Investigating cellular stress responses—a multidisciplinary approach from basic science to therapeutics—Report on the EuroSciCon (European Scientific Conferences) meeting

Katarzyna Bogunia-Kubik¹ and Gabriele Multhoff²

¹L. Hirsfeld Institute of Immunology and Experimental Therapy, Laboratory and Department of Clinical Immunology, Polish Academy of Sciences, Wrocław, Poland

²University Hospital rechts der Isar, Technical University Munich, Bldg 557, Room 1.33, Ismaningerstr. 22, 81675 Munich, Germany

Abstract The meeting on “Investigating cellular stress responses—a multidisciplinary approach from basic science to therapeutics” was held in London on 13 October 2006. The purpose of this 1-day meeting was to bring together European scientists investigating the immune biology of stress proteins and their potential clinical applications. The main topics included: the role of heat shock proteins (Hsps) in bacterial infections; the role of Hsps with a molecular mass of about 70 kDa in cancer therapy and in prediction of the clinical outcome following allogeneic hematopoietic stem cell transplantation; the quality and duration of stress as a danger signal for the initiation of a stress response; the mechanism of Hsp-protein interaction; and Hsp export from tumor cells in secretory granules.

INTRODUCTION

EuroSciCon (European Scientific Conferences), the Life Science Communication company, was founded in 2003 by Dr Shara Cohen, who identified the need for researchers to communicate in relatively small groups (a maximum of 100 delegates) for a short period of time (usually for 1 day) at a central European location on a specific topic so that they could develop new technologies that are required for cutting-edge research. The major goal of EuroSciCon is to provide a platform for scientists to interact, exchange information, and build up scientific networks. The agenda of the meetings is carefully planned and provides the opportunity for informal discussions to speakers, delegates, and commercial exhibitors. EuroSciCon meetings are attractive for a broad scientific community, including project leaders, laboratory managers,

quality control managers, postdoctoral fellows, PhD and MD students, and technical staff. Apart from research topics, the meetings also cover technically orientated areas, including improvements in “real-time PCR,” “viral techniques for gene transfer,” and “2D electrophoresis,” which are supported by commercial partners.

The EuroSciCon meeting “Investigating cellular stress responses—a multidisciplinary approach from basic science to therapeutics,” chaired by Katarzyna Bogunia-Kubik, was held in London on 13 October 2006. The purpose of this 1-day meeting was to bring together European scientists investigating the immune biology of stress proteins and their potential clinical applications. The first part of the meeting focused on the role of Hsps in bacterial infections. Kathy Triantafyllou (University of Sussex, Brighton, UK) has a long-term interest in studying innate immune responses to microbial pathogens and its relationship to lipopolysaccharide (LPS) receptors. The next talk, given by Brian Henderson (University College London, UK) discussed the interplay of both bacterial and host Hsps that have a considerable effect on the regula-

Correspondence to: Katarzyna Bogunia-Kubik, Tel: (+48) 71 3709960; Fax: (+48) 71 3371382; E-mail: bogunia@iitd.pan.wroc.pl.

Received 19 December 2006; Revised 9 February 2007; Accepted 13 February 2007.

tion of immune responses to infection. In the second part of the meeting, Gabriele Multhoff (Technical University Munich, Germany) and Katarzyna Bogunia-Kubik (L. Hirsfeld Institute of Immunology PAS, Wroclaw, Poland) presented data on the role of Hsps with a molecular mass of about 70 kDa in cancer therapy and in predicting the clinical outcome after allogeneic hematopoietic stem cell transplantation. John Williams (University of Chester, UK) emphasized that the quality and duration of stress is a danger signal for the initiation of a stress response. Two final talks were associated with the mechanism of Hsp-protein interaction, discussed by Pawel Stocki (University of Newcastle upon Tyne, UK), and Hsp export from tumor cells in secretory granules, discussed by Anton Evdonin (Institute of Cytology RAS, St. Petersburg, Russia).

HEAT SHOCK PROTEINS (HSPTS) AS MODULATORS OF THE INNATE IMMUNE RESPONSE, BY KATHY TRIANTAFILOU

Although Hsps are intracellular chaperones, it is becoming increasingly apparent that they participate in the generation of immune responses. Hsps seem to participate in acquired immune responses by enhancing processing and presentation of antigens via the major histocompatibility complex (MHC) class II pathway (Wang et al 2006), but also seem to be involved in the innate immune response because they have been found to be involved in the recognition of bacteria lipopolysaccharide (LPS) (Triantafilou et al 2001).

