement of heat shock proteins 27 and 70 and ubiquitin in the oxidative modification of proteins, as well as the execution of dexamethasone-induced apoptosis in Jurkat tumor cells under inhibition of heat shock protein 27. It was assessed how 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)isoxazole at a final concentration of 0.1 μM and/or dexamethasone at a final concentration of 10 μM affected cytoplasmic exposure of phosphatidylserine followed by annexin V binding, the number of FasL and TNFα receptors, and the reduction of mitochondrial membrane potential in cells. Their effects were also examined with respect to the levels of $OH^{\bullet}$ radicals, free sulfhydryl groups of cysteine in proteins, protein-bound glutathione, oxidized tryptophan residues, bi-tyrosine cross-links, carbonyl derivatives of proteins, ubiquitin, ubiquitin ligase, NFkB, Apaf-1, and heat shock proteins 27 and 70, as well as caspase-3 activity in Jurkat tumor cells.
The observed changes in the levels of heat shock proteins 27 and 70, ubiquitin, oxidative modifications of amino acid residues, and proteins were associated with the execution of apoptosis in Jurkat tumor cells. When exposed simultaneously to 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazole and dexamethasone, Jurkat tumor cells exhibited correlations between the activation of irreversible oxidative modifications and the reduction of reversible oxidative modifications of proteins, associated with the execution of apoptosis involving TNFα and Fas receptors.
The findings indicate that heat shock proteins 27 and 70, together with ubiquitin, participate in both reversible and irreversible oxidative modifications of amino acid residues and proteins, as well as in the execution of dexamethasone-induced apoptosis in Jurkat tumor cells when heat shock protein 27 is inhibited.

Keywords: heat shock proteins, ubiquitin, oxidative protein modification, Jurkat tumor cells, apoptosis, dexamethasone, 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazol

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Оригинальная исследовательская статья

РОЛЬ БЕЛКОВ ТЕПЛОВОГО ШОКА 27 И 70, УБИКВИТИНА В ОКИСЛИТЕЛЬНОЙ МОДИФИКАЦИИ ПРОТЕИНОВ И РЕАЛИЗАЦИИ ДЕКСАМЕТАЗОН-ИНДУЦИРОВАННОГО АПОПТОЗА ОПУХОЛЕВЫХ КЛЕТОК ЛИНИИ JURKAT В УСЛОВИЯХ ИНГИБИРОВАНИЯ БЕЛКА ТЕПЛОВОГО ШОКА 27

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Резюме: Актуальным в изучении патогенетических аспектов опухолевой трансформации является молекулярное управление окислительной модификацией белков-регуляторов и эффекторных ферментов апоптоза посредством их взаимодействия с убиквитином и протеинами теплового шока. Цель исследования — изучение молекулярных механизмов вклада протеинов теплового шока 27 и 70, убиквитина в окислительную модификацию белковых молекул и реализацию апоптоза опухолевых клеток линии Jurkat, стимулированного с помощью дексаметазона на фоне ингибирования протеина теплового шока 27. Изучено влияние 5-(5-этил-2-гидрокси-4-метоксифенил)-4-(4-метоксифенил)-изоксазола в конечной концентрации 0,1 мкМ и/или дексаметазона в конечной концентрации 10 мкМ на цитоплазматическую презентацию фосфатидилсерина с последующим связыванием с аннексином V, количество рецепторов к FasL и TNFα, снижение митохондриального потенциала клеток; на содержание OH•-радикала, свободных сульфгидрильных групп цистеина в протеинах, белково-связанного глутатиона, окисленных аминокислотных остатков триптофана, битирозиновых сшивок, карбонильных производных белков, убиквитина, убиквитинлигазы, NFkB, Apaf-1, протеинов теплового шока 27 и 70; на активность каспазы-3 опухолевых клеток линии Jurkat. Выявленное изменение содержания протеинов теплового шока 27 и 70, убиквитина, окислительной модификации аминокислотных радикалов и протеинов сопряжено с реализацией апоптоза опухолевых клеток линии Jurkat. В опухолевых клетках линии Jurkat при одновременном воздействии 5-(5-этил-2-гидрокси-4-метоксифенил)-4-(4-метоксифенил)-изоксазола и дексаметазона доказаны взаимосвязи активации необратимой и снижения обратимой окислительной модификации протеинов с реализацией апоптоза при участии цитоплазматических рецепторов TNFα и Fas. Полученные результаты свидетельствуют об участии протеинов теплового шока 27 и 70, убиквитина в обратимой и необратимой окислительной модификации аминокислотных радикалов и протеинов, реализации стимулированного дексаметазоном апоптоза в опухолевых клетках линии Jurkat на фоне ингибирования белка теплового шока 27.

