The IXth CSSI international symposium on heat shock proteins in biology and medicine: stress responses in health and disease

Alexandria Old Town, Alexandria, Virginia, November 10–13, 2018

Stuart K. Calderwood¹ · Elizabeth A. Repasky² · Len Neckers³ · Lawrence E. Hightower⁴

Published online: 15 January 2019

© Cell Stress Society International 2019

Abstract

The stress response has been studied now for over 50 years and is known to have significance in the survival of organisms in a challenging environment and in the healthy development of all known descendants of the last common universal ancestor (LUCA). This meeting was concentrated mostly on the responses of cells and organisms to environmental and cell stress including the impact of thermal stress, which was a major theme throughout this meeting. One emphasis was on the deployment of the heat shock response that permits damage to proteins to be detected and responded to by the abundant synthesis of heat shock proteins (HSPs). Speakers and presenters of posters responded to the questions of how are the HSPs rapidly induced by stressors? By which mechanisms are they regulated in the cell by protein-protein interactions or posttranslational modification? And, what are the consequences when these abundantly expressed proteins escape the confines of the cell and influence the extracellular microenvironment? Key among the questions was how does stress influence longevity and aging and what happens in terms of disease control (malignant, neurodegenerative) when stress responses become compromised? In this context, many presenters addressed the question of pharmacologically modifying the heat shock response and HSP functions and thus improving responses to a range of disease types.

Keywords CSSI symposium · Heat shock proteins · Stress responses · Cancer biology · Proteostasis · Caloristasis · Hsp90 · Hsp70

Introduction

The ninth international symposium in this series about heat shock proteins in biology and medicine was registered to maximum capacity at the Hilton Old Town Alexandria

Hotel this year (Supplementary Fig. 1). The series began in 1999 in Woods Hole, MA, at the Marine Biological Laboratory and the venue was moved in 2012 to Old Town, Alexandria, VA. There were 86 registrants from 12 countries for eight sessions and 33 posters. The program included a keynote address, three plenary talks, 32 invited speakers, and five speakers selected from the poster abstracts. The principal organizer and founder of this series Stuart Calderwood (Harvard Medical School, USA) opened the meeting and introduced our Keynote Speaker Jason Gestwicki (University of California San Francisco, USA). The theme of the Susan Lee Lindquist Science without Boundaries Session was “Stress Responses in the Natural Environment”. To honor the memory of Susan Lindquist, who connected seemingly unrelated research areas in unexpected ways, this session always includes a talk outside the scope of the session but one that emphasizes her broad range of research interests. This year’s talk was titled “Interaction and collaboration between Hsp90 and Hsp70 in *E. coli* and yeast” by Shannon Doyle (NCI/NIH, USA).

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12192-018-00966-w>) contains supplementary material, which is available to authorized users.

✉ Lawrence E. Hightower
lawrence.hightower@uconn.edu

¹ Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave, Boston, MA 02215, USA

² Roswell Park Cancer Center, Elm and Carlton Streets, Buffalo, NY 14263, USA

³ National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20850, USA

⁴ University of Connecticut, 91 North Eagleville Road, Storrs, CT 06269-3125, USA

Meeting report

We have discussed the content of the meeting under six headings that we have emphasized in the molecular response to proteotoxic stress, as suggested by the cartoon in (Fig. 1).

Keynote address

We first discuss the meeting keynote address, given at the commencement of the conference by Jason Gestwicki. Dr. Gestwicki gave an exciting presentation on the protein-protein interactions involved in the chaperone network, which will be key to understanding the complex pathways of protein folding (Supplementary Fig. 2). The maintenance of the proteome requires multiple interactions among a large number of chaperones of different families and multiple co-chaperones. Dr. Gestwicki discussed the principles by which decisions are made as to client protein fate, how proteins might be folded in an orderly manner and illustrated his unique chemical biology approach to these studies. This approach has of course another advantage in the potential for discovery of novel small

molecules that may become therapeutics and lead compounds for downstream development in protein folding disorders.

