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MEETING REVIEW

Report on the VIIth International Symposium on Heat Shock Proteins in Biology & Medicine

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Abstract This seventh symposium in a series on heat shock proteins in biology and medicine was held November 1–5, 2014, at the Hilton Hotel in Old Town Alexandria, Virginia. Approximately 70 participants including principal investigators, postdoctoral fellows, and graduate students were in attendance. The major themes were: new properties of heat shock proteins (HSPs) and heat shock factor (HSF) and role in the etiology of cancer, molecular chaperones in aging, extracellular HSPs in inflammation and immunity, role of heat shock and the heat shock response in immunity and cancer, protein aggregation disorders and HSP expression, and Hsp70 in blood cell differentiation. The next meeting is planned for the fall of 2016 in the same venue.

Keywords Heat shock proteins · Biology · Medicine · Cancer · Immunity · Aging · Molecular chaperones

Introduction

This symposium was the seventh symposium in a series presided over by Dr Stuart Calderwood aimed at exploring the association of molecular chaperones, heat shock proteins, and the heat shock response in physiological/pathological processes. The biochemistry and ultrastructure of molecular chaperones was not emphasized, as these topics are well represented at other meetings. The major themes were: new properties of heat shock proteins (HSPs) and heat shock factor (HSF) and role in the etiology of cancer, molecular chaperones in aging, extracellular HSPs in inflammation and immunity, role of heat shock and the heat shock response in immunity and cancer, protein aggregation disorders and HSP expression, and Hsp70 in blood cell differentiation. This report gives a thematic overview and does not include all the topics presented.

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New properties of HSPs and HSF, and role in the etiology of cancer

One of the exciting aspects of the meeting involved advances made in understanding the biology of Hsp90. In recent years, we have understood the molecular chaperone activities of Hsp90 mostly in terms of its biochemistry, cooperative interactions with cochaperones. However, Dr Len Neckers (NCI/NIH), the conference keynote speaker, has opened up new areas in our understanding of this chaperone by characterizing the role of posttranslational modification (PTM) in terms of phosphorylation, acetylation, and sumoylation in Hsp90 biology. One particularly intriguing possibility is that altered signaling mechanisms characteristic of cancer may target such PTMs, and this could contribute to the "addiction to chaperones" observed in malignant cells. (Also discussed later by Dr Mehdi Mollapour, SUNY Upstate Medical University).

In addition, interesting differences in properties of the two Hsp90 isoforms have been detected. Dr Wei Li (University of Southern California) has shown that Hsp90a can be released into the extracellular environment and there take part in cell regulation, mediating for instance wound healing effects. In addition, proteomic studies carried out by Thomas Prince (NCI/NIH) in the Neckers lab indicate that Hsp90β may be

more dedicated to "housekeeping" molecular chaperone functions while Hsp90α may play more glamorous roles in cell regulation. These distinctions might not be anticipated based on the rather minimal sequence differences between the Hsp90s but offer keen insights into the biology of this chaperone. Finally, Dr Tim Haystead (Duke University) discussed the approach of targeting ectopically expressed Hsp90 for imaging and treatment.

Another PTM with implications in the stress response is the modification of intracellular proteins by monosaccharides of O-linked β-N- acetylglucosamine (O-GlcNAc). Dr Natasha Zachara (Johns Hopkins University School of Medicine) discussed targets for this modification and roles in cytoprotection.

The poster session was also rich in Hsp90 studies, mostly from the Neckers lab—presentations by Kristin Beebe et al. (NCI/NIH) *Posttranslational modification state of Hsp90 differentially affects binding of small molecule inhibitors*; Toshiki Kijima et al. (NCI/NIH), *Defined interactions between HSF1 and Hsp90*; T. Prince et al. (NCI/NIH) *Hsp90 and tyrosine kinase inhibitors*: *A synergistic approach towards combating cancer*; Andrew W. Truman (University of Chicago) *Quantitive ptoteomics of the yeast Hsp70/Hsp90 interactomes during DNA damage reveals chaperone-dependent regulation of ribonucleotide reductase. Inhibition of Hsp90 via C-domain induces temporally distinct phosphorylation patterns*; and Diana M. Dunn (SUNY Upstate Medical University) *Phosphorylation of human Hsp90 threonine 115 modulates chaperone function and drug sensitivity.*