Ключевые слова: белки теплового шока, убиквитин, окислительная модификация белков, опухолевые клетки Jurkat, апоптоз, дексаметазон, 5-(5-этил-2-гидрокси-4-метоксифенил)-4-(4-метоксифенил)-изоксазол

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Носарева О. Л., Степовая Е. А., Литвинова Л. С., Юрова К. А., Спирина Л. В. Роль белков теплового шока 27 и 70, убиквитина в окислительной модификации протеинов и реализации дексаметазон-индуцированного апоптоза опухолевых клеток линии Jurkat в условиях ингибирования белка теплового шока 27. *Задачи биомедицины*. 2025;1(2):31—41. https://doi.org/10.5922/ATB-2025-1-2-2

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Introduction

The malfunctioning of programmed cell death is a crucial contributor to the emergence and advancement of tumors, as well as the development of resistance to chemotherapy [1; 2]. Cancer cells are perpetually exposed to oxidative stress, and heat shock proteins (HSPs) function as molecular chaperones, preventing damage to biomolecules caused by reactive oxygen species within cells [3—7]. The protein refolding process within cells serves as a critical regulator of essential cellular functions, encompassing phenomena such as proliferation, differentiation, and programmed cell death, known as apoptosis [5; 8]. The upregulation of HSPs production in cancer cells may potentially augment their capacity for survival [4; 9—11].

Oxidative stress overactivation in cancer cells leads to activation of molecular mechanisms that inhibit apoptosis, with the functional state of protein molecules playing a crucial role. One molecular mechanism that disrupts apoptosis is the oxidative modification of macromolecules, with proteins playing a crucial role in the initiation, execution, and regulation of programmed cell death in cancer cells [12—14].

Hsp27, Hsp70, and ubiquitin play a critical role in protein folding, refolding, and proteolysis, and also contribute to the regulation of functional activity of both regulatory and effector proteins within intracellular environments, including those involved in cell death processes. The specific role of Hsp27 in apoptosis inhibition is linked to its engagement in the phosphorylation of MEK/ERK signaling cascades and the subsequent inhibition of p53 activity [15; 16]. Furthermore, it has been demonstrated that this HSP contributes to the chemoresistance initiation in the treatment of various cancers, particularly in cases where there is an enhancement in the Hsp27 expression associated with a change in the topoisomerase II activity [16]. The influence of Hsp70 on the inhibition of apoptosis is determined by its interaction with a wide range of molecules, from unfolded to initially folded and aggregated proteins, thereby providing a cytoprotective role. Elevated expression of Hsp70 in tumor cells may promote oncogenesis by enhancing resistance to chemotherapy [17]. Activation of oxidative stress results in a marked increase in the quantity of oxidatively modified proteins in cancer cells, which can alter their functional properties or target them for degradation through ubiquitination. Comprising 76 amino acids, ubiquitin not only directly mediates the covalent modification of proteins (ubiquitination) but is also subject to phosphorylation, acetylation, and other chemical modifications. These modifications play a crucial role in regulating cellular processes, including apoptosis, in eukaryotic organisms [18—20].

In view of the above, it is essential to examine the roles of molecules such as chaperones Hsp27 and Hsp70, along with ubiquitin, in protein oxidation and the induction of apoptosis in cancer cells.

This study aims to elucidate the molecular mechanisms underlying the involvement of heat shock proteins 27 and 70, and ubiquitin, in the oxidative modification of proteins and the execution of dexamethasone-induced apoptosis in Jurkat tumor cells under inhibition of heat shock protein 27.

Materials and methods

In order to achieve the objectives of this study, a Jurkat human T-lymphoblastic leukemia cell line obtained from the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg, Russia) was used. Tumor cells were cultured by the suspension method [21], and only cultures exhibiting more than 95 % cell viability were included in the experiments.

To study the Hsp27 role in oxidative modification of proteins and dexamethasone-induced apoptosis, cells were incubated for 18 hours at 37 °C in an atmosphere

containing 5 % CO_2 with the following additives: The Hsp27 inhibitor—5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazole (KRIBB3) from Sigma-Aldrich (USA), at a final concentration of 0.1 μM [22] and/or apoptosis inducer, dexamethasone (DEX), (Sigma-Aldrich, USA) at a concentration of 10 μM [21].

