VIIIth International Symposium on Heat Shock Proteins in Biology and Medicine: Stress Responses in Health and Disease

Hilton Alexandria Old Town, Alexandria, Virginia
October 29-November 2, 2016

ORGANIZING COMMITTEE

Principal Organizer
Stuart K. Calderwood, Harvard Medical School

Co-organizers
Larry Hightower, University of Connecticut
Elizabeth Repasky, Roswell Park Cancer Institute
Len Neckers, National Cancer Institute

Keynote Speaker
Stuart K. Calderwood, Harvard Medical School

Plenary Speakers:
Wei Li, University of Southern California
Michael Sherman, Boston University Medical School
Carmen Garrido, INSERM U866, University of Burgundy
László Vigh, Hungarian Academy of Science
PROGRAM

SATURDAY OCTOBER 29

2:30 – 4:30 Registration - Plaza Foyer

4:30 Opening of meeting - Washington-Jefferson Room

   Welcoming remarks – Stuart K. Calderwood, Harvard Medical School
   Presentation of CSSI awards – Larry Hightower, University of Connecticut
   Remarks – Elizabeth Repasky, Roswell Park Cancer Institute
   Remarks – Len Neckers, National Cancer Institute
   Meeting Sponsor – Ariel Louwrier, StressMarq Biosciences Inc.

5:30 Keynote Address: Stuart K. Calderwood - Washington-Jefferson Room

6:30 – 7:30 Reception - Plaza Foyer
SUNDAY OCTOBER 30

8:00 – 9:00 Breakfast - Plaza Foyer

ALL SESSIONS ARE IN THE WASHINGTON-JEFFERSON ROOM

9:00 – 9:45 Plenary Lecture: Wei Li

10:00 – 12:00 Session 1: Comparative Biology of Cancer and Longevity
Larry Hightower
Heike Gruber
Rochelle Buffenstein
Melody S. Clark

12:00 – 1:00 Lunch - Plaza Foyer

1:00 – 3:00 Session 2: Hsps and Proteostasis
Yatrik Shah
Kazue Hashimoto-Torii
Brian C. Freeman
Yihong Ye

3:00 – 3:30 Tea and Coffee Break – Plaza Foyer

3:30 – 5:30 Session 3: Extracellular HSPs
Jennifer Isaacs
Ayesha Murshid and Thiago J. Borges
Ed O’Brien
Michael W. Graner

6:00 – 7:30 POSTERS – Plaza Foyer

NB: All posters in the program will be presented at this poster session. Late abstracts not included in the abstract booklet will be available on the CSSI website.
8:00 – 9:00 Breakfast – Plaza Foyer

ALL SESSIONS ARE IN THE WASHINGTON-JEFFERSON ROOM

9:00 – 9:45 Plenary Lecture: Michael Sherman

10:00 – 12:00 Session 4: Stress, Metabolism and Immunosuppression
Elizabeth Repasky
Connie J. Rogers
Caroline Le Poole
Sharon S. Evans

12:00 – 1:00 Lunch – Plaza Foyer

1:00 – 3:30 Session 5: Molecular Chaperones and Cancer
(Note extended time)
Len Neckers
Julia Yaglom
Jane Trepel
Irina Guzhova
Boris Margulis

3:30 – 4:00 Tea and Coffee Break – Plaza Foyer

4:00 – 6:00 Session 6: HSF1 in Health and Disease
M. Gabriella Santoro
Lea Sistonen
Nahid F. Mivechi
Valérie Mezger

7:00 – 10:00 Banquet dinner – Plaza Foyer
TUESDAY NOVEMBER 1

8:00 – 9:00 Breakfast – Plaza Foyer

9:00 – 9:45 Plenary Lecture – Carmen Garrido

ALL SESSIONS ARE IN THE WASHINGTON-JEFFERSON ROOM

10:00 – 12:00 Session 7: HSPs Modulate the Immune Response
Cristina Bonorino
Xiang-Yang (Shawn) and John Subjeck (jointly)
Kamal Moudgil
Michael A. Lynes