Stress responses and the changing world environment—living in interesting times

This meeting is traditionally held at the time of transition from fall to winter, certainly turning the thoughts of the audience to the stress of climate change in nature. Melody Clark (British Antarctic Survey, UK) addressed the meeting with the question—Is there a universal stress response? She indicated the urgency of the question in the rapidly changing Antarctic climate and outlined the difficulties of finding a universal molecular response to stress. The predicament of Antarctic species was further indicated by the studies of Kevin Bilyk (Western Kentucky University, USA) showing loss of the transcriptomic HSP response in Antarctic fish. However, adaptation to an Antarctic environment does not inevitably lead to abandoning the heat shock response and studies in the crustacean *Branchinecta gaini* that inhabits fresh water lakes in Antarctica indicated an intact heat shock response (Marcelo Gonzalez, INACH, Punta Arenas, Chile). With many

Fig. 1 The heat shock response and its role in tissue physiology and pathology. (1) Cells and organisms are subjected to protein folding stress and may succumb or deploy the homeostatic heat shock response. (2) Heat shock factors (HSF) are induced by the stress and lead to rapid and abundant synthesis of a number of families of HSPs. (3) The cytoplasm is flooded with HSPs depicted here as Hsp27, 70, and 90 which mediate folding or destruction of damaged proteins. (4) These

chaperones can combine in multiple complexes to carry out optimal folding of the proteome. (5) Failure of this response can lead to aging, cancer, and degeneration of differentiated tissues. (6) Chaperones can escape the cytoplasm and mediate multiple effects through binding to receptors on adjacent tissues, including those that influence subsequent immune responses

Antarctic species facing extinction in the near future, the studies take on increasing urgency.

The influence of environmental temperature in the health of mammals and humans was also addressed by Elizabeth Repasky (Roswell Park Cancer Center, USA) who showed that cancer-bearing animals undergo deterioration in their anti-tumor immune response when they are housed at even mildly sub-thermoneutral temperatures. These findings by Repasky et al. may thus have implications on the connection of mammalian well-being to outside temperatures in disease states. They indicate an unusual sensitivity of homeotherms to ambient temperature. (Mohan Tulapurkar and Jeff Hasday, University of Maryland at Baltimore, USA) indicated a potential role for the stress kinase p38 in responses to fever. Recent studies by Sharon Evans (Roswell Park Cancer Center) have previously shown a role of fever range hyperthermia in permitting activated T cells to enter the tumor microenvironment. New studies presented show that studies myeloid suppressor cells (MDSC) can cleave the L-selectin homing receptor on T cells, excluding them from the tumor space. The laboratory of Dr. Ling-Jia Qian, (Institute of Military Cognitive and Brain Sciences, Beijing, China) showed an effect of circulating homocysteine on brain function after its elevation in the plasma of stressed rats. These effects were mediated by effects on DNA methylation of key genes in the brain. These effects may mediate stress-induced cognitive impairment.

Two presentations indicated HSR-linked responses to environmental radiation. Dr. Xiaoreng Wang (Chinese PLA General Hospital, Beijing) showed a role for the ER stress response protein calreticulin in protection from microwaves. Stuart Calderwood showed that mammary cancer cells undergo a wound healing type response to ionizing irradiation that involved regulation by HSF1, prostaglandin E2, and Hsp72. These studies indicate a connection between the proteotoxic stress response, the DNA damage response, processes involved in wound healing and radiation effects on metastasis.

HSF1—the fastest factor in mammalian transcription

HSF1 is a unique factor both in the rapidity that it can be activated and bound to chromatin (within seconds at elevated temperature) and the abundance of its transcriptional products. Thus, increasing our understanding of its regulation in the cell is key to appreciating how the HSR participates in healthy existence and disease by responding to protein stress. In this context, Sandy Westerheide, (University of South Florida, USA) showed a novel signaling pathway leading to HSF1 activation involving the deacetylating molecule SIRT1. She showed that while HSF1 can be activated by its deacetylation through SIRT1, this effect is moderated in *C. elegans* by the regulatory molecule CCAR2 that antagonizes the effects of SIRT1. In addition to HSF1, mammalian cells also express a range of HSF1 family paralogs (HSF2-5). Two interesting

presentations implicated roles for HSF2 in key aspects of human pathobiology. Lea Sistonen (Åbo Akademi University, Turku, Finland) showed a role for HSF2 in cell adhesion, a key process in tumor progression in prostate cancer. In addition, the laboratory of Valérie Mezger (Université Paris Diderot, France) implicated the involvement of HSF2 in neural development, highlighting roles for acetylation of the factor in regulation. Continuing the theme of acetylation/deacetylation, histone deacetylase inhibitors were shown to facilitate HSP induction in motor neurons (Heather Durham, McGill University, Canada). In addition to acetylation, another modification-phosphorylation at multiple sites regulates HSF1 activity as shown by Laureano de la Vega (University of Dundee, UK). Gabriella Santoro (University of Rome Tor Vergata, Italy) has characterized novel transcriptional targets for HSF1 in cancer and concentrated here on the role of AIRAP which plays a role in dampening the rates of apoptosis in cancer development under HSF1 regulation. Avoidance of programmed cell death is an essential step in carcinogenesis. Novel pathways of HSF1 action were also indicated by the findings of new intracellular protein partners and use of chemical biology approaches in the studies of Petr Muller (Masaryk Memorial Cancer Institute, Brno, Czech Republic), Eileen Burchfiel and Dennis Thiele (Duke University), and Bushu Dong, Dennis Thiele (Duke University). In addition, Mohammad Shahid (George Washington University, USA) described new constructs to study lineage tracing of cells subjected to stress and HSF1 induction with relevance to studying stress-induced birth defects and mental retardation.