After incubation, the cells were rinsed three times with 0.01 M sodium phosphate buffer (pH 7.4; Amresco, USA) and then used to assess the number of cells displaying cytoplasmic exposure of phosphatidylserine followed by annexin V binding, as well as the expression of FasL and TNFα receptors. Cells exhibiting reduced mitochondrial potential were identified by flow cytometry following the manufacturer's instructions. To assess NFκB, Apaf1, Hsp27, Hsp70, ubiquitin, and ubiquitin ligase content, a cell lysate was prepared using protease inhibitors from Amresco, USA. To determine the concentration of free cysteine sulfhydryl groups in proteins and protein-bound glutathione, cell lysates were deproteinized with a 5 % solution of sulfosalicylic acid from Sigma-Aldrich, USA. To assess the activity of caspase-3 and the content of oxidized tryptophan, bityrosine, carbonyl derivatives of proteins, and $OH^{\bullet}$ radicals, the cell suspension was resuspended in a buffer containing 1 % Triton X-100 from Sigma-Aldrich, USA.

In previous studies [21; 23], a detailed methodology was provided for determining the number of cells positive for annexin V, expressing FasL and TNFα receptors on their cytoplasmic surface, and exhibiting reduced mitochondrial potential. Methods were described for assessing levels of $OH^{\bullet}$ radicals, NFkB, Apaf-1, ubiquitin, ubiquitin ligase, free SH groups in proteins, protein-bound glutathione, oxidized tryptophan, bityrosine, carbonyl derivatives of proteins, total protein content, and caspase-3 activity.

Western blot analysis was employed to measure the content of Hsp27 and Hsp70. Primary monoclonal antibodies specific to the phosphorylated form of Hsp27 (Sigma-Aldrich, USA) were used at a 1:2000 dilution. For Hsp70, antibodies from Sigma-Aldrich (USA) were used at a 1:1000 dilution. For comparative analysis, β-actin, a cytoskeletal protein, served as the control. Data were processed using ImageJ2x software (version 2.1.4.7), developed by Wayne Rasband at the National Institutes of Health (USA).

Statistical analysis was carried out using Statistica 6.0 software for Windows. The Shapiro—Wilk test was applied to assess the normality of data distribution. The results indicated that the data did not follow a normal distribution at a significance level of $p < 0.05$; therefore, the median (Me), first quartile (Q_1) and the third quartile (Q_3) were calculated. To assess the statistical significance of differences between independent samples, the nonparametric Mann—Whitney U test with Bonferroni correction was applied. Correlations between the indicators were evaluated using Spearman's correlation analysis at a significance level of $p < 0.05$.

Results and discussion

Maintaining the balance of cellular processes is essential for cellular health. Using specialized molecular detectors, critical metabolic parameters are carefully controlled and kept within acceptable ranges. These molecules are signaling proteins that change their conformational structure in response to reactive oxygen species. Any factors that disrupt cellular balance trigger internal mechanisms designed to restore the equilibrium of biochemical processes within the cell [5].

Tumor cells exhibit excessive proliferation, disrupted differentiation, and defective apoptosis regulation. Therefore, there is an urgent need to investigate their metabolism at the molecular level. Heat shock proteins offer promising molecular targets for developing technologies that can regulate apoptosis in tumor cells [15; 24].

Establishing the relationships between the levels of Hsp27, Hsp70, and ubiquitin, oxidatively modified proteins and amino acid residues, and apoptosis in Jurkat tumor cells treated with 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazole and dexamethasone had scientific relevance.

The role of Hsp27 is linked to oxidative stress and activation of oxidation modifications of proteins and amino acid residues, as revealed by our study of dexamethasone-mediated apoptosis induction in the Jurkat cell line. When combined, DEX and KRIBB3 increased the levels of $OH^{\bullet}$ radicals by 551.5 % ($p < 0.05$) compared to untreated cells (523.00 [415.10—719.37] nmol/mg protein). Additionally, in this group, a decrease of 85.7 % ($p < 0.05$) was observed in the concentration of free sulfhydryl groups in cysteine residues of proteins compared to the levels in the intact group (Fig. 1). A rise in intracellular concentration of hydroxyl radicals was accompanied by oxidative modifications of proteins. Specifically, simultaneous administration of DEX and KRIBB3 resulted in a significant 207.3 % increase ($p < 0.05$) in protein carbonyl content, accompanied by a 31 % decrease ($p < 0.05$) in protein-bound glutathione. Moreover, ubiquitin levels were reduced by 24.6 % ($p < 0.05$) compared to untreated cells (Fig. 1, 2). The levels of oxidized amino acid, such as tryptophan, bityrosine and ubiquitin ligase were comparable to those observed in the intact cell group. Thus, the simultaneous addition of DEX and KRIBB3 resulted in a significant increase in the number of annexin-positive cells by 1165.4 % ($p < 0.05$), cells with reduced mitochondrial potential by 302.5 % ($p < 0.05$), Fas-positive cells by 1844.2 % ($p < 0.05$), and TNF RI-positive cells by 676.8 % ($p < 0.05$). NFκB levels increased by 131.5 % ($p < 0.05$) and caspase-3 activity by 319.7 % ($p < 0.05$), while Apaf-1 concentration decreased by 46.6 % ($p < 0.05$). The levels of Hsp27 and Hsp70 were comparable to those observed in the intact cell group (Fig. 2, 3).

