HSP90 INHIBITOR GELDANAMYCIN AS A RADIATION RESPONSE MODIFICATOR IN HUMAN BLOOD CELLS

Katia Stankova, Gergana Savova, Vladimir Nikolov and Rayna Boteva

National Center of Radiobiology and Radiation Protection, Georgi Sofiyski 3, Sofia1606, Bulgaria

Heat shock protein 90 (Hsp90) is a highly conserved molecular chaperone, involved in the folding, assembly, stabilization and activation of numerous proteins with unrelated amino acid sequences and functions. Geldanamycin (GA), a natural benzoquinone, can inhibit the chaperone activity of Hsp90. It has been shown that GA can produce superoxide anions and increase the intracellular oxidative stress, which, in addition to the direct inhibition of Hsp90, might also contribute to the modifying effects of the inhibitor on the early response in human mononuclear cells exposed to ionizing radiation. The present study shows that GA antagonizes the radiation-induced suppression on MnSOD and catalase, key enzymes of the radical scavenging systems. By significantly up-regulating catalase levels over the entire range of doses from 0.5 to 4 Gy, the inhibitor of Hsp90 exerted adaptive protection and modified the early radiation response of the human blood cells.

Key words: molecular chaperone Hsp90; geldanamycin, human lymphocytes; radiation response; antioxidant enzymes.

INTRODUCTION

Heat shock protein 90 (Hsp90) is a highly conserved molecular chaperone, involved in the folding, assembly, stabilization and activation of several hundreds of proteins referred to as Hsp90 “clients” (Zhao et al. 2005, Neckers 2007, Wandinger et al. 2008, Trepel et al. 2010, Samant et al. 2012, Sharma et al. 2012). Belonging to the clients of Hsp90 are proteins with unrelated amino acid sequences and functions, involved in signal transduction, protein trafficking, receptor maturation and innate and adaptive immunity (Taipale et al. 2010) as well as numerous oncoproteins. Hsp90 has been found to be up-regulated in a number of cancer types in comparison with normal tissues and this has been associated with poor prognosis and resistance to therapy (Yano et al, 1999, Becker et al. 2004). Due to its essential role in the survival of cancer cells, Hsp90 is an important therapeutic target which has led to the development of Hsp90 inhibitors applicable for cancer treatment. As a weak ATP-ase, Hsp90 uses the energy generated in a complex cycle of adenosine triphosphate (ATP) binding and hydrolysis to assist protein folding and
maturation. Benzoquinones, such as the natural product geldanamycin (GA), and its derivatives 17-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-demethoxy-17-N,N-dimethylaminoethylaminogeldanamycin (17-DMAG) are inhibitors of Hsp90 and exert their pro-apoptotic effects on malignant cells through inhibition of ATP binding in the ATP/ADP-binding pocket of Hsp90. These inhibitors bind to the ATP-binding center of Hsp90 with higher affinity compared to the natural nucleotides thus blocking its chaperone activity, leaving the substrates of Hsp90 in an unfolded state, prone to proteolytic degradation. Therefore, the inhibition of Hsp90 has been proved selectively lethal to tumor cells (Lin et al. 2008), and the inhibitors of Hsp90, including GA and its derivatives have been used either on their own, or in combination with chemotherapy and/or radiotherapy in the treatment of solid tumors such as breast, prostate and lung cancers, malignant melanoma as well as of hematological malignancies like chronic lymphocytic leukemia (CLL), CML, AML and multiple myeloma (Didelot et al. 2007, Banerji 2009, Kabakov et al. 2010).

In addition to serving as molecular chaperones, Hsp90 and other chaperones have been implicated in autoimmune diseases, antigen presentation, and tumor immunity. Studies have demonstrated that Hsp90 plays a role in generating antigen-specific T-cell responses and activating the innate immune system (Tamura et al. 1997, Hauet-Broere et al. 2006, Van Eden et al. 2007). Hsp90 has been also found to induce the production of pro-inflammatory cytokines in autoimmune and chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus (Ripley et al. 2001, Rice et al. 2008). Quite recently, in accordance with the effects of the molecular chaperone on the immune system, it has been demonstrated that Hsp90 inhibitors at clinically relevant concentrations can modulate adaptive immune responses both on the level of dendritic cells (DC) activation and T cell proliferation (Trojandt et al. 2014).