At least 3 cell surface molecules have been recognized as components of the mammalian signaling receptor for LPS: CD14, TLR4, and MD2. Hsps have been suggested to be part of this activation cluster, possibly acting as additional LPS transfer molecules. We have previously shown that the innate recognition of LPS takes place within lipid rafts, and Hsp70 interacts with TLR4 within membrane microdomains (Triantafilou and Triantafilou 2003, 2004; Triantafilou et al 2002, 2004). Although Hsps have been shown to be able to bind LPS and to be involved in the innate recognition of bacterial products (Triantafilou and Triantafilou 2004; Triantafilou et al 2001, 2004), the possible mechanism for their participation in innate immunity has not been elucidated yet.

In this study, we attempted to elucidate these mechanisms and found that addition of exogenous soluble Hsp70 was able to diminish LPS-induced cytokine responses, suggesting that Hsp70 plays a crucial role in modulating the innate recognition of LPS. The Hsp70 was human recombinant Hsp70, and the cytokine studied was tumor necrosis factor (TNF)- α (in human monocytes). Furthermore, to determine the molecular basis of LPS recognition by Hsp70, we generated recombinant truncated

products of Hsp70 (C- and N-terminal fragments), as well as site-directed mutagenesis within the N-terminal domain. Our data suggest that the C-terminus of Hsp70 does not play a role in LPS binding and that LPS binds to the N-terminal ATPase domain of Hsp70. In particular, it seems that the base of the ATP-binding cleft is crucial for LPS binding.

Because we have found that Hsp70 is able to associate with LPS and toll-like receptor 4 (TLR4) and to modulate LPS-induced responses, the question that remains is whether this is a common feature of Hsps and whether they are able to interact and modulate all TLR-induced responses. This hypothesis seems to be supported by recent findings that Hsp10, like Hsp70, is able to modulate LPS-induced responses (Johnson et al 2005; Vanags et al 2006), thus suggesting that Hsps have the capacity to interact with TLRs and interfere with ligand binding, as well as the subsequent inflammatory response. Thus, what we are left contemplating is: Are Hsps endogenous modulators of the innate immune response? The findings so far seem to hint that the answer to this question promises to be an exciting one.

STRESS WARS: ROLE OF CELL STRESS PROTEINS IN BACTERIAL INFECTION, BY BRIAN HENDERSON

Bacterial infection involves an intricate interaction between the invading bacterium and the cells of the host. It is now becoming clear that over and above the normal up-regulation of expression of cell stress proteins (CSPs; infection being stressful for bug and host), their moonlighting actions are increasingly seen to play a role in infection. Moonlighting is a term that refers to proteins having multiple actions. A good example of this are the reports that host CSPs such as Hsp70, BiP, Gp96, and Hsp90 can function as receptors for bacterial components such as lipopolysaccharide, for bacteria themselves, and even for various pathogenic viruses (Henderson and Pockley 2005). In addition, many host CSPs function as modulators of inflammation, with Hsp10 and BiP currently in clinical trial for the treatment of the chronic inflammatory disease rheumatoid arthritis. One key question is whether bacterial homologues interfere with the signaling actions of host CSPs.

Bacterial CSPs also have a number of interesting moonlighting functions. For example, a number of bacteria appear to use Hsp60, Hsp70, or both as adhesins for binding to the host. Peptidyl prolyl isomerases (PPIs) of a number of bacteria have been shown to be involved in bacterial virulence. *Helicobacter pylori*, the cause of stomach ulceration, releases a soluble PPI, which causes apoptosis of gastric cells and might therefore function as a

cause of ulceration. Other bacterial CSPs with cell signaling actions include Hsp10, Hsp60, and Hsp70.

In addition to being virulence factors, it now appears that certain CSPs could be therapeutic targets for antibacterial agents. Perhaps the most exciting example is the Hsp100 family of proteins. It has recently been reported that inhibition of the activity of these proteins by specific depsipeptides can kill gram-positive bacteria, both in vitro and in vivo.

It is early days, but the evidence is emerging to support the hypothesis that CSPs play important roles in the interactions between bacteria and their hosts in health and disease.