FREE AFTERNOON
8:00 – 9:00 Breakfast – Plaza Foyer

9:00 – 9:45 Plenary Lecture – Laszlo Vigh

10:00 – 12:00 Session 8: Small HSPs and Human Diseases
Robert M. Tanguay
Carmen Garrido
Serena Carra
Zarah Batulan

12:15 Closing Remarks by Organizers
SATURDAY OCTOBER 29: KEYNOTE ADDRESS

Extracellular Molecular Chaperones in Cancer Vaccines and Radioimmunotherapy

Stuart K. Calderwood

Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215; scalderw@bidmc.harvard.edu

Molecular chaperones have proven to be excellent agents in design of vaccines as they are able to bind a wide spectrum of tumor antigens, giving rise to polyvalent vaccines that target the cancer antigenic fingerprint. However, many HSPs lack the ability to effectively trigger innate immunity which is an important event in overcoming tolerance to antigens. We aimed to combine molecular chaperone vaccines (Hsp70 DC-fusion vaccines) with radiotherapy to remedy this deficiency. Radiation kills tumor cells through death pathways leading to necrosis and can thus trigger an inflammatory response in tumors that amplifies innate immunity. The combined treatment would thus be predicted to give local tumor killing and immunostimulation to target local and metastatic cancer cells.

In basic research studies investigating this combination, we made the unexpected finding that mammary cancer cells surviving an initial 6Gy radiotherapy treatment became highly resistant to further treatments. These cells additionally exhibited a cancer stem cell (CSC)-like phenotype, with increases in CSC markers Sca1, CD44 and ALDH1. The cells additionally exhibited a highly increased capacity to invade and metastasize to the lymph nodes and lungs of tumor bearing mice. Investigation of mechanisms involved in this phenotype showed that the effects of radiation were mediated by secreted products, and conditioned medium from irradiated cells could confer stem-likeness and invasiveness on recipient cultures. These effects of radiation or conditioned medium could be entirely reversed by inhibitors of cyclooxygenase 2 (Cox2). Furthermore we showed that Cox2 product prostaglandin E2 (PGE2) was responsible for mediating these effects through its receptor EP4 and was reversed by EP4 antagonists. This appeared to be a common response to radiation and was observed in 4T1, MMT and MCF7 mammary cancer and MC38 colon carcinoma.

We next aimed to remedy these effects by combining radiation and Hsp70 DC-fusion vaccines. The vaccines were prepared using previously irradiated MMT tumors enriched in the CSC-like cells. We combined treatment with two 6Gy doses of radiotherapy and three vaccinations with Hsp70 fusion vaccine. Although neither arm of the treatment was effective individually, we observed synergistic control of primary and secondary mammary carcinomas and decreased spread of CSC like cells to the lungs after radioimmunotherapy. Future studies will explore the enhanced vaccine design as well as targeting Cox2 and PGE2 receptors in radioimmunotherapy treatment.

These experiments indicated the flexibility and effectiveness of molecular chaperone vaccines, which may be adapted to targeting aggressive treatment-resistant tumor sub-populations.
SUNDAY OCTOBER 30

PLENARY LECTURE

Wei Li
Secreted Heat Shock Protein-90alpha (Hsp90α) in Wound Healing and Cancer

Wei Li

USC-Norris Cancer Center and Department of Dermatology, University of Southern California Keck School of Medicine, Los Angeles, California, USA; wli@usc.edu

