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Enhancement of complement-induced cell lysis: a novel mechanism for the anticancer effects of Hsp90 inhibitors

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Abstract

Molecular chaperones (heat shock proteins, Hsp-s) play a pleiotropic role in immunological functions. Hsp-s participate in the presentation of peptide antigens, folding of several immunologically important proteins, such as the MHC, and in the maintenance of the activation-competent conformation of key signaling molecules (mostly serine/threonine and tyrosine kinases) of B and T cells activation. The most abundant cytoplasmic chaperone, Hsp90, is in the center of these processes. In recent years Hsp90 inhibitors emerged as very promising anticancer agents. Not surprisingly, Hsp90 inhibitors behave as immunosuppressants, and also cause an induction of superoxide production. Here we extend our previous data by showing the enhancement of complement-induced lysis of several types of tumor cells after Hsp90 inhibition. This novel mechanism may significantly contribute to the anticancer effects of Hsp90 inhibitors in vivo.

Keywords: Complement system; Heat shock proteins; Hsp90; Geldanamycin; Jurkat T lymphocytes; Molecular chaperones

1. Molecular chaperones and the immune system

Chaperones are ubiquitous, highly conserved proteins, which either work passively, by preventing the aggregation of damaged proteins, which expose "sticky" hydrophobic surfaces or utilize an active, ATP-driven conformational process to unfold their target proteins, and thus giving the targets a new chance to repeat parts of the folding process [1]. Chaperones probably played a major role in the molecular evolution of modern enzymes [2,3] and in the development of modern cells having a stable genetic material [4]. Environmental stress leads to the expression of most chaperones, which therefore are also called heat shock, or stress proteins.

Chaperones play a complex role in the mediation and maintenance of immune functions (Fig. 1) [5]. Cytoplasmic chaperones, such as the 90 kDa heat shock protein, Hsp90 participate in the assembly and "feeding" of the proteasomes; proteasome cap-structures themselves have chaperone activity to unfold irreversibly damaged proteins, and to direct them to the active sites of the proteasomal cavity, where antigenic peptides are generated [6–9]. Chaperones both in the cytoplasm and in the endoplasmic reticulum participate in transporting, trimming and presenting antigenic peptides to the MHC-I molecules [10–13]. Extracellular chaperones and their peptide-cargo, released as a result of cell death, and taken up by antigen presenting cells (APCs) through chaperone receptors [5,14,15] and are involved in the cross-presentation of chaperoned peptides on MHC molecules of APCs. Chaperones are also involved in assistance and quality control of the MHC-I complex, T and B cell receptors and many other key proteins of immune signaling [5,16–19].

Besides their complex role in the activation of adaptive immunity, extracellular chaperones may also act as natural adjuvants activating cells of the innate immune system leading to cytokine release, up-regulation of MHC-II complexes in antigen presenting cells as well as to increased dendritic cell maturation. These effects are proposed to be mediated by surface receptors of released, extracellular molecular chaperones, such as the scavenger receptor CD91, CD36 and the Toll-like receptors 2 and 4. A major element of the activation signals utilizes the NF-κB pathway [15].