It has also been found that GA and its derivatives can produce superoxide anions and increase the intracellular oxidative stress, which might also contribute to the antitumor effect of the inhibitor (Dikalov et al. 2002, Sreedhar et al. 2003). The pro-oxidant properties of GA have been explained by its electrophilic nature allowing the inhibitor to react with and deplete cellular glutathione (GSH) (Cysyk et al. 2006) and to oxidize thiol groups of critical cellular proteins including reactive cysteine residues of Hsp90. As the chaperone has been suggested to play a role in the maintenance of the redox status of the cytosol (Nardai et al. 2000), oxidizing cysteines of Hsp90, GA can inhibit the ability of Hsp90 to regulate cellular oxidative stress and disrupt its signaling networks (Clark et al. 2009), thus, contributing to cellular oxidative and unfolded protein responses.

Ionizing radiation is a strong inducer of reactive oxygen and nitrogen species - ROS and RNS, respectively (O’Neill and Wardman, 2009). Depending on their concentration, reactivity and distribution, these
species may mediate either adaptive/protective responses or genomic instability in the progeny of irradiated cells and their neighbors (bystander effects) (Azzam and Little, 2004, Brooks 2005, de Toledo and Azzam, 2006). Since GA can produce superoxide anions and increase the intracellular oxidative stress, the pre-treatment of the cells with GA may induce protective adaption in irradiated cells. To test this hypothesis, the present study analyses the impact of Hsp90 inhibition by GA on the early radiation response in human blood cells and provides evidence for a significant, dose-independent up-regulation of catalase in a broad range of doses from 0.5 to 4 Gy indicating induction of adaptive response by the reagent.

MATERIALS AND METHODS

Isolation and treatment of peripheral blood mononuclear cells (PBMC)

PBMC were isolated from 3-5 ml of venous blood from healthy volunteer donors. Isolation was performed by density gradient, as previously described (Stankova et al. 2011, Ivanova et al. 2010, Stankova et al. 2013) and cells were re-suspended in RPMI 1640 medium (Sigma, St. Louis, MO, USA), supplemented with 10% fetal calf serum (Sigma, St. Louis, MO, USA). For each experiment, cells from 3-5 donors were pooled right after the isolation, brought to a concentration of $2 \times 10^6$ cells/ml and split into samples which were subjected to different treatments: (A) 2 hours of incubation, followed by in vitro irradiation ($^{60}$Co gamma source, dose rate 7.5 Gy/min, Gitawa Ltd, Sofia, Bulgaria), and incubation for another 2 hours; (B) 4 hours of incubation in the presence of 0.1 μM GA (Sigma St Louis, MO, USA); (C) 2 hours of incubation in the presence of 0.1 μM GA, followed by irradiation (as described), and incubation for another 2 hours; (D) control samples, incubated for 4 hours without GA and non-irradiated. As the GA stock has been dissolved in DMSO (Sigma St Louis, MO, USA), amounts of DMSO corresponding to the volume of the chemical in the treated samples were added to all control samples.

Approval for the studies was obtained from the institutional review body, and informed consent from volunteer donors was obtained in accordance with the Declaration of Helsinki.

Toxicity of treatments with GA and/or radiation exposure

Toxic effects due to the treatment of the cells with GA and/or irradiation were analyzed using the fluorescent dye calcein AM (Molecular Probes Inc., USA), as it has been previously described (Stankova et al. 2013). Changes in the cellular viability were determined in three independent experiments and calculated as the percentage ratio of the fluorescence emissions of the treated and/or irradiated samples versus non-treated, non-irradiated controls. The levels of apoptotic cells were determined using the commercially available Caspase 3 colorimetric assay kit (Sigma
St Louis, MO, USA) according to the manufacturer’s instructions for the 96-well plate microassay method (Stankova et al. 2013). Apoptosis was analyzed 4 and 24 h after treatment with GA and 22 h post-irradiation.