EXOSOMAL EXPORT OF HSP70, BY GABRIELE MÜLTHOFF

Depending on their intracellular, membrane-bound, extracellular, or combination of locations, heat shock proteins (Hsps) either mediate protection against stress-induced cell damage or act as danger signals, stimulating the adaptive and innate immune system (Gehrmann et al 2004; Nylandsted et al 2004; Maguire et al 2005). Nearly all cell types, including tumors, have the capacity for an active release of Hsp70 in detergent-soluble vesicles (Baussero et al 2005; Gastpar et al 2005; Lancaster and Febbraio 2005). Biophysical properties, including floating properties (1.17g/ml) correlating with a maximum acetylcholine esterase activity, characterized them as exosomes. Profiling of luminal proteins revealed that tumor-derived exosomes contain cytosolic proteins but lack endoplasmic reticulum (ER)-resident proteins. The intracellular pathway of exosomes from the endosomal compartment to the plasma membrane is documented by an enrichment of the small GTPase Rab-4. A proteomic profiling revealed that the exosomal surface reflects that of the plasma membranes of tumors from which they are derived. In particular, exosomes originating from Hsp70 plasma membrane-positive tumors present Hsp70 on their exosomal surface. In line with these findings, only Hsp70 surface-positive exosomes, but not their negative counterparts, had the capacity to stimulate the innate immune system in a similar manner as soluble Hsp70 protein (Gastpar et al 2005). These data provide an explanation as to how the innate immune system might become activated by tumor-derived exosomal Hsp70 in vivo (Triantafyllou et al 2006).

Recently, a direct interaction of Hsp70s with phosphatidylserine in preapoptotic cells could be identified by the group of Antonio DeMaio (Arispe et al 2004). To characterize the localization of Hsp70 in plasma membranes and exosomes generated from viable tumor cells that differ in their capacity to present Hsp70 on the surface, we currently perform lipid analysis by mass spectrometry. Lipid analysis did reveal that Hsp70 is associated with

phosphatidylserine (PS) in the plasma membrane. This finding is supported by work of the group of Antonio DeMaio (Arispe et al 2004). We hypothesize that Hsp70 is shuffled into the plasma membrane together with PS. Because a disequilibrium exists in the content of PS between the outer and inner leaflet of the plasma membrane, we have to analyze the exact location of Hsp70 (in preparation). Further analyses are ongoing as to whether other lipids are involved in the interaction of Hsp70 in the plasma membrane.

HSP70-HOM GENE POLYMORPHISM AND THE RISK OF POSTTRANSPLANT COMPLICATIONS IN THE RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELLS, BY KATARZYNA BOGUNIA-KUBIK

A number of genetic polymorphisms have been shown to predict the clinical outcome after allogeneic hematopoietic stem cell transplants (HSCTs). The involvement of heat shock protein 70 (Hsp70) in peptide binding and presentation could suggest their potential role in the alloreactive processes resulting in graft vs host disease (GvHD). Hsp70s have been shown to be involved in the pathology of GvHD in animal models (Goral et al 2000). More recent studies in humans have documented an association between the increased levels of antibodies to 70 and 90 kDa Hsps in serum and the incidence of acute GvHD (Goral et al 2002). Furthermore, a correlation between the expression of an inducible form of Hsp70 and the degree of graft vs host reaction in an in vitro skin explant model has been observed (Jarvis et al 2003), although no significant association with the *Hsp70-1* or *Hsp70-2* genes coding for inducible Hsp70s was reported (Dickinson et al, in press).

Interestingly, association of the *Hsp70-hom* (+2763 G/A) gene polymorphism (the third of the Hsp70 genes located on chromosome 6 within the MHC class III region) with the risk for generation of toxic lesions and acute GvHD was recently demonstrated in Polish recipients of allogeneic hematopoietic stem cell transplants (Bogunia-Kubik and Lange 2005). In this study, a mixed cohort of patients receiving transplants at the Lower Silesian Center for Cellular Transplantation in Wrocław with human leukocyte antigen (HLA)-matched sibling donors, haploidentical family members, or matched unrelated donors were investigated. The presence of the *Hsp70-hom* AA homozygous genotype in recipients was found to be associated with an increased risk for acute GvHD, whereas no correlation was observed when genotypes of donors were analyzed.