Another key level of regulation of the stress response was indicated by the studies of Albena Dinkova-Kostova (University of Dundee) involving the transcription factor Nrf2 that regulates an overlapping family of stress genes with HSF1. Interactions between these factors may be key to understanding processes in longevity, neurodegenerative diseases, and for stress conditioning humans.

Molecular chaperoning

The business end of the heat shock response involves the ability of the abundantly expressed molecular chaperones to fold target proteins denatured in disease or after stress or permit them to enter the pathways of proteolysis. Effective folding by most chaperones requires the cooperation of accessory proteins known as co-chaperones. Michael Sherman (Ariel University, Israel) showed a novel pathway co-regulated by Hsp70 and co-chaperone Bag3 involving the Hippo pathway and stress kinases required in protein folding. In addition, the laboratory of Mehdi Mollapour (SUNY Upstate Medical University, USA) showed a novel role for chaperones Tsc2 and FNIPs in the chaperoning of tumor suppressors.

A key to translating these studies to human disease is discovery of small molecules that might up- or downregulate the

activities of chaperones aberrantly expressed in disease. Len Neckers (NIH/NCI), who pioneered the early studies of naturally occurring Hsp90 inhibitors, chaired a session entitled “Clinically relevant inhibitors of Hsp90 and Hsp70.” Recent studies have aimed to target each functional domain in the chaperones in order to maximize therapeutic effectiveness and minimize toxicity in human tissues. In this session, Jason Gestwicki (University of California San Francisco), Tim Haystead (Duke University), Brian Blagg (University of Notre Dame), and Shelli McAlpine (University of New South Wales, Australia) demonstrated novel approaches to the chemical targeting of Hsp70 and Hsp90, offering a new world of agents that will help in mechanistic studies of chaperones as well as indicating potential future avenues for future drug discovery.

Earlier in the meeting, Len Neckers and Jane Trepel (NIH/NCI) had presented their recent studies on reconceptualizing Hsp90 as a molecular target, based on feedback from previous clinical trials and advances in chaperone biology. They described the entry of the first Hsp90 drug conjugate into a clinical trial. Jane Trepel next presented some interesting findings on development of an Hsp40 inhibitor that promoted anticancer activity through its impact on dynamic interplay between the Hsp70 and Hsp90 complexes. Andrew Truman and colleagues (University of North Carolina Charlotte, USA) also took the approach of targeting the Hsp70 co-chaperone HDJ2 as a potentially less toxic approach to chaperone-based therapy.

HSP interactions in the cell—assembling the chaperone machine

As mentioned above, Jason Gestwicki opened the meeting with a discussion of the key chaperone/co-chaperone interactions involved in assembling chaperone machines in the cell. A similar theme was pursued by Shannon Doyle (NIH/NCI) who showed interesting studies on interactions between Hsp70 and Hsp90. Tsc1, a protein formerly thought to be involved in TOR kinase regulation was found to play an inhibitory role in the ATPase activity of Hsp90 by interference with Aha1 co-chaperone activity (Sarah Backe, Mollapour Laboratory, SUNY Upstate Medical University). Co-chaperones may also regulate HSF1 activity and HOP was shown to negatively regulate the factor as well as mRNA processing (Abantika Chakraborty, Edkins Laboratory, Rhodes University, South Africa). The Hsp70 co-chaperone Yd1/HDJ2 was shown to regulate ribonucleotide reductase in yeast (Laura Knighton, Truman Laboratory, University of North Carolina Charlotte).

In addition to protein-protein interactions, it is becoming clear that posttranslational modifications (PTMs) may play key roles in regulation of chaperone and co-chaperone properties that may be key to the functioning of chaperone

machines. Important regulatory PTMs for Hsp90, Hsp60, and co-chaperones were discussed in several presentations (Wanping Xu (NIH), Mehdi Mollapour (SUNY Upstate Medical University)); Antonella Marino Gammazza, Francesco Cappello, (University of Palermo, Italy).