Hsp70 is also emerging as a factor in cell regulation, exhibiting properties beyond a narrow role in chaperoning. Dr Michael Sherman (Boston University) showed a key role for Hsp72 in mammary cancer, and this property did not seem to depend on alterations in protein folding. Instead, Hsp72 appeared to function through its co-chaperone Bag3, a major regulatory molecule in cell signaling. In addition, a presentation by Stuart Calderwood (Harvard Medical School) that included work by Jianlin Gong showed that Hsp72 is required for tumor initiation and metastasis in murine spontaneous breast cancer. These effects appeared to be partially mediated through regulation of expression of the protoooncogene *c-Met*, a key player in invasion and metastasis in cancer. We anticipate advances in understanding of the roles of individual members of the Hsp70 family, as is currently emerging for Hsp90. The prospect of targeting Hsp70 with small molecule inhibitors was elegantly discussed by Maureen Murphy (The Wistar Institute), who introduced a novel class of drugs that could selectively kill cancer cells by inhibiting Hsp70 function. In a related topic, Dr Mathias P. Mayer (University of Heidelberg) showed a detailed analysis of the activities of inhibitors targeting various domains in Hsp70.

Dr Takanori Eguchi (Harvard Medical School) then described his studies showing an unconventional role for the extracellular protease MMP3 as a nuclear protein that could trigger molecular chaperone synthesis (HspA7) in mammary cancer. Interestingly, a role in cancer for the Hsp70 co-chaperone Hsp40 was also shown by Dr Jane Trepel (NCI/NIH).

One presentation that stood apart was that of Dr Carmen Garrido (INSERM U866) who has shown very impressive studies indicating a key role for Hsp70 in hematopoiesis, acting through the factor GATA1. This role appeared to depend on nuclear localization of Hsp70, and Dr Garrido is attempting to study the role of PTM, particularly phosphorylation in this function/localization of Hsp70. This continued the theme of HSP PTM and regulation in the cell.

Molecular chaperones in aging

A symposium on molecular chaperones in aging was organized by Dr Shelley Buffenstein (University of Texas Health Science Center San Antonio). This symposium featured some fascinating studies on the naked mole rat (NMR), a rodent with a remarkable lifespan based on size (32 years compared to 3 years in the comparably sized mouse). This has permitted comparative biology studies that have uncovered important aspects of the aging process in mammals. Dr Buffenstein showed that one aspect of the proteotoxic response was enhanced in NMR—proteasome activity that was resistant to oxidative stress as well as conventional proteasome inhibitors. Such proteasome resistance appeared to be conferred by Hsp70 and Hsp40. Karl Rodriguez (also from the UTHSC San Antonio) stressed the importance of Hsp25 in the longevity of NMR. This small HSP is expressed to very high levels in this organism. Kenneth B. Storey (Carleton University) finally gave an encyclopedic presentation entitled "HeatShock Proteins and Hypometabolism in Nature", discussing the multiple roles of chaperones in hibernation and other processes involving a step down in metabolism.

Protein aggregation disorders and HSP expression

Michael Sherman (Boston University) chaired a lively and highly diverse session on protein aggregation disorders and HSPs. Gary Jones (Maynooth University) discussed his studies on the roles of Hsp104, Hsp70, and Hsp40 in prion propagation in yeast, concentrating on Hsp70. The Hsp complex was able to dissolve prions in yeast. Daniel Kaganovich (Hebrew University) then continued in a yeast theme, discussing a further strategy for resolving proteotoxic stress involving asymmetric cell division in which damaged proteins and mitochondria remain with the mother cell after mitosis. Nava Zaarur (Boston University) then discussed the role of aggresome particles in resolving aggregated proteins, in this

case in eucaryotes. Alberto Macario (University of Maryland School of Medicine) discussed the role of chaperonins in proteotoxic disorders dealing with the effects of a pathogenic mutation of human CCT5 on its intrinsic properties. Dr Elaine C. Lee (University of Connecticut) discussed another type of stress. She showed significant roles for chaperones in osmotic stress responses of *Caenorhabditis elegans* models of polyglutamine diseases.