**Levels of exogenous ROS after treatment and/or irradiation of PBMC**

Changes in the intracellular levels of the reactive oxygen species (ROS) were analyzed by the fluorescent dye 2’,7’-dichlorodihydro fluorescein diacetate (DCDHF-DA, Molecular probes, Life Technologies Corporation) as it has been already described by Stankova et al. 2013. Data represents relative increase in DCDHF fluorescence, reflecting changes in the intracellular ROS content in comparison to the non-treated, non-irradiated controls.

**Western Blot analysis**

Protein levels of the antioxidant enzymes MnSOD (manganese superoxide dismutase) and catalase, NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and Nrf2 (nuclear factor (erythroid-derived 2)-like 2) were determined by Western blots in whole-cell extracts as described (Ivanova et al. 2010, Stankova et al. 2013). Blots were visualized with 3, 3’-diaminobenzidine (DAB) peroxidase substrate (Sigma St Louis, MO, USA). Optical densities of the protein bands were analyzed using the Labworks 4.0 software package (UVP Bioimaging Systems, Cambridge, UK). Changes in protein levels are expressed relative to the level of the non-treated, non-irradiated controls and represent the average values of at least five independent experiments performed with different pools of patients’ PBMC.

**Statistics**

All datapoints represent the mean and standard deviation from at least three independent experiments, performed with different pools of patients’ PBMC. The experimental data was analyzed by the ANOVA (ANalysis Of VAriance) method. The contribution of each group was characterized by Scheffe test for multiple comparisons. Possible cooperative effects between the factors (GA and radiation) were analyzed by factorial ANOVA, followed by multiple endpoint Post-hoc comparison with the Scheffe test and p < 0.05 was considered statistically significant (de Sa 2007).

**RESULTS AND DISCUSSION**

**Effects of GA on the viability of PBMC**

Treatment of PBMC with 0.1 μM GA and/or radiation exposure to doses ranging up to 4 Gy did not affect significantly the viability of PBMC
Geldanamycin modifies antioxidant responses of blood cells as determined by the calcein AM assay (Figure 1, \( F_{5/22} = 2.42, p = 0.07 \)). The observation was confirmed by the Caspase-3 activity-based apoptotic test (Figure 2), applied 4 h and 24 h after the \textit{in vitro} exposure of the cells to GA and/or 3 Gy of gamma rays (\( \gamma \)-rays). As seen in Figure 2, apoptosis

![Figure 1](image1.png)

**FIGURE 1.** Dose-dependence of cellular viability, measured 2 h post-irradiation in lymphocytes pre-treated for 2 h or not treated with GA and exposed to doses ranging from 0.5 to 4 Gy of gamma rays. Exposure to 4 Gy induced statistically significant reduction in the viability of non-treated lymphocytes (\( F_{5/22} = 2.54, p = 0.0395 \)). Pre-treatment with GA abolished the IR-induced reduction in the viability which remained close to those of the non-treated, non-irradiated controls. Error bars represent the standard deviation (+/−SD) of the mean for \( n=3 \) independent experiments performed with different pools of patients’ PBMC.

![Figure 2](image2.png)

**FIGURE 2.** Effects of the Hsp90 inhibitor GA on human PBMC. PBMC were treated for 4 h and 24 h with GA and/or exposed to 3 Gy of gamma rays. Factorial ANOVA, followed by multiple endpoint Post-hoc comparison with the Scheffe test revealed statistically significant differences (*\( p<0.01 \)) between lymphocytes treated with GA and irradiated versus non-treated but irradiated lymphocytes, measured 24 h post-irradiation.
measured 24 h post-exposure, was lowest (10-11%) in the control lymphocytes, not exposed to GA or radiation. In vitro irradiation of the cells with 3 Gy slightly increased the apoptotic levels and the magnitude of the effect was dependent on whether the cells were pre-treated with GA or not (Figure 2).