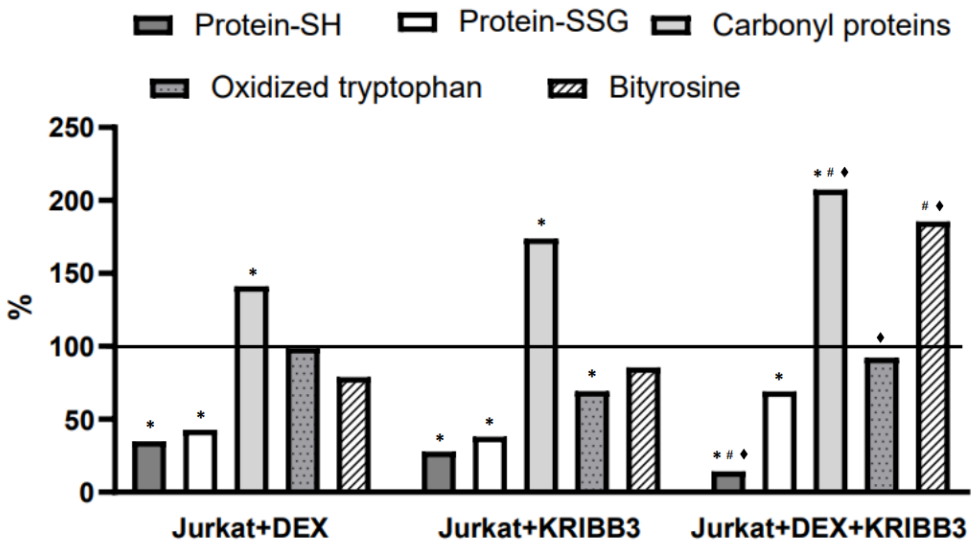

Fig. 1. Influence of the heat shock protein 27 inhibitor on indicators of oxidative modification of proteins and amino acids during dexamethasone-induced apoptosis in Jurkat tumor cells, %

Note: DEX stands for dexamethasone; KRIBB3, 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazole; Protein-SH, free SH groups of protein; Protein-SSG, protein-bound glutathione; Carbonyl proteins, protein carbonyl derivatives; Oxidized tryptophan, oxidized tryptophan residues; Bityrosine, dityrosine; * indicates statistically significant differences ($p < 0.05$) compared to the intact Jurkat group; # indicates statistically significant differences ($p < 0.05$) compared to the Jurkat + DEX group; ♦ indicates statistically significant differences ($p < 0.05$) compared to the Jurkat + KRIBB3 group. The sample size is 5. Values for the intact Jurkat group were set at 100 % (protein-SH content: 1.26 [1.08—1.34] nmol/mg protein; protein-SSG content: 0.42 [0.40—0.57] nmol/mg protein; protein carbonyl derivative content: 0.382 [0.379—0.388] nmol/mg protein; oxidized tryptophan content: 25.19 [22.14—38.30] units/mg protein; dityrosine content: 0.76 [0.51—1.52] units/mg protein).