The association of recipient *Hsp70-hom* AA homozygosity with GvHD was also seen in the next 2-center study, involving 158 patients: 62 receiving transplants in

Wroclaw and 96 receiving transplants in Newcastle, UK (Bogunia-Kubik et al 2006). This group comprised only recipients grafted from HLA-matched sibling donors. As mentioned previously, the incidence of acute GvHD was significantly higher among AA homozygous patients compared with those carrying the G allele and was independent from the origin of the recipient cohort (ie, Wroclaw or Newcastle transplant centers). These results confirmed the association between the polymorphic features of the *Hsp70-hom* gene and susceptibility to GvHD.

HEAT SHOCK PROTEINS AS DANGER SIGNALS, BY JOHN WILLIAMS

The talk focused on the contrasting roles of Hsps in disease processes. Two of these related to the use of Hsps in developing novel therapies. Homocysteine is a recognized risk factor for coronary heart disease and Alzheimer's disease (McAddon et al 2001). Folate directly protects endothelial cells from homocysteine-induced damage. With the use of chemical and siRNA inhibition, it was shown that Hsp32 and Hsp70 are essential for this protection. The system has been expanded to a number of novel therapeutic compounds. Treatments that increase cellular Hsps are being used in combination with a variety of chemotherapeutic agents, and in many cases, carefully timed heat treatments have allowed significant reduction in the concentration of the compound used to induce cell death. Evidence was then presented for the role of Hsp70 and Hsp60 as danger signals to the immune system. This focused on the release of Hsp70 and Hsp60 from living cells (Hunter-Lavin et al 2004; Davies et al 2006). This release is regulated by factors common to infection and other stressors. The talk concluded with a brief consideration of how understanding the manipulation of cellular stress to regulate Hsp concentration and localization can be used to develop new therapies.

HSP70 CHAPERONE MACHINE REMODELS PROTEIN AGGREGATES AT THE INITIAL STEP OF HSP70-HSP100-DEPENDENT DISAGGREGATION, BY PAWEŁ STOCKI

The proper conformation of proteins is challenged by stress conditions. Exposure of cells to extreme heat shock conditions results in a massive protein aggregation. The process of solubilization and reactivation of aggregated proteins in cells is mediated by 2 cooperating chaperone systems: Hsp70 (DnaK, DnaJ and GrpE) and Hsp100 (ClpB). With green fluorescent protein (GFP) as an experimental substrate model, we were able to examine the disaggregation process dependent on both Hsp70 and Hsp100 by real-time fluorescence measurements. To characterize the individual steps during the disaggregation

reaction, the chaperonin mutant GroEL D87K, called "trap," was used. Its characteristic feature, permanent binding to the nonnative proteins, allowed us to exhibit acid denaturation, in which monomeric GFP binds to the GroEL trap, which blocks its spontaneous folding. Real-time fluorescence measurements showed that the GroEL trap efficiently inhibits Hsp70/Hsp100-dependent disaggregation of heat-aggregated GFP, making it a unique molecular tool for investigation of this process. Sedimentation analysis of protein complexes formed during the GFP disaggregation process showed that the presence of the Hsp70 system, but not that of Hsp100, is required for formation of the stable protein complexes containing monomeric, unfolded GFP and the GroEL trap.

Our results suggest that the Hsp70 chaperone system monomerizes aggregated GFP at the initial step of the disaggregation reaction. These monomers aggregate secondarily in the absence of Hsp100.

SECRETED HSP70 INDUCES EGFR TRANSACTIVATION IN A431 CELLS, BY ANTON EVDONIN AND N.D. MEDVEDEVA

Although initially Hsp70 was thought to be a typical intracellular protein, recent studies demonstrated that it is being released into the blood or the conditioned medium of cultured cells under stress conditions (Guzhova et al 2001; Campisi et al 2003; Hunter-Lavin et al 2004). The purpose of this study was to identify cellular organelles involved in the release of Hsp70 from A431 carcinoma cells and to define the function of the secreted protein. We determined that Hsp70 is present in granules located at the periphery of cells, which were subjected to heat shock. The Hsp70-containing granules did not carry markers specific to lipid bodies, endosomes, or lysosomes. However, they were positive for the marker of secretory granules chromogranin A. The colocalization of Hsp70 with CGA in these granules and a parallel increase at the extracellular level of these 2 proteins suggest that the release of Hsp70 is mediated by secretory-like granules.