**GA increases the cellular oxidative stress and up-regulates key antioxidant enzymes as part of the early radiation response in PBMC**

It has been demonstrated that GA can increase the intracellular superoxide formation by enzymatic and non-enzymatic redox pathways (Dikalov et al. 2002). Accordingly, we found that 0.1 μM GA elevated the intracellular ROS in PBMC up to 145-150% \( (F_{5/12} = 62.068, \ p<0.0001) \). Exposure of the cells to different doses of gamma ionizing radiation (IR) ranging from 0.5 to 4 Gy, in addition to their treatment with GA, led to dramatic increase of ROS which reached supra-physiological levels, exceeding several-fold the levels of the endogenous ROS. The effect was linearly dose-dependent \( (r^2 = 0.99) \) and statistically significant (Figure 3, \( F_{1/16} = 9101.914, \ p<0.0001 \)) as revealed by the linear regression analysis of the data. Cells pretreated with GA, generated higher levels of ROS in the whole dose range from 0.5 to 4 Gy (Figure 3) which, however, were shown to attenuate faster as compared to the decay kinetics of ROS measured in the cells which were not pre-treated with the reagent.

**FIGURE 3.** Effects of Hsp90 inhibition on intracellular ROS. Dose-dependence of ROS levels in PBMC either pre-treated for 2 h or not treated with GA and exposed to different doses of gamma radiation, analyzed 2 h post-irradiation. Data represents a percentage ratio of the emission of DCDHF-DA at 535 nm (excitation at 485 nm) measured in samples pre-treated with GA and/or irradiated versus the emission of DCDHF-DA in non-treated and non-irradiated controls. Error bars indicate the standard deviation (+/-SD) of the mean for \( n=3 \) independent experiments performed with different pools of patients’ PBMC.
Geldanamycin modifies antioxidant responses of blood cells

(Figure 4) and approaching equal levels at later time points post-irradiation (Figure 4). This might reflect some GA-induced stimulation of the cellular antioxidant defense due to up-regulation of certain antioxidant enzymes. Indeed, as seen in Figure 5, the reagent up-regulated MnSOD and catalase levels up to 130 and 115%, respectively. The stimulating effect of GA on the antioxidant enzymes was also well pronounced in the irradiated cells; the reagent effectively counteracted the radiation-induced suppression on MnSOD and catalase over the entire dose range (from 0.5 to 4 Gy). Maintaining the MnSOD levels higher or equal to those in the control (Figure 5a), and dramatically up-regulating catalase (Figure 5b; $F_{4/29}=4.798$, $p=0.0043$), the reagent efficiently antagonized the radiation-induced suppression on the two enzymes and contributed to the antioxidant capacity of the cells in the early stages of their radiation response. Notably, the stimulating effect of GA on catalase was non-linear and dose-independent, thus suggesting induction of protective adaptation upon pre-treatment of the cells with the reagent.

Since the stress-response NF-κB and Nrf2 pathways contribute to cellular redox homeostasis by inducing the expression of antioxidant genes, including those of MnSOD and catalase (Kensler et al. 2007, Ahmed and Li, 2008, Holley et al. 2010), and on the other hand Hsp90 is directly involved in the regulation of these two pathways (Hertlein et al. 2010, Niture and Jaiswal, 2010), the current study aimed to also analyze the effects of GA and/or irradiation on the protein levels of NF-κB and Nrf2.

**FIGURE 4.** Effects of Hsp90 inhibition on intracellular ROS. Kinetics of ROS decay in PBMC, pre-treated or not treated with GA, and exposed to 4 Gy of gamma rays. Data represent a percentage ratio of the emission of DCDHF-DA at 535 nm (excitation at 485 nm) measured in samples pre-treated with GA and/or irradiated versus the emission of DCDHF-DA in non-treated and non-irradiated controls. Error bars indicate the standard deviation (+/-SD) of the mean for n=3 independent experiments performed with different pools of patients’ PBMC.
It was found that NF-κB and Nrf2 levels remained similar to those in the control cells which were not exposed to GA and/or radiation (data not shown), in accordance with other recent studies which have demonstrated that NF-κB and Nrf2 are highly expressed in CLL PBMC but not in the normal PBMC (Hertlein et al. 2010, Wu et al. 2010). The Hsp90 inhibitor has been identified as a potent inducer of the endoplasmic reticulum (ER) stress and unfolded protein responses leading to disruption of mitochondrial homeostasis (Lawson et al. 1998). Thus, pathways, connected with the unfolded protein responses may be predominantly involved in the up-regulation of the antioxidant enzymes MnSOD and catalase observed here. The superoxide, accumulated in the cells pre-treated with