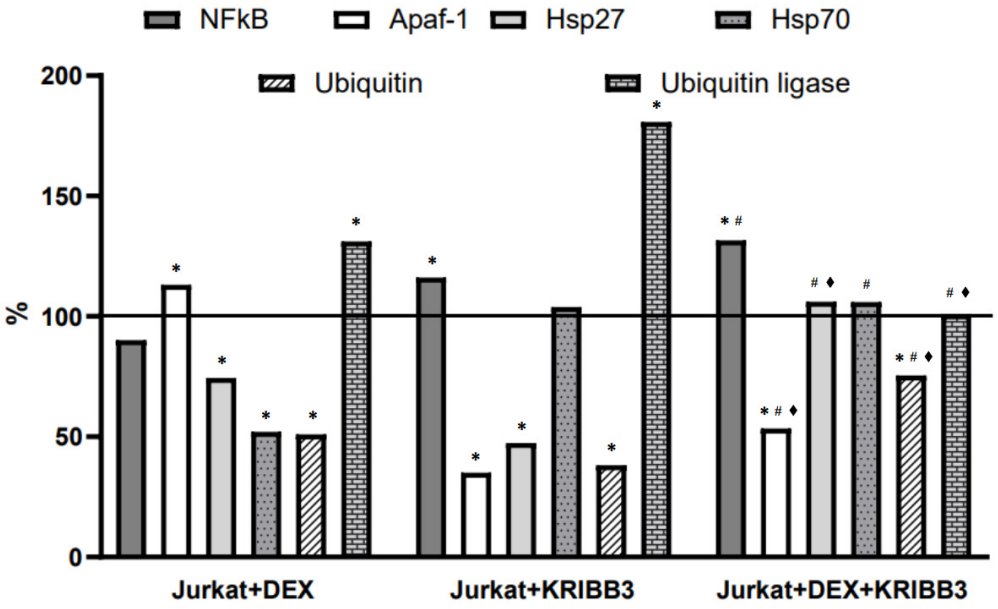

Fig. 2. Influence of the heat shock protein 27 inhibitor on the levels of NFκB, Apaf-1, ubiquitin, ubiquitin ligase, and heat shock proteins 27 and 70 during dexamethasone-induced apoptosis in Jurkat tumor cells, %

Note: DEX, dexamethasone; KRIBB3, 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazole; Hsp27, heat shock protein 27; Hsp70, heat shock protein 70; Ubiquitin, ubiquitin; Ubiquitin ligase, ubiquitin ligase. * indicates statistically significant differences ($p < 0.05$) compared to the intact Jurkat group; # indicates statistically significant differences ($p < 0.05$) compared to the Jurkat + DEX group; ♦ indicates statistically significant differences ($p < 0.05$) compared to the Jurkat + KRIBB3 group. The sample size 5. Values for the intact Jurkat group were set at 100 % (NFκB: 3.106 [3.095—3.128] units; Apaf-1: 1.884 [1.856—1.917] units; Hsp27: 2.890 [2.340—2.993] units; Hsp70: 4.614 [4.416—4.710] units; ubiquitin: 3.177 [3.099—3.412] units; ubiquitin ligase: 1.672 [1.588—1.711] units).

One of the most reactive oxidative species is the hydroxyl radical, which can produce radical protein compounds through the involvement of amino acid radicals, such as tyrosine, tryptophan, histidine, and phenylalanine. In tumor cells, elevated levels of protein carbonyl derivatives indicate irreversible oxidative damage to proteins. Yet, cysteine residues in amino acids can undergo reversible oxidative modification, followed by changes in their functional activity. The findings suggest that Hsp27, Hsp70, and ubiquitin are involved in initiating these oxidative changes and facilitating irreversible protein degradation. Effector and regulatory proteins of apoptosis act as targets during this process.

Tumor cells respond to both internal and external environmental factors by controlling the rate and direction of intracellular processes through changes in protein content and activity. These alterations in protein functional states enable the cell to rapidly respond to stimuli during the early stages of exposure. This process depends on post-translational protein modifications, with chaperones playing a critical role in their regulation [7; 24]. Carbonylation, ubiquitination, glutathionylation, phosphorylation, acetylation, nitrosylation, and glycosylation are chemical reactions that modify amino acid side chains, resulting in changes to protein structure and function [25]. To sustain their metabolic processes and ensure survival, tumor cells must undergo substantial metabolic alterations, which require a high concentration of chaperones activated in response to oxidative stress. Therefore, heat shock proteins, a specific class of molecular chaperones, may contribute to cancer development [15].