Extracellular HSPs, inflammation, and immunity

Although it is now generally accepted that HSPs can escape the confines of the cell, many questions still remain regarding their extracellular properties, particularly with regard to their immune effects. These questions include: whether HSPs are mostly immunostimulatory or immunosuppressive, whether they can induce sterile inflammation, and what structures on the immune cells recognize the HSPs. Dr Cristina Bonorino (Pontifícia Universidade Católica do Rio Grande do Sul) chaired a symposium "HSP as modulators of immunity: prokaryotic meets eukaryotic" featuring presentations by Robert Binder (University of Pittsburgh), Eckhart R. Podack (University of Miami), Renata Pasqualini (University of New Mexico Medical School), and Cristina Bonorino. In short, the talks indicated that while the prokaryotic chaperone DNA-PK can be immunosppressive and prolong the lifetime of transplanted tissues and reduce the morbidity of arthritis (Drs Bonorino and Kamal Moudgil (University of Maryland School of Medicine)), HSPs can also be immunostimulatory and act as cancer vaccines when associated with cancer antigens (Drs Binder and Podack). In the discussion, it was stressed that these effects may be related to HSP dose, with low doses of HSP antigen complex favoring immunity while higher doses may lead to immunoregulatory effects (Dr Binder). Most parties agreed that much future study is required to resolve all these issues. It was also suggested, inspired by the presentation of Dr Neckers, that HSP PTMs might also be playing roles in shading the immune effects of HSPs (Dr Bonorino). In the next session, Drs Shawn Wang (Virginia Commonwealth University School of Medicine) and John Subjeck (Roswell Park Cancer Institute) discussed the molecular foundations of their highly effective large HSP vaccines that are now in clinical trial for tumor immunotherapy. They indicated that the high avidity for antigen of the larger HSPs might be key for effectiveness. Although the nature of HSP receptors is still not fully resolved, Ayesha Murshid (Harvard Medical School) made a strong case for the scavenger receptor SREC-I as a key molecule in the effects of HSPs on immune cells. As many of the HSPs are in large families, it has not been clear whether all members of Hsp90 or Hsp70 can function outside the cell. Dr Wei Li (University of Southern California) showed that HSP90 family member Hsp90α is the major secreted factor while Dr John Williams (University of Chester) showed potent extracellular effects for human HSP70 isoform HSPA1A. Extracellular roles are not restricted to Hsp90, and Edward O'Brien (Libin Cardiovascular Institute of Alberta/University of Calgary) discussed the extracellular role of heat shock protein 27 (HSPB1) in inflammatory vascular disease. Another lively issue is whether HSPs are released as free proteins, packaged in exosomes, or whether both forms co-exist. This issue was discussed by Monika Fleshner (University of Colorado) and Antonio De Maio (University of California San Diego). Dr De Maio brought up the interesting scenario of Hsp70 binding directly to lipid membranes and perhaps forming membrane channels (Ryan White, University of Maryland).

HSPs are evidently not the only types of stress proteins that can function in the extracellular milieu, as indicated by Dr Michael A. Lynes (University of Connecticut). In a presentation entitled *Therapeutic manipulation of the stress response during inflammatory disease*, Dr Lynes showed a significant role for extracellular metallothionen in inflammatory bowel disease. Along those lines, Dr George Perdrizet (University of California San Diego) discussed the use of hyperbaric oxygen for enhanced wound healing in diabetic neuropathy, showing impressive clinical findings.