**FIGURE 5.** Effects of Hsp90 inhibition on radiation-induced changes in MnSOD (5a) and catalase (5b) in PBMC pre-treated for 2 h with geldanamycin. Changes in protein levels are presented relative to the protein levels in non-treated, non-irradiated PBMC which were referred to as 100% (dotted lines). Error bars represent the standard deviation (+/-SD) of the mean for n=5 independent experiments performed with different pools of patients’ PBMC.
Geldanamycin modifies antioxidant responses of blood cells

GA, may act as a mediator of adaptive ROS signal which activates pathways linking the cellular antioxidant response to unfolded protein response (Sarbassov and Sabatini 2005) as shown by the concerted up-regulation of key enzymes of the radical scavenging systems such as MnSOD and catalase. This can modify significantly the early dose response and exert protective effects on the blood cells exposed to lethal stress, such as an acute exposure to high doses of ionizing radiation. Similar protective effect has been demonstrated in normal human keratinocytes, where the overexpression of catalase has been shown to decrease significantly the UV-induced apoptosis after reducing the caspase-9 activation and p53 levels (Rezvani et al. 2006).

CONCLUSION

We have shown that GA, inhibitor of Hsp90, can induce changes in ROS signaling and protein homeostasis, and promote protective radio adaptation in normal human lymphocytes. GA up-regulates the protein levels of MnSOD and catalase, two key antioxidant enzymes. The elevation in MnSOD levels induced by GA completely compensated the radiation-induced down-regulation of the enzyme levels. The reagent increased dramatically the protein levels of catalase in the irradiated cells exposed to doses up to 1 Gy and the elevation remained constant at higher doses ranging from 1 to 4 Gy, as part of the early dose-response of lymphocytes. This effect of GA on catalase seems of particular interest as it is nonlinear and may suggest a role of GA as a priming agent inducing protective adaptation of the blood cells to radiation exposure and lowering radiation injury.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

We are grateful to Elena Zaharieva for the critical reading of the text and helpful discussion. Part of the work was supported financially by the Bulgarian Ministry for Education and Science, grant G-3-10/05.

REFERENCES

Ahmed KM, and Li JJ. 2008. NF-kappa B-mediated adaptive resistance to ionizing radiation. Free Radic Biol Med. 44:1-13, 10.1016/j.freeradbiomed.2007.09.022
Azzam EI, and Little JB. 2004. The radiation-induced bystander effect: evidence and significance. Hum Exp Toxicol 23:61–65 [PubMed: 15070061]
Banerji U. 2009. Heat shock protein 90 as a drug target: some like it hot. Clin Cancer Res. 15:9-14, 10.1158/1078-0432.CCR-08-0132
Becker B, Muhlhoff G, Farkas B, Wild PJ, Landthaler M, Stolz W, and Vogt T. 2004. Induction of Hsp90 protein expression in malignant melanomas and melanoma metastases. Exp Dermatol 13:27–32.

Brooks AL. 2005. Paradigm shifts in radiation biology: their impact on intervention for radiation-induced disease. Radiat Res 164:454–461 [PubMed: 16187749]

Clark CB, Rane MJ, El Mehdí D, Miller CJ, Sachlben LR Jr, and Gozal E. 2009. Role of oxidative stress in geldanamycin-induced cytotoxicity and disruption of Hsp90 signaling complex. Free Radic Biol Med 47:1440-1449

Cysyk RL, Parker RJ, Barchi JJ Jr, Steeg PS, Hartman NR, and Strong JM. 2006. Reaction of geldanamycin and C17-substituted analogues with glutathione: product identifications and pharmacological implications. Chem Res Toxicol 19:376-381

de Sa JPM. 2007. Applied Statistic: Using SPSS, STATISTICA, MATLAB, in, Springer, Berlin

de Toledo SM, and Azzam EI. 2006. Adaptive and bystander responses in human and rodent cell cultures exposed to low level ionizing radiation: the impact of linear energy transfer. Dose Response 4:291–301 [PubMed: 18648584]

Didelot C, Laneau D, Brunet M, Joly AL, De Thonel A, Chiosis G, and Garrido C. 2007. Anti-cancer therapeutic approaches based on intracellular and extracellular heat shock proteins, Curr Med Chem 14:2839-2847