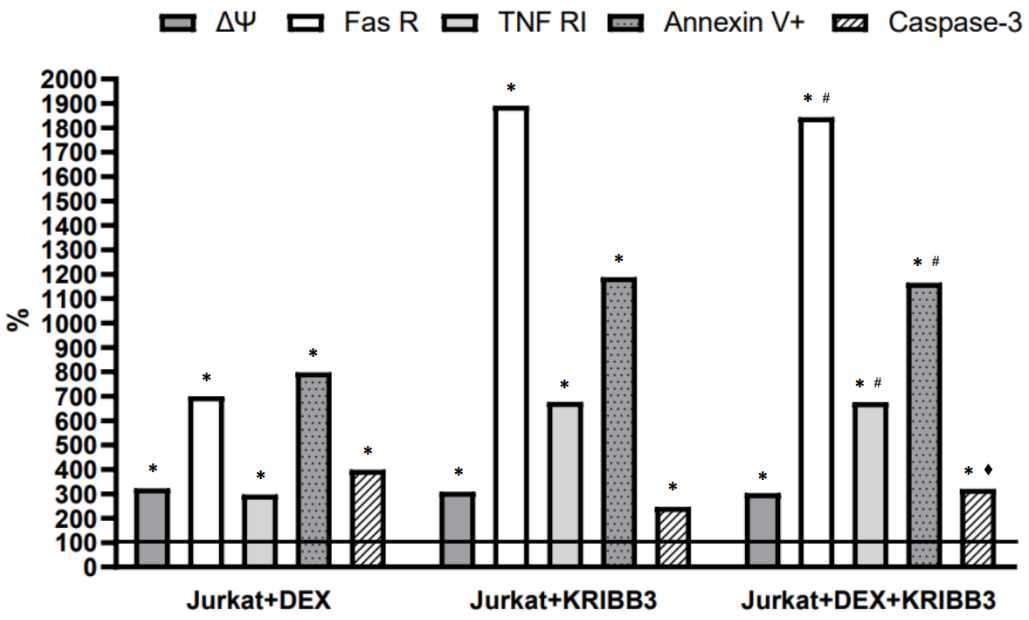

Fig. 3. Influence of the heat shock protein 27 inhibitor on apoptosis-related indicators during dexamethasone-induced apoptosis in Jurkat tumor cells, %

Note: DEX, dexamethasone; KRIBB3, 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazole; ΔΨ, cells with reduced mitochondrial potential; Fas R, Fas-positive cells; TNF RI, TNF RI-positive cells; Annexin V+, annexin-positive cells; Caspase-3, caspase-3. * indicates statistically significant differences ($p < 0.05$) compared to the intact Jurkat group; # indicates statistically significant differences ($p < 0.05$) compared to the Jurkat + DEX group; ♦ indicates statistically significant differences ($p < 0.05$) compared to the Jurkat + KRIBB3 group. The sample size is 5. Values for the intact Jurkat group were set at 100 % (cells with reduced mitochondrial potential ΔΨ: 18.00 [15.10—19.00] %; Fas-positive cells: 4.30 [2.12—8.90] %; TNF RI-positive cells: 9.56 [7.32—14.20] %; annexin-positive cells: 5.20 [4.00—5.60] %; caspase-3 activity: 36.58 [22.66—43.89] pmol/min × mg protein).

Analysis of Jurkat tumor cells treated simultaneously with DEX and KRIBB3 revealed a significant 129.4 % increase in caspase-3 activity ($p < 0.05$). Apaf-1 levels increased by 152.7 % ($p < 0.05$), protein carbonyl derivatives by 119.3 % ($p < 0.05$), oxidized tryptophan by 133 % ($p < 0.05$), bi-tyrosine by 216.9 % ($p < 0.05$), Hsp27 by 223.5 % ($p < 0.05$), and ubiquitin by 197.8 % ($p < 0.05$), whereas free sulfhydryl groups decreased by 48.6 % ($p < 0.05$) and ubiquitin ligase by 44.2 % ($p < 0.05$). Compared to the group cultured with the Hsp27 inhibitor alone, decreases were observed in the number of annexin-, Fas-, and TNF RI-positive cells, cells with reduced mitochondrial potential, NFκB and hydroxyl radical levels, protein-bound glutathione, and Hsp70 (Fig. 1—3).

Simultaneous exposure of Jurkat tumor cells to DEX and KRIBB3 in the incubation medium resulted in a significant increase in annexin-positive cells by 146 % ($p < 0.05$), Fas-positive cells by 263.5 % ($p < 0.05$), TNF RI-positive cells by 227.4 % ($p < 0.05$), and NFκB by 146 % ($p < 0.05$). In addition, levels of hydroxyl radicals increased by 170.8 % ($p < 0.05$), protein carbonyl derivatives by 146.9 % ($p < 0.05$), bi-tyrosine by 235 % ($p < 0.05$), Hsp27 by 142.4 % ($p < 0.05$), Hsp70 by 203.7 % ($p < 0.05$), and ubiquitin by 147.8 % ($p < 0.05$). Moreover, in the cell group cultured with the apoptosis inducer alone, Apaf-1 levels decreased by 52.7 % ($p < 0.05$), free protein SH groups by 59.1 %

($p < 0.05$), and ubiquitin ligase by 23.1 % ($p < 0.05$), while caspase-3 activity, the number of cells with reduced mitochondrial potential, and levels of protein-bound glutathione and oxidized tryptophan remained comparable (Fig. 1—3).