Decay of HSP regulation, aging, cancer and degenerative diseases

One of the illuminating aspects of the meeting was a study of the role of the HSR using the naked mole rat (NMR) model (Rochelle Buffenstein, Calico Life Sciences, USA). The NMR has an unprecedentedly prolonged lifespan compared to rodents of similar size (laboratory mouse). These animals display remarkably expanded expression of a number of stress responses including the HSR, increased numbers of HSP genes and reduced activity of the TOST pathway (Vikram Narayan, Calico Life Sciences, Rochelle Buffenstein). Changes in HSP expression have also been associated with diseases of age such as cancer and neurodegeneration. Ben Lang, (Harvard Medical School) showed that knockdown of the genes encoding Hsp72 reduced metastasis in mammary cancer and decreased expression of extracellular matrix gene such as collagens associated with metastasis. Michael Sherman had shown that Hsp72 cooperates with co-chaperone Bag3 in mammary carcinogenesis. Most evidence suggested that the HSR is expanded in cancer, is associated with poor prognosis and offers a target for therapy. One rather neglected area in the study of cancer is the role of Hsp60. However, Alberto Macario (University of Maryland at Baltimore-Institute of Marine and Environmental Technology, USA) showed evidence for a role for Hsp60 in thyroid cancer and described novel posttranslational modifications that may be involved in its regulation in the cell. In addition, Merin Roy and Mehdi Mollapour (SUNY Upstate Medical University) showed a role for Hsp90 co-chaperone PP5 in kidney cancer. In contrast to the predominantly pro-malignant roles of most chaperones and co-chaperones, HSP-BP1 appears to be suppressive in terms of tumorigenesis and higher levels of this protein are associated with a good prognosis (Cristina Bonorino, University of California San Diego, USA).

A number of neurodegenerative diseases appear to be protein folding disorders, triggered by proteins with dominantly aggregative conformations. Claudia Marino (University of Texas Medical Branch, Galveston, USA) showed that overexpressing Hsp60 could protect against amyloid beta in the APP mouse model of Alzheimer's disease. Eileen Burchfiel and Dennis Thiele (Duke University) discussed a role for the repressive function of HSF1 in Huntington's disease. Jie Qian (Chinese Academy of Medical Science, Beijing) also described a role for Hsp70 in protecting cognitive impairment in the hippocampus through its ability to suppress the

apoptotic cascade. Chaperones may however play a more sinister role in neuronal development and Irene Zohn (Children's National Medical Center, Washington, DC, USA) showed that excessive Hsp90 could lead to neural tube closure defects in the Hect D mouse model of anencephaly.

HSPs and the extracellular stress response

Extracellular roles for molecular chaperones finally have become accepted. The original function of extracellular HSPs (HSPe) was thought to be in inflammation and immunity where they appeared to function as danger signals and components of vaccines. That theme was continued in the meeting with Xiangyang (Shawn) Wang (Virginia Commonwealth University, USA) showing that Grp170-based vaccines coupled to an inflammatory mediator (flagellin) could overcome resistance to immune checkpoint inhibitors and lead to tumor destruction. In addition, Kamal Moudgil (University of Maryland) showed that Hsp65 could induce arthritis through generation of a T cell response. Although mammalian HSPs possess largely pro-inflammatory properties, it seems the opposite with prokaryotic HSPs and Thiago Borges, (PUCRS, Porto Alegre, Brazil/Harvard Medical School) showed that Mycobacterial Hsp70 (DnaK) had a profoundly anti-inflammatory effect and could suppress immunity in the context of transplant, allergic asthma, and sepsis. Dinesh Jaishankar (Lurie Comprehensive Cancer Center, Chicago, USA) showed that a single point mutation in Hsp70 (Hsp70iQ43A) produced a mutant chaperone that could trigger a humoral response to melanoma while removing unwanted autoimmune effects. The data indicate that chaperones may be engineered to foster desirable physiological effects.