Dikalov S, Landmesser U, and Harrison DG. 2002. Geldanamycin leads to superoxide formation by enzymatic and non-enzymatic redox cycling. Implications for studies of Hsp90 and endothelial cell nitric-oxide synthase. J Biol Chem 277:25480-25485, 10.1074/jbc.M203271200

Hauet-Broere F, Wieten L, Guichelaar T, Berlo S, Van der Zee R, and Van Eden W. 2006. Heat shock proteins induce T cell regulation of chronic inflammation. Ann Rheum Dis 65:iii65–iii68

Hertlein E, Wagner AJ, Jones J, Lin TS, Maddocks KJ, Towns WH, Goettl VM, Zhang X, Jazouli D, Raymond CA, West DA, Croce CM, Byrd JC, and Johnson AJ. 2010. 17-DMAG targets the nuclear factor-kappaB family of proteins to induce apoptosis in chronic lymphocytic leukemia: clinical implications of HSP90 inhibition. Blood 116:45-53, 10.1182/blood-2010-01-263756

Holley AK, Xu Y, St Clair DK, and St Clair WH. 2010. RelB regulates manganese superoxide dismutase gene and resistance to ionizing radiation of prostate cancer cells. Ann N Y Acad Sci 1201:129-136, 10.1111/j.1749-6632.2010.05613.x

Ivanova KG, Stankova KG, Nikolov VN, Georgieva RT, Minkova KM, Gigova LG, Rupova IT, and Boteva RB. 2010. The biliprotein C-phycocyanin modulates the early radiation response: a pilot study. Mutat Res 695:40-45, 10.1016/j.mrgentox.2009.11.002

Kabakov AE, Kudryavtsev VA, and Gabai VL. 2010. Hsp90 inhibitors as promising agents for radiotherapy. J Mol Med (Berl) 88:241-247, 10.1007/s00109-009-0562-0

Kensler TW, Wakabayashi N, and Biswal S. 2007. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev Pharmacol Toxicol 47:89-116, 10.1146/annurev.pharmtox.46.120604.141046

Lennon CP, Brewer JW, and Hendershot LM. 1998. Geldanamycin, an hsp90/GRP94-binding drug, induces increased transcription of endoplasmic reticulum (ER) chaperones via the ER stress pathway. J Cell Physiol 174:170-179, 10.1002/(SICI)1097-4652(199802). Lin K, Rockliffe N, Johnson GG, Sherrington PD, and Pettitt AR. 2008. Hsp90 inhibition has opposing effects on wild-type and mutant p53 and induces p21 expression and cytotoxicity irrespective of p53/ATM status in chronic lymphocytic leukaemia cells. Oncogene 27:2445-2455, 10.1038/sj.onc.1210893

Nardai G, Sass B, Eber J, Orosz G, and Csermely P. 2000. Reactive cysteines of the 90-kDa heat shock protein, Hsp90. Arch Biochem Biophys 384:59-67.

Neckers L. 2007. Heat shock protein 90: the cancer chaperone. J Biosci 32:517-530

O’Neill P, and Wardman P. 2009. Radiation chemistry comes before radiation biology. Int J Radiat Biol 85:9-25 [PubMed: 19205982]

O’Neill P, and Wardman P. 2009. Radiation chemistry comes before radiation biology. Int J Radiat Biol 85:9-25 [PubMed: 19205982]

Niture SK, and Jaiswal AK. 2010. Hsp90 interaction with INrf2(Keap1) mediates stress-induced Nrf2 activation. J Biol Chem 285:38585-38595, 10.1074/jbc.M110.175802

Rezvani HR, Mazurier F, Cario-Andre M, Pain C, Ged C, Taieb A, and de Verneuil H. 2006. Protective effects of catalase overexpression on UVB-induced apoptosis in normal human keratinocytes. J Biol Chem 281:17999-18007, 10.1074/jbc.M100536200