Dexamethasone alone induces apoptosis in Jurkat tumor cells by activating both receptor-mediated and mitochondrial pathways. This process is accompanied by the accumulation of oxidatively modified proteins, as evidenced by positive correlations:

— between oxidized tryptophan levels and the proportion of Fas-positive cells ($r = +0.89$, $p < 0.05$);

— between oxidized tryptophan levels and Apaf-1 expression ($r = +0.89$, $p < 0.05$).

When the heat shock protein 27 inhibitor KRIBB3 is added to the culture medium of Jurkat tumor cells, a negative correlation is observed between bi-tyrosine content and NF-κB expression ($r = -0.94$, $p < 0.05$).

Under the combined treatment of dexamethasone (apoptosis inducer) and KRIBB3 (Hsp27 inhibitor) in Jurkat cells, the following correlations were identified:

— between TNF RI-positive cell numbers and protein-bound glutathione levels ($r = -0.94$, $p < 0.05$);

— between bi-tyrosine content and oxidized tryptophan ($r = +0.89$, $p < 0.05$).

The results indicate that Hsp27 inhibition markedly enhances apoptosis induction via the receptor pathway under DEX treatment, highlighting a key role for Hsp27 in both irreversible and reversible oxidative modifications of apoptotic proteins, Fas receptors, and TNF RI.

Conclusions

The study demonstrated that the combined treatment of Jurkat tumor cells with 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazole and dexamethasone alters the levels of Hsp27, Hsp70, and ubiquitin, and induces oxidative stress, as evidenced by increased hydroxyl radicals along with elevated bi-tyrosine, protein carbonyl derivatives, and oxidized tryptophan. In addition, protein-bound glutathione levels decrease. These findings suggest an increase in irreversible protein modifications and a decrease in reversible modifications. Thus, inhibition of Hsp27 with KRIBB3 combined with apoptosis induction by DEX revealed the molecular mechanisms through which Hsp27, Hsp70, and ubiquitin predominantly modulate the receptor-mediated apoptosis pathway in Jurkat tumor cells, involving oxidatively modified proteins.

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Conflict of interest. The authors declare absence of the conflict of interest.

Финансирование. Исследование проводилось без внешнего финансирования.

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References

1. Pistritto G., Trisciuoglio D., Ceci C., Garufi A., D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY). 2016, 8(4), 603—619, DOI: 10.18632/aging.100934.

2. Kulbay M., Paimboeuf A., Ozdemir D., Bernier J. Review of cancer cell resistance mechanisms to apoptosis and actual targeted therapies. Journal of cellular biochemistry. 2022, 123(11), 1736—1761, DOI: 10.1002/jcb.30173.

3. Hayes J. D., Dinkova-Kostova A. T., Tew K. D. Oxidative Stress in Cancer. Cancer Cell. 2020, 38(2), 167—197, DOI: 10.1016/j.ccell.2020.06.001.

4. Wu J., Liu T., Rios Z., Mei Q., Lin X., Cao S. Heat Shock Proteins and Cancer. Trends in pharmacological sciences. 2017, 38(3), 226—256, DOI: 10.1016/j.tips.2016.11.009.

5. Ahmed K., Zaidi S. F., Mati-Ur-Rehman, Rehman R., Kondo T. Hyperthermia and protein homeostasis: Cytoprotection and cell death. Journal of thermal biology. 2020, 91, 102615, DOI: 10.1016/j.jtherbio.2020.102615.

6. Szyller J., Bil-Lula I. Heat Shock Proteins in Oxidative Stress and Ischemia/Reperfusion Injury and Benefits from Physical Exercises: A Review to the Current Knowledge. Oxidative medicine and cellular longevity. 2021, 2021, 6678457, DOI: 10.1155/2021/6678457.

7. Lang B. J., Prince T. L., Okusha Y., Bunch H., Calderwood S. K. Heat shock proteins in cell signaling and cancer. Biochimica et biophysica acta. Molecular cell research. 2022, 1869(3), 119187, DOI: 10.1016/j.bbamcr.2021.119187.

8. Takakuwa J. E., Nitika, Knighton L. E., Truman A. W. Oligomerization of Hsp70: Current Perspectives on Regulation and Function. Frontiers in molecular biosciences. 2019, 6, 81, DOI: 10.3389/fmolb.2019.00081.

9. Nosareva O. L., Stepovaya E. A., Ryazantseva N. V., Zakirova E. V., Mazunin I. O., Litvinova L. S. et al. Disruption of Expression of mRNA Hsp27 and Ubiquitin as a Mechanism of Escaping from Apotosis of Jurkat Line Tumor Cells. Bulletin of Siberian medicine. 2015, 14(1), 66—72, DOI: 10.20538/1682-0363-2015-1-66-72.