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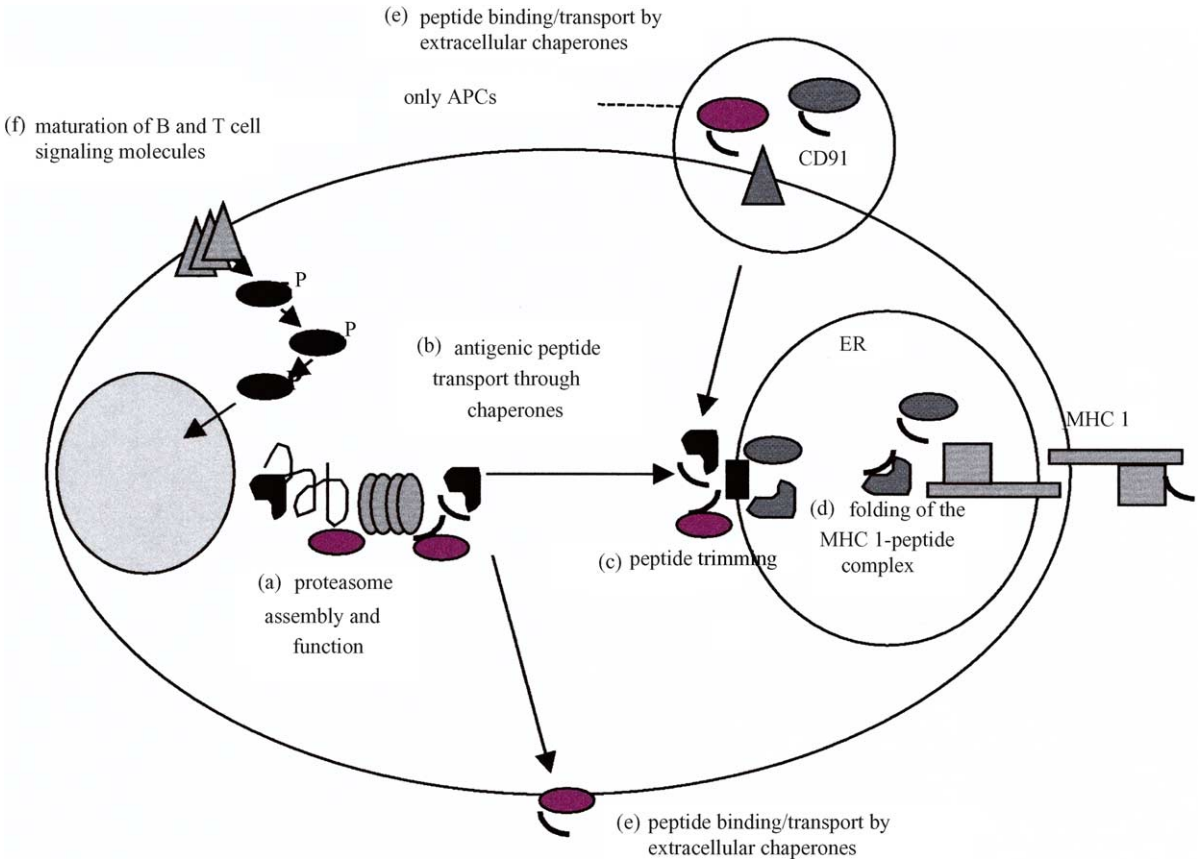

Fig. 1. Involvement of molecular chaperones in immune function: (a) proteasome assembly and function; (b) antigenic peptide transport through the chaperone relay-line; (c) peptide trimming; (d) folding of the MHC-I-peptide complex; (e) peptide binding/transport by extracellular chaperones; (f) participation in the conformational maturation of various proteins (mostly serine/threonine and tyrosine kinases) involved in B and T cells signaling.

2. Hsp90 inhibition as a novel path for anticancer therapies

Geldanamycin, a benzoquinonoid ansamycin analogue of herbimycin A, was originally developed as a tyrosine kinase inhibitor, but later it was shown to bind specifically to the unique N-terminal ATP/ADP-binding site of Hsp90-homologues [20,21]. Geldanamycin does not inhibit purified tyrosine kinases, but it induces the degradation of both tyrosine and serine/threonine kinases by the proteasome in vivo [20,22]. Thus its “kinase inhibition” is mediated by the premature disruption of Hsp90-kinase complexes. The geldanamycin analogue, 17-allyl-17-dimethoxygeldanamycin (17 AAG) is currently undergoing phase II clinical trials as an anticancer agent [23]. Other, highly potent Hsp90 inhibitors, such as radicicol [24] and purine scaffold inhibitors [25] have also been developed, and their clinical use is currently explored.

