Heat shock proteins in physiology and pathology: the Berlin meeting

Alexzander A. A. Asea,¹ Valeria Milani,² and Stuart K. Calderwood³

¹Scott & White Memorial Hospital and Clinic, Texas A&M University Health Science Center, Temple, TX 76508, USA

²Clinical Cooperation Group on Hyperthermia, Internal Medicine Department III, Klinikum Grosshadern, and Ludwig-Maximilians-University, 81377 Munich, Germany

³Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

The IIIrd International Symposium on Heat Shock Proteins in Biology and Medicine took place at Charité University in Berlin, Germany, on 23–25 May 2006. This Symposium, concentrating on the physiological and pathological correlations associated with heat shock proteins and the stress response was organized in collaboration with the European Society for Hyperthermic Oncology (ESHO) and the Cell Stress Society International (Fig 1). It was the third in a series that began at Woods Hole, MA, USA, in 2000. The meeting attracted over 100 participants and included 41 oral presentations and 20 posters. It was most appropriate that the auditorium (Fig 2) was near the entrance to the Rudolf Virchow Krankenhaus (Fig 3), named for the father of modern cellular pathology.

The meeting began with a return to roots with the session **Molecular Chaperones**. **Bernd Bukau** (University of Heidelberg, Germany) gave a beautiful presentation showing a novel function for Hsp110 family members as nucleotide exchange factors for Hsp70 proteins. He showed that the yeast *hsp110* homolog Sse1p is a powerful nuclear exchange factor for yeast Hsp70 family members Ssa1 and Ssb1 and that cells deficient in Sse1p are sensitive to stress. **Lawrence Hightower** (University of Connecticut, USA) then spoke regarding members of the human Hsp70 family focusing on the *hsp70B'* isoform, the most strictly stress-inducible of the Hsp70 members. His studies indicate that *hsp70B'* performs a unique role in the stress response and is reciprocally regulated with other members of the Hsp70 family. The focus next switched to the small HSP in a presentation by **Robert Tanguay** (Laval University, Canada). Dr. Tanguay has shown in a number of publications in recent years a connection between the HSP and longevity. Indeed,

Hsp22, 23, 26, and 27 each appear to play a role in extending lifespan which could be related to their effectiveness in combating the effects of oxidative stress. The transcriptional regulator of the heat shock response, heat shock factor (HSF) was also discussed because a number of mouse model studies indicate intriguing roles for HSF family members in development, human disease, and longevity.

The meeting then switched focus to the role of **Heat Shock Proteins in Inflammation & Immunology**. **Antonio DeMaio** (University of California San Diego, USA) showed data indicating Hsp70 binding to intracellular membranes containing the lipids phosphatidylserine and cholesterol and linked these interactions to a role for Hsp70 in endocytosis through both coated-pit and caveolin-based pathways. The membrane theme was continued by **Laszlo Vigh** (Hungarian Academy of Sciences, Hungary) whose experiments suggest a link between membrane perturbations and induction of the heat shock response. **John Williams** (University of Chester, UK) then presented some intriguing studies indicating a role for Hsp70 release into the intracellular microenvironment after exposure of macrophages to pathogen-derived molecules such as lipopolysaccharides and GroEL. These agents induce Hsp70 release, suggesting a role for extracellular HSP as danger signals for disease and infection.

The session **Heat Shock Proteins in Exercise Immunophysiology** continued with the common theme of elucidating the role of extracellular HSPs. **Alexzander Asea** (Scott and White Clinic and Texas A&M University Health Science Center College of Medicine, USA) presented data showing the 2 mechanisms by which intracellular heat shock proteins are released into the extracellular milieu: a passive mechanism based on cell/tissue necrosis or trauma, and an active mechanism based on controlled physiological release. In addition to certain

Correspondence to: Stuart K. Calderwood, E-mail: scalderw@bidmc.harvard.edu.