Role of temperature in immunity and cancer

Dr Betsy Repasky (Roswell Park Cancer Institute) gave a keynote address entitled *Reducing adrenergic stress in mice reveals new relationships between thermoregulatory metabolism and immunosuppression*. Dr Repasky has uncovered a fascinating relationship between temperature and immunity, indicating much greater tumor immunity in animals kept at a thermoneutral temperature. Cold stress-induced release of adrenergic hormones was shown to underlie immunosuppression at low ambient temperatures. This has enormous implication for the way in which we plan experiments and for immunotherapy. Dr Sharon Evans (Roswell Park Cancer Institute), in another exciting keynote speech, explored the role of fever range hyperthermia in tumor immunity through extravasation into the tumor space. She stressed the central role of interleukin 6 in mediating uptake of CD8+ T cells into the tumor, a key step in tumor control. Dr Jeff Hasday (University of Maryland) followed up his long-term studies of heat shock/fever-induced inflammatory responses showing a key role for the stress kinase p38 MAP kinase and preliminary studies aimed at selecting p38 targeting drugs. Dr Tim Crul (Hungarian Academy of Sciences), from the laboratory of Laszlo Vigh, gave an interesting talk regarding changes in membrane lipids as triggers for thermal events. He showed that limited cholesterol deprivation reprograms the heat

inducibility of the major stress proteins parallel with enhancing the acquired thermotolerance in B16 melanoma cells.

Heat shock factors

As the primary transcriptional regulator of HSPs, heat shock factor 1 (HSF1) is suspected to have many roles in physiology and pathology. In addition at least three other HSFs exist—HSF 2, 3, and 4, and these molecules may play auxiliary or regulatory roles in chaperone expression. Dr Lea Sistonen (Abo Akademi University) has studied extensively the interplay of HSF1 and HSF2 in chaperone expression. In this report, she showed opposing effects of HSF2 and HSF1 activity in mitotic and interphase cells. Indeed, HSF2 appeared to block RNA Pol II occupancy of HSP genes under these circumstances. Dr Heeyoun Bunch (Harvard Medical School) described studies aimed to discover novel activators of *hsp70* transcription. She has shown that *hsp70* is repressed by TRIM28/ Kap1, an agent that confers polymerase II pausing on *hsp* genes. HSF1 acts to reverse pausing by a mechanism involving the phosphorylation of TRIM28. Trim 28 is a repressor of some types of cancer and may function to antagonize HSF1/Hsp72 and suppress cancer. Dr Gabriella Santoro (University of Rome) also spoke on the complexity of heat shock factors in cancer, describing her seminal studies showing prostaglandin induction of HSF1 as well as novel HSF1-induced gene products. There has been much emphasis recently on the role of HSF1 in cancer, breast cancer in particular. Dr Nahid Mivechi (Georgia Regents University Cancer Center) emphasized hepatocellular carcinoma in a talk entitled: *HSF1 deficient hepatocytes exhibited reduced gluconeogenesis through altered mitochondria biogenesis*. She showed that *hsf1−/−* mice have reduced liver carcinogenesis, associated with increased metabolic rate and reduced ATP. The studies indicate a key role for *hsf1*. The studies of Dr Ming Tan (University of South Alabama) also indicated roles for HSF1 in breast cancer cell pathology, in this case through mediation of cytoprotective autophagy. Dr Pranoti Mandrekar (University of Massachusetts) showed the involvement of HSF1 in another pathology, alcoholic liver disease. Her studies indicated a pathway involving HSF1 induction of Hsp90 and enhanced signaling through toll like receptor 4, leading to cytokine production and liver pathology. Dr William Balch (Scripps Research Institute) provided a powerful conceptual synthesis of many of the themes in the meeting under the banner of proteostasis. He discussed the successful application of the concepts of proteostasis and the proteostatic code to the focus of his laboratory on cystic fibrosis.

Additional abstracts and topics are available on the Program page at http://oldtown.cellstressresponses.org

Student poster award winners sponsored by the Cell Stress Society International. Left to right: Stuart K. Calderwood, main organizer; Toshiki Kijima, NCI/NIH; Benjamin Lang, Beth Israel Deaconess Medical Center/Harvard University; Thomas Prince, NCI/NIH; Jennifer Groves, Johns Hopkins University School of Medicine; and Lawrence Hightower, Secretary-Treasurer, CSSI. Harvey Schwartz was recognized by Thomas Prince for his work on his poster.

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