10. Saini J., Sharma P. K. Clinical, Prognostic and Therapeutic Significance of Heat Shock Proteins in Cancer. Current drug targets. 2018, 19(13), 1478—1490, DOI: 10.2174/1389450118 66617082312124.

11. Boliukh I., Rombel-Bryzek A., Żuk O., Radecka B. The role of heat shock proteins in neoplastic processes and the research on their importance in the diagnosis and treatment of cancer. Contemporary oncology (Poznań, Poland). 2021, 25(2), 73—79, DOI: 10.5114/wo.2021.106006.

12. Nosareva O. L., Stepovaya E. A., Shakhristova E. V., Alekseeva O. N., Kuzmenko D. I., Sadykova A. A. et al. The role of redox status and oxidative modification of proteins in implementing apoptosis in human blood lymphocytes in norm and under experimental oxidative stress. Rossiĭskii fiziologicheskiĭ zhurnal imeni I. M. Sechenova. 2019, 105(3), 327—338, DOI: 10.1134/S0869813919030063.

13. Dilek O. Current Probes for Imaging Carbonylation in Cellular Systems and Their Relevance to Progression of Diseases. Technology in cancer research & treatment. 2022, 21, 15330338221137303, DOI: 10.1177/15330338221137303.

14. Wang H., Yang L., Liu M., Luo J. Protein post-translational modifications in the regulation of cancer hallmarks. Cancer gene therapy. 2023, 30(4), 529—547, DOI: 10.1038/s41417-022-00464-3.

15. Regimbeau M., Abrey J., Vautrot V., Causse S., Gobbo J., Garrido C. Heat shock proteins and exosomes in cancer theranostics. Seminars in cancer biology. 2022, 86(Pt 1), 46—57, DOI: 10.1016/j.semcancer.2021.07.014.

16. Lampros M., Vlachos N., Voulgaris S., Alexiou G. A. The Role of Hsp27 in Chemotherapy Resistance. Biomedicines. 2022, 10(4), 897, DOI: 10.3390/biomedicines10040897.

17. Kumar S., Stokes J., Singh U. P., Scissum Gunn K., Acharya A., Manne U. et al. Targeting Hsp70: A possible therapy for cancer. Cancer letters. 2016, 374(1), 156—166, DOI: 10.1016/j.canlet.2016.01.056.

18. Swatek K. N., Komander D. Ubiquitin modifications. Cell research. 2016, 26(4), 399—422, DOI: 10.1038/cr.2016.39.

19. Song L., Luo Z. Q. Post-translational regulation of ubiquitin signaling. The Journal of cell biology. 2019, 218(6), 1776—1786, DOI: 10.1083/jcb.201902074.

20. Dikic I., Schulman B. A. An expanded lexicon for the ubiquitin code. Nature reviews. Molecular cell biology. 2023, 24(4), 273—287, DOI: 10.1038/s41580-022-00543-1.

21. Nosareva O. L., Stepovaya E. A., Ryazantseva N. V., Shakhristova E. V., Orlov D. S., Novitsky V. V. The role of ubiquitin in regulation of apoptosis in Jurkat cells. Bulletin of Siberian medicine. 2018, 17(3), 96—104, DOI: 10.20538/1682-0363-2018-3-96-104.

22. Ryazantseva N. V., Stepovaya E. A., Nosareva O. L., Konovalova E. V., Orlov D. S., Naumova A. I. et al. Role of heat shock protein 27 in regulation of glutathione system and apoptosis of Jurkat tumor cells and blood lymphocytes. Bulletin of experimental biology and medicine. 2014, 158(9), 366—369, DOI: 10.1007/s10517-015-2766-3.

23. Nosareva O. L., Stepovaya E. A., Ryazantseva N. V., Shakhristova E. V., Egorova M. Y., Novitsky V. V. The Role of the Glutathione System in Oxidative Modification of Proteins and Dysregulation of Apoptosis in Jurkat Tumor Cells. Bulletin of experimental biology and medicine. 2017, 164(8), 228—231, DOI: 10.1007/s10517-017-3957- x.

24. Kurop M. K., Huyen C. M., Kelly J. H., Blagg B. S. J. The heat shock response and small molecule regulators. European journal of medicinal chemistry. 2021, 226, 113846, DOI: 10.1016/j.ejmech.2021.113846.

25. Dubinina E. E. Products of oxygen metabolism in the functional activity of cells (life and death, creation and destruction). Physiological, clinical and biochemical aspects. Medical Press: St. Petersburg. 2006, ISBN 5-85474-072-9.

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