However, multiple new roles for HSPe beyond the immune response have recently been uncovered. Wei Li (University of Southern California, USA) showed some of his illuminating studies indicating that HSP90e is a potent mediator of wound healing due to its profound ability to promote the mobility of cells entering the wound to accelerate wound closure. In addition, secreted HSP90e possesses the ability to promote tumor formation. Another unrelated property of HSP90e was the ability to protect neurons from neuroinflammation mediated cell death (Ayesha Murshid, Harvard Medical School). In this system, HSP90e appeared to function by promoting beta amyloid processing through the autophagy pathway. Co-chaperones have also been shown to have extracellular functions, including those of a novel isoform of HOP (Gregory Blatch, University of Notre Dame, Australia) and HSPBP1 (Cristina Bonorino, University of California San Diego). In addition, a function for extracellular chaperone TIMP-2 was shown by Alexander Baker-Williams and Dimitra Bourboulia, SUNY Upstate Medical University. TIMP-2 interacts with HSP90e to promote its interaction with matrix metalloprotease-2 to facilitate client interactions. Perhaps at the next meeting we will

be discussing protein-protein interactions within the extracellular chaperone network. These moonlighting extracellular properties are also seen in other families of stress proteins and Michael Lynes (University of Connecticut, USA) showed similar effects of circulating metallothionein in mediating inflammation.

Two key remaining questions regarding extracellular chaperones are (1) How are these leaderless proteins secreted? and (2) What is the nature of the receptors mediating the effects of HSPe on target cells. Michael Graner (University of Colorado, USA) discussed the role of extracellular lipid vesicles such as exosomes as carriers of HSPe. Indeed Wei Li had shown HSP90e to be present on the outer surface of exosomes where it may exert its effects on cells involved in wound healing and tumor cells. Ayesha Murshid had also shown in her studies that tumor resident macrophages (microglia) could secrete HSP90e in exosomes and that this effect was increased by cell stress. Antonio De Maio (University of California San Diego) showed next that the lipid components of the extracellular vesicles may also play a signaling role in addition to embedded HSPs. Multiple receptors have been shown to bind HSPe and further studies along these lines may resolve some of the conflicting findings of inflammatory vs. anti-inflammatory effects of the HSPs as well as the wide diversity of physiological effects recently attributed to extracellular chaperones.

Concluding remarks

We had a spirited poster session with lots of interactions over posters that were uniformly of high quality (Supplementary Figs. 3 and 4). This made for a happily difficult task for the organizers to select five posters for presentation as brief talks. The selected presenters were Abantika Chakraborty (Adrienne Edkins lab at Rhodes University, South Africa), Eileen Burchfiel (Dennis Thiele lab, Duke University), Sarah Backe (Mehdi Mollapour lab, SUNY Upstate Medical University), Irene Zohn (Children's National Medical Center) (Supplementary Fig. 5), and Nitika, presenting joint work done with Laura Knighton (Andrew Truman Lab, University of North Carolina) (Supplementary Fig. 6).

Awards and recognition were given during the symposium banquet. Two CSSI senior fellows (Ian Brown, University of Toronto, Canada and John Subjeck, Roswell Park Cancer Center), five CSSI fellows (Francesco Cappello, Melody Clark, Jason Gestwicki, Michael Graner, Jane Trepel) (Supplementary Fig. 7), and one CSSI corporate fellow (Ariel Louwrier, StressMarq Biosciences, Canada) (Supplementary Fig. 8) were inducted by President Gabriella Santoro on behalf of the Executive Council of the Cell Stress Society International. The most cited paper, already with more than 30 citations, published in *Cell Stress & Chaperones* in 2017 was recognized. This is also the most downloaded paper,

a review article written by Erik Zuiderweg, Lawrence E. Hightower, and Jason E. Gestwicki entitled “The remarkable multivalency of the Hsp70 chaperones” (*Cell Stress and Chaperones*, 2017, 22:173–189).

There were several “firsts” at this meeting. An entire session and plenary talk by Melody Clark was devoted to environmental stress in recognition of the rapidly developing impact of global warming on the biota of our planet. The first combined session on proteostasis and caloristasis was held in an effort to better understand the overlapping roles they have in cellular protein and energy homeostasis. A number of excellent talks during the sessions using immunological approaches convinced us that the special issue of *Cell Stress and Chaperones* for the 2019 volume should be “Molecular Chaperones and Cancer Immunotherapy.” We are pleased to

announce that we will begin accepting manuscripts at the SpringerNature Editorial Manager site of *Cell Stress and Chaperones* for original articles, reviews, and perspective/reflections articles on January 1, 2019. The Guest Editor is Professor Cristina Bonorino.

Acknowledgements We wish to thank our sponsoring organizations including the Cell Stress Society International, Springer Nature, MGM Resorts International, and StressMarq Biosciences for their continued support without which this symposium would not have been possible. A special thanks to CSSI General Secretary Helen Neumann who handled all of the details of meeting organization.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.