Due to the key role of Hsp90 in a number of signaling processes including cyclin-dependent kinases at the checkpoints of the cell cycle, cell survival signals, such as those mediated by the Akt kinase, as well as several tumor-specific alterations, such as telomerase activation, the Bcr-Abl kinase, mutant p53, etc., Hsp90 inhibitors affect numerous cellular processes simultaneously [23]. In this way they can efficiently attack tumor cells, and strongly diminish their chances for survival [26]. The Hsp90 inhibitor, 17AAG preferentially accumulates in tumor cells [27] and binds to the co-chaperone-complexed Hsp90 of tumor cells with a 100-fold higher affinity than to the uncomplexed Hsp90 form of normal cells [28].

3. Hsp90 inhibitors as immunosuppressants

Not surprisingly, Hsp90 inhibitors act as immunosuppressants. Geldanamycin blocks T cell signaling by counteracting both the T cell receptor- [17,18] and CD28-mediated [16] signal transduction. Inhibition of Hsp90 leads to a block in IL-2 secretion, IL-2 receptor expression, and the proliferation of stimulated T lymphocytes. Moreover, geldanamycin decreases the amount and the phosphorylation of Lck and Raf-1 kinases and prevents the activation of the ERK-2 kinase. Geldanamycin also disrupts the T cell receptor-mediated activation of NF-AT. On the contrary, geldanamycin treatment does not affect the activation of lysophosphatide acyltransferase, a plasma membrane enzyme coupled to the T cell receptor after T cell stimulation

[16–18]. Hsp90 inhibition also inhibits B cell responses [19]. Several well established immunosuppressants, such as deoxyspergualine or mizobirine were shown to interact with other major chaperones, like Hsp70 or Hsp60, respectively [29,30]. Compromised chaperone function leads to a serious impairment of immune responses.

4. Enhanced cell lysis as a novel consequence of Hsp90 inhibition

In recent studies [31,32] we analyzed the involvement of Hsp90 in the maintenance of cellular integrity using partial cell lysis as a measure. Inhibition of Hsp90 by geldanamycin, radicicol, cisplatin and novobiocin induced a significant acceleration of detergent- and hypotonic shock-induced cell lysis both in red blood cells and in the Jurkat T lymphoid cell line. The concentration and time-dependence of cell lysis-acceleration was in agreement with the Hsp90 inhibition characteristics of the N-terminal inhibitors, geldanamycin and radicicol. Hsp90 appeared as an important factor in the maintenance of cellular integrity.

5. The dual role of Hsp90 inhibitors: superoxide production and Hsp90 inhibition

Interestingly, later experiments of the above studies [32] demonstrated that the geldanamycin-induced additional lysis of Jurkat cells strongly depended on the experimental conditions, namely, if cells were mildly shaken during the experiment or not. At this time the first results of geldanamycin-induced superoxide generation appeared. In these studies geldanamycin-induced superoxide production independent of Hsp90 inhibition, due to the redox cycling of the quinoidal structure of the drug [33,34]. What if cell shaking introduced more oxygen to the medium, which helped superoxide formation besides a potential mechanical damage of the cytoskeleton? These results turned our attention to examine the contribution of superoxide-related versus Hsp-related events to diminished cellular integrity after Hsp90 inhibition. Using various Hsp90 inhibitors as well as newly developed anti-Hsp90 hammerhead ribozymes [32] we demonstrated that besides an increase in membrane fragility by geldanamycin-induced superoxides, inhibition or lack of Hsp90 *alone* also results in a compromised cellular integrity. The contribution of superoxide-induced lipid peroxidation and the bona fide Hsp90 inhibition to the enhanced cellular lysis was roughly 50% each, irrespectively from the fact, if we measured the extent of superoxide-mediated events by the addition of reducing agents or that of Hsp90-dependent events by ribozyme treatment [32]. The agreement between our independently obtained data gave us confidence that we indeed see a complex phenomenon after using Hsp90 inhibitors.