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Fig 1. The organizers (left to right): Stuart Calderwood, Valeria Milani, Alexander Asea, and Larry Hightower (CSSI photographer).

proinflammatory cytokines, physiological stress and mild exercise also stimulate the active release of Hsp70. **Elvira Fehrenbach** (University of Tübingen, Germany) continued this common theme and presented work on Hsp70 reaction to exercise in peripheral blood under normal and hypoxic environmental conditions, showing that moderate hypoxia has no effect on exercise-induced extracellular heat shock protein. However, it down-regulated post-interval training concentrations of intracellular heat shock proteins. Parallel increases in extracellular heat shock protein and cytokine in the plasma could support the role of Hsp70 as a chaperokine in the exercise-induced stress response. **Mark A. Febbraio** (Royal Melbourne Institute of Technology, Australia) presented data in which he showed that messenger RNA (mRNA) expression of Hsp72 is associated with insulin sensitivity in humans. Hsp70 is pivotal in protecting against obesity-induced insulin resistance, in part by attenuating fat feeding-induced JNK up-regulation in skeletal muscles. **Peter Csermely** (Semmelweis University, Hungary) ended the session by presenting data on molecular chaperones and network models in cell regulations and drug targeting. He concluded that network studies might help us in identifying and designing multitarget drugs that could efficiently target polygenic diseases and stabilize the cells in a simultaneous fashion.

The Keynote address was given by **Stuart K. Calderwood** (Beth Israel Deaconess Medical Center & Harvard Medical School, USA).

The next day, the theme of extracellular properties of HSP was continued in a subsequent session dealing with **Heat Shock Proteins in Tumor Immunity**. The issue of danger signals was further addressed by **Hansjoerg**

Schild (University of Mainz, Germany), who showed that the chaperone Gp96 (*grp94*) is able to interact with bacterial danger signals, including ligands for the toll-like receptors (TLR). Gp96 could enhance interaction of TLR2 ligands and TLR4 ligands with their cognate receptors and thus enhance the danger signal response. A complex interaction of HSP, bacterial products, and inflammatory cells is thus indicated. Three presentations, including these from **Ralf Dressel** (Göttingen, Germany), **Valeria Milani** (Ludwig-Maximilians-University, Germany), and **Gabriele Multhoff** (Regensburg University, Germany), dealt with the role of natural killer (NK) cells in killing tumor cells displaying Hsp70 on the cell surface. It is not clear whether tumor HSP interacts with NK receptors such as CD94 directly or whether it exerts its effects indirectly through cell surface proteins MICA and MICB, which are ligands for the NKG2D receptor. Impressive preclinical data were presented indicating that incubation of NK cells with Hsp70-derived peptide TKD activates NK killing of tumor cells. **Noriyuki Sato** (Sapporo Medical University, Japan) showed some exciting studies showing a role for Hsp90 in antigen presentation to dendritic cells. Hsp90 was able to cross present peptides and the whole ovalbumin protein, leading to cross-priming of cytolytic CD8⁺ lymphocytes. This has major implications in tumor immunotherapy.

The session on **Heat Shock Proteins in Cardiovascular Disease** was opened by **Graham Pockley** (University of Sheffield, UK), who demonstrated that circulating Hsp60 levels are not associated with cardiovascular risk factors such as body mass index, blood pressure, and smoking status. Circulating Hsp60 is elevated in a subpopulation of patients with acute coronary syndromes and levels are

Fig 2. Audience during symposium in the auditorium of Universitätsklinikum Charité, Medizinische Fakultät der Humboldt Universität zu Berlin, Campus Virchow-Klinikum.

associated with early atherosclerosis and serum concentrations of proinflammatory cytokines. In contrast, Hsp70 levels are not associated with very low density lipoprotein and triglyceride concentrations. **Richard Heads** (King's College London School of Medicine, UK) followed this presentation by showing data about a unique protein known as Brn3b. Brn3 POU factors may play an important role in specification, proliferation, differentiation, and survival of cardiomyocytes and in the development and maintenance of other important structures within the heart. **Harm H. Kampinga** (The Netherlands Cancer Institute, The Netherlands) presented data that supports the hypothesis that Hsp27 plays an important role as an endogenous suppressor of atrial fibrillation. It promotes remodeling, which could break the progressive atrial fibrillation self-perpetuating cycle.