The initial steps of heat stress in A431 cells were previously characterized by ligand-independent epidermal growth factor receptor (EGFR) transactivation via an unknown mechanism and concomitant secretion of Hsp70 (Evdonin et al 2005). The depletion of Hsp70 from the conditioned medium of heated cells abolishes EGFR transactivation, indicating that the secreted protein is essential for EGFR transactivation. Analysis of the effect of pure Hsp70, which induced EGFR transactivation and activation of EGFR-dependent signaling pathways, proved this finding. Both heat stress and pure Hsp70 stimulate activation of TLR2/4 and their association with EGFR. These results suggested that the secreted Hsp70 mediates

cross-communication of TLR and EGFR signaling systems in A431 cells. These results provide new insight into the function of extracellular Hsp70 secreted from epithelial cells during heat stress.

ACKNOWLEDGMENTS

The authors are grateful to all speakers that provided summaries of their talks.

Kathy Triantafilou

Infection and Immunity Group
School of Life Sciences
University of Sussex
Falmer, Brighton, BN1 9QG, UK

Brian Henderson

Division of Microbial Diseases
UCL Eastman Dental Institute
University College London
256 Gray's Inn Road
London WC1X 8LD, UK

Gabriele Multhoff

Technical University Munich
Department of Radiotherapy and Radiooncology
Ismaningerstr. 22
81675 Munich, Germany

Katarzyna Bogunia-Kubik

Laboratory and Department of Clinical Immunology
L. Hirszfeld Institute of Immunology and Experimental
Therapy
Polish Academy of Sciences
R. Weigl 12
53-114 Wrocław, Poland

John H.H. Williams

Chester Centre of Stress Research
Department of Biological Sciences
University of Chester
Parkgate Road
Chester CH1 4BJ, UK

Pawel Stocki

Haematological Sciences
School of Clinical and Laboratory Sciences,
University of Newcastle Medical School
Newcastle upon Tyne, UK

Anton L. Evdonin and N.D. Medvedeva

Institute of Cytology
Russian Academy of Sciences
St. Petersburg 196064, Russia

REFERENCES

Arispe N, Doh M, Simakova O, Kurganov B, DeMaio A. 2004. Hsc70 and Hsp70 interact with phosphatidylserine on the surface of

- PC12 cells resulting in a decrease of cell viability. *FASEB J* 18: 1636–1645.
- Bausero M, Gastpar R, Multhoff G, Asea A. 2005. Alternative mechanisms by which IFN-gamma enhances tumor recognition: active release of Hsp72. *J Immunol* 175: 2900–2912.
- Bogunia-Kubik K, Dickinson A, Uklejewska A, Jarvis M, Lange A. 2006. *HSP70-hom* gene polymorphism as a prognostic marker of graft-versus-host disease. *Transplantation* 82: 1116–1117.
- Bogunia-Kubik K, Lange A. 2005. *HSP70-hom* gene polymorphism in allogeneic hematopoietic stem-cell transplant recipients correlates with the development of acute graft-versus-host disease. *Transplantation* 79: 815–820.
- Campisi J, Leem TH, Fleshner M. 2003. Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. *Cell Stress Chaperones* 8: 272–286.
- Davies EL, Bacelar MMFVG, Marshall MJ, Johnson E, Wardle TD, Andrew SM, Williams JHH. 2006. Heat shock proteins form part of a danger signal cascade in response to LPS and GroEL. *Clin Exp Immunol* 145: 183–189.
- Dickinson AM, Jarvis M, Stocki P, Bogunia-Kubik K. The potential role of HSP70 in predicting outcome in haematopoietic stem cell transplants—genomic and functional studies. In: *Heat Shock Proteins in Biology and Medicine*, ed Radons J. Publisher, City, Country, in press.
- Evdonin AL, Tsupkina NV, Nikol'skii NN, Medvedeva ND. 2005. Transactivation of the EGF receptor in A431 human carcinoma cells upon exposure to heat shock. *Dokl Biol Sci* 401: 158–159.
- Gastpar R, Gehrmann M, Bausero M, Asea A, Gross C, Schroeder J, Multhoff G. 2005. Hsp70 surface-positive tumor-exosomes stimulate migratory and cytolytic activity of NK cells. *Cancer Res* 65: 5238–5247.
- Gehrmann M, Marienhagen J, Eichholtz-Wirth H, et al. 2004. Dual function of membrane-bound Hsp70, Bag-4 and Hsp40: protection against radiation-induced effects and target structure for NK cells. *Cell Death Differ* 12: 38–51.
- Goral J, Mathews HL, Nadler SG, Clancy J. 2000. Reduced levels of Hsp70 result in a therapeutic effect of 15-deoxyspergualin on acute graft-versus-host disease in (DA × LEW) F1 rats. *Immunobiology* 202: 254–266.
- Goral J, Shenoy S, Mohanakumar T, Clancy J. 2002. Antibodies to 70 kD and 90 kD heat shock proteins are associated with graft-versus-host disease in peripheral blood stem cell transplant recipients. *Clin Exp Immunol* 127: 553–559.
- Guzhova I, Kislyakova K, Moskaliova O, Fridlanskaya I, Tytell M, Cheetham M, Margulis B. 2001. In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. *Brain Res* 914: 66–73.
- Henderson B, Pockley AG. 2005. *Molecular Chaperones and Cell Signalling*. Cambridge University Press, Cambridge, UK.
- Hunter-Lavin C, Davies EL, Bacelar MMFVG, Marshall MG, Andrew SM, Williams JHH. 2004. Hsp70 release from peripheral blood mononuclear cells. *Biochem Biophys Res Commun* 324: 511–517.
- Jarvis M, Marzolini M, Wang XN, Jackson G, Sviland L, Dickinson AM. 2003. Heat shock protein 70: correlation of expression with degree of graft-versus-host response and clinical graft-versus-host disease. *Transplantation* 76: 849–853.
- Johnson BJ, Le TT, Dobbin CA, et al. 2005. Heat shock protein 10 inhibits lipopolysaccharide-induced inflammatory mediator production. *J Biol Chem* 280: 4037–4047.
- Lancaster GI, Febbraio MA. 2005. Exosome-dependent trafficking of Hsp70. *J Biol Chem* 280: 23349–23355.
- Maguire M, Poole S, Coates AR, Tormay P, Wheeler-Jones C, Henderson B. 2005. Comparative cell signalling activity of ultrapure