6. Enhancement of complement mediated cell lysis as a physiologically relevant action of Hsp90 inhibitors

To assess the decreased cell stability after the inhibition of Hsp90 function in experiments, which are more relevant to physiological conditions than mild detergent treatment or hypotonic shock, we have examined the effect of Hsp90 inhibitors and the disruption of Hsp90 by anti-Hsp90 ribozyme on hypoxia-induced and complement-mediated cytolysis of Jurkat cells [32]. Both conditions mimicked quite well the lytic conditions usual for tumor cells experiencing both hypoxia and immune attacks.

Our results demonstrated a clear enhancement of cell lysis under both conditions after any type of Hsp90 inhibition used [32]. In Fig. 2 the dependence of Jurkat cell lysis on the dilution of human serum (panel A) and on geldanamycin concentration (panel B) is shown. The extent of cell lysis reaches saturation, if examined as a function of either parameters. The half-maximal geldanamycin concentration, which is around 1.5 μM, shows a good agreement with the concentration dependence of other geldanamycin-induced effects in Jurkat cells [16,17,32]. Mouse serum induced a much larger lysis of Jurkat cells than human serum (data not shown). The complement-induced lysis rate was elevated in anti-Hsp90

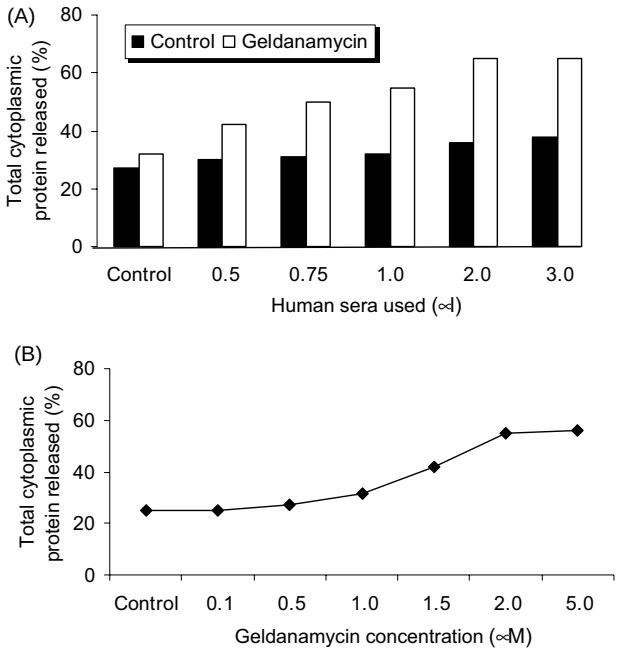

Fig. 2. Effect of geldanamycin on complement-induced cytolysis. (A) Jurkat cells in 0.5 ml volume of RPMI 1640-complete medium at a density of 2×10^6 ml^{-1} were pre-incubated with 2 μM geldanamycin (open bars) or without geldanamycin (filled bars) for 2 h at 37 °C, and were subjected to increasing concentration of 1:5 diluted human serum as shown in the panel for 10 min at 30 °C as described in [32]. Cell lysis was measured by estimating the percent of total cytoplasmic protein released using the Bradford method. (B) 2×10^6 Jurkat cells were pre-incubated with various concentrations of geldanamycin as shown in the panel for 2 h at 37 °C and were subsequently subjected to immune lysis with human serum at 1:5 dilution. Data are representatives of three experiments.