In the session **HSF1 Regulation and Role in Disease**, **Valérie Metzger** (École Normale Supérieure, France) showed intriguing mouse knockout studies indicating a role for HSF2 in neuronal migration in the brain cortex. **Nahid Mivechi** (Medical College of Georgia, USA) described a number of mouse models with disruptions of *hsf1*, *hsf2* and *hsf4* genes indicating roles for *hsf* family members in thermotolerance, cell transformation and lens development in the eye. **Lea Sistonen** (Åbo Akademi University, Finland) next gave a comprehensive overview of HSF regulation in the cell, focusing on the role of phosphorylation and interaction between HSF family members. Finally, **Richard Voellmy** (University of Miami School of Medicine, USA) reported on the use of HSP-based promoters in gene therapy and the targeted expression of therapeutic genes. This exciting presentation indicated how the *hsp70B'* promoter could be engineered to give strictly inducible expression of therapeutic genes.

The **Heat Shock Proteins in Cancer** session was opened by **Daniel R. Ciocca** (National University of Cuyo Medical School, Argentina), who presented a pilot clinical study with a therapeutic vaccine based on hydroxyapatite ceramic particles and self-antigens in cancer patients. The

conclusion of the study was that the vaccine works adequately, mainly in patients in which the tumor is still growing at the primary site and in which the tumor mass has been decreased by surgery. **Vishwanie Budhram-Mahadeo** (King's College London School of Medicine, UK)

Fig 3. Entrance of the Rudolf Virchow Krankenhaus, established 1906.

presented data that shows a strong correlation between elevated levels of Brn3b and Hsp27 in human breast tumors compared with benign growth. Interestingly, Brn3a and 3b are also expressed in specific regions of the developing heart, and both transcriptional factors transactivate the Hsp27 promoter in isolated cardiomyocytes. Therefore, the regulation of Hsp27 and cell survival by Brn3a and 3b could be a common mechanism in regulating growth and survival in different cell types, including cancer cells. **Vladimir Gabai** (Boston University Medical School in Boston, USA) presented data that demonstrated that the down-regulation of Hsp27 or Hsp72 by small interfering RNA (siRNA) sensitizes tumor cells to doxorubicin and gamma and ultraviolet (UV) radiation. Sensitization to doxorubicin was associated with aggravated senescence, whereas sensitization to gamma and UV radiation was due to increased rate of mitotic catastrophe.

Ian Brown (University of Toronto, Canada) opened the **Heat Shock Proteins and Cell Death** session showing that prior heat shock protects neurons in the rat retina from bright light-induced cell death. **Cinzia Dello Russo** (Catholic University Medical School, Italy) presented a study in which she and her colleagues characterized the effect of 17-allylamino-17-demethoxy-geldanamycin (17-AAG), a less toxic derivative of the natural occurring Hsp90 inhibitor geldanamycin on glial inflammatory responses and the development of experimental autoimmune encephalomyelitis. Incubation of astrocytes or microglia with 17-AAG dose dependently reduced the li-

popolysaccharide-dependent induction of inducible nitric oxide synthase expression and IL-1 β production. **Marja Jäättelä** (Danish Cancer Society, Denmark) presented data that shows that depletion of Hsp70 in cancer cells triggers the expression of macrophage inhibitory cytokine-1 (MIC-1) and MIC-1-dependent G1 cell cycle arrest. They showed that Hsp70 depletion also triggers a delayed MIC-1-independent cell death and identified lens epithelial-derived growth factor as an Hsp70-regulated survival protein with oncogenic potential. **Michael Sherman** (Boston University School of Medicine, USA) demonstrated that the effects of Hsp70 depletion on senescence and p53 did not result from proteotoxic stress, DNA instability, or activation of ATM/ATR pathways. Instead, depletion of Hsp70 reduced stability and activity of the p53 inhibitor Mdm2.

The session **Hyperthermia and Thermotolerance** was opened by **Elizabeth Repasky** (Roswell Park Cancer Institute, USA), who presented data on the development of new approaches with heat and heat shock proteins to improve cancer immunotherapy. **Gloria Li** (Memorial Sloan-Kettering Cancer Research Center, USA) showed data from the first human tumor xenograft model that allows the detection of hypoxia-induced molecular events and mapping of their distribution in vivo by noninvasive positron emission tomography imaging. **Mark Hurwitz** (Dana-Farber Cancer Institute, USA) presented data on Hsp70 and prostate cancer and proposed translational opportunities from a clinical perspective.