- recombinant chaperonin 60 proteins from prokaryotes and eukaryotes. *Immunology* 115: 231–238.
- McCaddon A, Hudson P, Davies G, Hughes A, Williams JHH, Wilkinson C. 2001. Homocysteine and cognitive decline in healthy elderly. *Dementia Geriatr Cognit Disord* 12: 309–313.
- Nylandsted J, Hansen MG, Danielewich A, et al. 2004. Hsp70 promotes tumor cell survival by inhibiting lysosomal membrane permeabilization. *J Exp Med* 200: 425–435.
- Triantafilou K, Triantafilou M, Dedrick RL. 2001. A CD14-independent LPS receptor cluster. *Nat Immunol* 4: 338–345.
- Triantafilou M, Brandenburg K, Gutschmann T, Seydel U, Triantafilou K. 2002. Innate recognition of bacteria: engagement of multiple receptors. *Crit Rev Immunol* 22: 251–268.
- Triantafilou M, Brandenburg K, Kusumoto S, Fukase K, Mackie A, Seydel U, Triantafilou K. 2004. Combinational clustering of receptors following stimulation by bacterial products determines LPS responses. *Biochem J* 381: 527–536.
- Triantafilou M, Gamper FG, Haston RM, Mouratis MA, Morath S, Hartung T, Triantafilou K. 2006. Membrane-sorting of toll-like receptor TLR-2/6 and TLR-2/1 heterodimers at the cell surface determines heterotypic associations with CD36 and intracellular targeting. *J Biol Chem* 281: 31002–31011.
- Triantafilou M, Triantafilou K. 2003. Receptor cluster formation during activation by bacterial products. *J Endotoxin Res* 9: 331–335.
- Triantafilou M, Triantafilou K. 2004. Heat-shock protein 70 and heat-shock protein 90 associate with toll-like receptor 4 in response to bacterial lipopolysaccharide. *Biochem Soc Trans* 32: 636–639.
- Vanags D, Williams B, Johnson B, Hall S, Nash P, Taylor A, Weiss J, Feeney D. 2006. Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial. *Lancet* 368: 855–863.
- Wang R, Kovalchin JT, Muhlenkamp P, Chandawarkar RY. 2006. Exogenous heat shock protein 70 binds macrophage lipid raft microdomain and stimulates phagocytosis, processing, and MHC-II presentation of antigens. *Blood* 107: 1636–1642.
- Zietkiewicz S, Lewandowska A, Stocki P, Liberek K. 2006. Hsp70 chaperone machine remodels protein aggregates at the initial step of Hsp70-Hsp100-dependent disaggregation. *J Biol Chem* 281: 7022–7029.