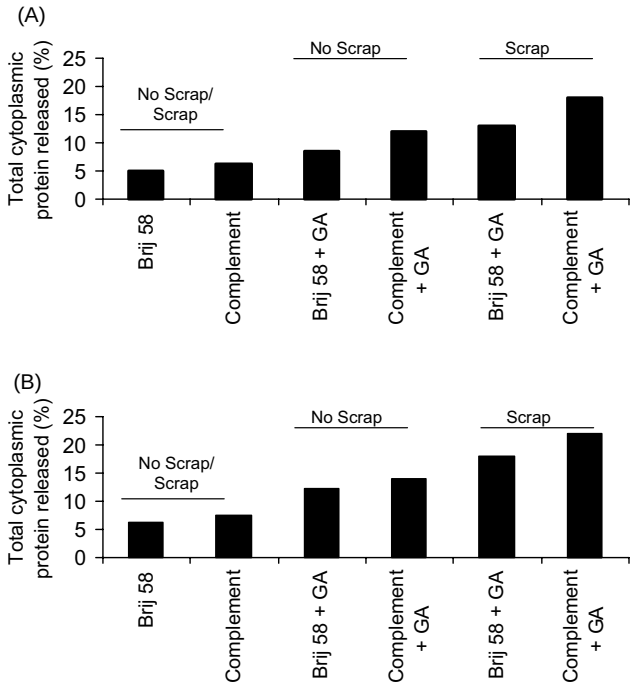

Fig. 3. Geldanamycin-enhanced cytolysis of human cervical carcinoma (HeLa; A) and SV40 transformed monkey kidney (Cos7; B) cells. HeLa and Cos7 cells in 0.5 ml volume of MEMNEAA and DMEM-complete medium, respectively, at a density of 2×10^6 cells/ml were treated with 2 μM geldanamycin (GA) for 2 h at 37 °C. Cells were washed twice with PBS, and were scraped with a rubber policeman (special care was taken not to disrupt the cells). One set was maintained without scrapping. Cells were lysed either with 0.005% Brij 58 or with 5 μl of 1:5 diluted human serum for 10 min at 30 °C, and were spun at 2000 rpm for 10 min. The supernatant was used to measure the extent of cytolysis by Bradford protein estimation. Percent of total cytoplasmic proteins released was expressed after deducting the control lysis value and by normalizing the data for complete lysis achieved after a 3 min sonication. Data are representatives of three experiments.

ribozyme-treated Jurkat cells, if compared to vector-treated cells [32].

Extending the above observations we have analyzed the geldanamycin-induced enhancement of complement lysis in two other tumor cell lines: HeLa and Cos7 cells (Fig. 3). Though the absolute sensitivity of these cells was different from that of Jurkat cells, in both cell lines inhibition of Hsp90 induced a marked increase in complement-induced cell lysis, and the relative extent of geldanamycin-induced additional lysis was similar to that observed with Jurkat cells, which showed the generality of our findings.

Our data suggest the contribution of Hsp90 in complement-mediated cell lysis. In agreement with the concept that chaperones play a role in the resistance against complement lysis of tumor cells Fishelson et al. [35] have demonstrated that the inhibition of Hsp70 by the immunosuppressant Hsp70 inhibitor, deoxyspergualine [29] enhances the complement-mediated lysis of K562 human erythroleukemia cells. On the contrary, stress-induced elevation of Hsp70 conferred elevated resistance of K562 cells against complement attack [35]. Heat shock proteins seem to have a general role in regulating the sensitivity level of various tumor cells against complement-induced lysis.

As we have briefly summarized earlier, the role of heat shock proteins in natural cell reactivity is well demonstrated [15]. Similarly, immune cell-mediated lysis is also associated with the production of superoxides [36]. Our earlier results showed that both pathways contribute to the Hsp90 inhibitor-enhanced cell lysis [32]. Sodium arsenite was shown to sensitize Jurkat cells for immune mediated cytolysis [37]. However, in this case the complexity of the stress response both in tumor and by-stander cells as well as the relative toxicity makes the treatment non-suitable for selective tumor therapy. On the contrary, selective depletion of Hsp90 seems to be an effective mode of cell sensitization to both hypoxia- and immune-mediated cell lysis, which adds a novel element to the mechanism of action of Hsp90 inhibitor drug candidates. This phenomenon may help the immune system to attack tumor cells. Similarly, a lysis sensitization may cause a shift from tumor cell apoptosis to necrosis, which gives a further help for the activation of the immune system [38].

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