Rice JW, Veal JM, Fadden RP, Barabasz AF, Partridge JM, Barta TE, Dubois LG, Huang KH, Mabbutt SR, Silinski MA, Steed PM, and Hall SE. 2008. Small molecule inhibitors of Hsp90 potently affect inflammatory disease pathways and exhibit activity in models of rheumatoid arthritis. Arthritis Rheum. 58:3765–3775
Geldanamycin modifies antioxidant responses of blood cells

Ripley BJM, Isenberg DA, and Latchman DS. 2001. Elevated levels of the 90 kDa heat shock protein (hsp90) in SLE correlate with levels of IL-6 and autoantibodies to hsp90. J Autoimmun 17:341–346

Samant RS, Clarke PA, and Workman P. 2012. The expanding proteome of the molecular chaperone HSP90. Cell Cycle 11: 1301-1308, 10.4161/cc.19722

Sarbassov DD, and Sabatini DM. 2005. Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex. J Biol Chem 280:39505-39509, 10.1074/jbc.M506096200

Sharma K, Vabulas RM, Macék B, Pinkert S, Cox J, Mann M, and Hartl FU. 2012. Quantitative proteomics reveals that Hsp90 inhibition preferentially targets kinases and the DNA damage response. Mol Cell Proteomics 11:M111 01456-4, 10.1074/mcp.M111.014564

Sreedhar AS, Mihaly K, Pato B, Schneider T, Stetak A, Kis-Petik K, Fidy J, Simonics T, Maraz A, and Csermely P. 2003. Hsp90 inhibition accelerates cell lysis. Anti-Hsp90 ribozyme reveals a complex mechanism of Hsp90 inhibitors involving both superoxide- and Hsp90-dependent events. J Biol Chem 278:35231-35240

Stankova K, Ivanova K, Nikolov V, Aneva N, Georgieva R, and Boteva R. 2013. Proteasome inhibition protects human peripheral blood mononuclear cells from radiation-induced oxidative stress. Int J Radiat Biol 89:493-500, 10.3109/09553002.2013.782451

Stankova K, Ivanova K, Nikolov V, Georgieva R, Minkova KM, Gigova LG, Rupova IT, and Boteva R. 2011. The biliprotein C-phycocyanin modulates the DNA damage response in lymphocytes from nuclear power plant workers. In: Tsvetkov PV (ed), Nuclear Power - Operation, Safety and Environment, pp. 327-340. InTech, Rijeka, Croatia

Tamura Y, Peng P, Liu K, Daou M, and Srivastava PK. 1997. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 278:117–120, 10.1038/nrm2918

Taipale M, Jarosz DF, and Lindquist S. 2010. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat Rev Mol Cell Biol 11:515–528. [PubMed: 20531426] Trepel J, Mollapour M, Giaccone G, and Neckers L. 2010 Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 10:537-549, 10.1038/nrc2887

Trojandt S, Reske-Kunz AB, and Bros M. 2014. Geldanamycin-mediated inhibition of heat shock protein 90 partially activates dendritic cells, but interferes with their full maturation, accompanied by impaired upregulation of RelB. J Exp Clin Cancer Res 33:16

Van Eden W, Wick G, Albani S, and Cohen I. 2007. Stress, heat shock proteins, and autoimmunity: how immune responses to heat shock proteins are to be used for the control of chronic inflammatory diseases. Ann NY Acad Sci 1113:217–237

Wandinger SK, Richter K, and Buchner J. The Hsp90 chaperone machinery. 2008. J Biol Chem 283:18473-18477, 10.1074/jbc.R800007200

Wu RP, Hayashi T, Cottam HB, Jin G, Yao S, Wu CC, Rosenbach MD, Corr M, Schwab RB, and Carson DA. 2010. Nrf2 responses and the therapeutic selectivity of electrophilic compounds in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 107:7479-7484, 10.1073/pnas.1002890107

Yano M, Naito Z, Yokoyama M, Shiraki Y, Ishiwata T, Inokuchi M, and Asano G. 1999. Expression of hsp90 and cyclin D1 in human breast cancer. Cancer Lett 137: 45–51

Zhao R, Davey M, Hsu YC, Kaplanek P, Tong A, Parsons AB, Krogan N, Cagney G, Mai D, Greenblatt J, Boone C, Emili A, and Honney WA. 2005. Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. Cell 120:715-727, 10.1016/j.cell.2004.12.024