Around 2007, we were searching for a “critical growth factor” that drives skin wound closure from conditioned medium of human keratinocytes under hypoxia. Conventional protein purification with a sensitive cell motility assay unequivocally identified Hsp90α, by surprise. We demonstrated three unique properties that functionally distinguish Hsp90α from the growth factor expected by the conventional wisdom. The new therapeutic entity resides in a 115-aa fragment called F-5 located in the middle domain of Hsp90α and independent of the N-terminal ATPase. The secreted Hsp90α (but not HSP90β) utilizes the cell surface LDL Receptor-related Protein-1 (LRP-1) to transmit its signal to the Akt pathway to promote both cell survival and cell migration under stress. Topical application of F-5 accelerates closure of acute, burn and diabetic skin wounds. Hsp90α is secreted through the extracellular vesicle/exosome pathway in cells in response to stress. We have recently identified a key and previously unrecognized regulator of this cellular exosome secretion pathway. The usefulness of secreted Hsp90α is also recognized by certain tumors, including those with constitutive accumulation of HIF-1α. These tumor cells use secreted Hsp90α to survive hostile hypoxia, invade residing tissue and promote tumor metastasis in mice. Two evolutionarily conserved lysine residues, lys-270 and lys-277, only found in the Hsp90α subfamily determine the extracellular Hsp90α function. A newly constructed monoclonal antibody, 1G6-D7, against the dual lysine region of secreted Hsp90α inhibits both de novo tumor formation and expansion of already formed tumors in mice. These findings suggest an alternative therapeutic approach to target Hsp90 in cancer, i.e. non-enzymatic activity of the tumor-secreted Hsp90α, instead of the enzymatic activity of intracellular Hsp90α and Hsp90β.
SUNDAY OCTOBER 30

SESSION 1: COMPARATIVE BIOLOGY OF CANCER AND LONGEVITY

SPEAKERS

Larry Hightower
Heike Gruber
Rochelle Buffenstein
Melody S. Clark

POSTER

Mercy Manley
Session 1: Comparative Biology of Cancer and Longevity

Type of Presentation: Oral Presentation

Inspiration for the Session “Comparative Biology of Cancer and Longevity”

Lawrence E. Hightower, Dept. of Molecular and Cell Biology, University of Connecticut Storrs, CT 06269; lawrence.hightower@uconn.edu

It is my pleasure to introduce this new session at our 2016 symposium. Stuart Calderwood invited Kenneth Storey and Rochelle Buffenstein to talk about their unusual animal systems at the 2014 Old Town meeting. Both speakers raised considerable interest and both followed up their talks with papers in our journal. Shelley Buffenstein wrote about determinants of longevity in the chaperone-protein degradation network of naked mole rats (Cell Stress & Chaperones (2016) 21:453-466) and Ken Storey published a study about hibernating ground squirrels and transcription factor regulation (Cell Stress & Chaperones (2016) 221:883-894).

Earlier this year, Time Magazine produced an article titled “A menagerie of super-agers”, a collection of remarkably long-lived animals that scientists are studying in search of clues to extend human longevity. I saw two animals in the article the inclusion of which I appreciated immediately. One was the naked mole rat, thanks to Dr. Buffenstein’s presentation. The article noted that these rodents can live up to 30 years and they exhibit very low cancer rates, even those exposed to carcinogens. Researchers are working now to find the molecules responsible.

The second animal was an elephant. Both Asian and African elephants have very low rates of cancer, a counterintuitive statistic. Large, long-lived animals have correspondingly more cells (increased body size) and larger numbers of cell divisions (increased species life span), each of which create spontaneous mutations leading to higher rates of cancer than found in smaller animals. As a result of a study by Joshua Schiffman and colleagues (JAMA (2015) 314:1850-1860), interest is now focused on TP53, a gene encoding the protein p53, a key tumor suppressor protein found mutated in many human cancers. Humans have 1 copy of this gene represented by 2 alleles whereas elephants have 20 copies represented by 40 alleles. DNA damage (mutation) induces p53, a protein involved in driving damaged cells into apoptosis instead of transformation into cancer cells. Summary slides from João Pedro de Magalhães summarizing genes possibly involved in evolution of longevity and cancer resistance of Bowhead Whales, another very large mammal that can live more than 200 years, will be shown (Cell Reports (2015) 10:112-122).

The Time article also described an Arctic clam Arctica islandica that can live 500 years or more, the longest-lived non-colonial animal known. Heike Gruber will tell us about telomere-independent aging and other metabolic adaptations in this species (AGE (2015) 37:90). This reminded me of several papers published in Cell Stress & Chaperones by Melody S. Clark and colleagues about stress responses in Antarctic limpets and clams, which we will hear about as well (CSAC (2008) 13:39-49). Recent genomic and proteomic approaches make possible the study of animals with extreme adaptations to their environments and evaluation of their relevance to human biology.

Session sponsored by MGM Resorts International.
Molecular and cellular hallmarks of aging in the long-lived ocean quahog, *Arctica islandica*

Heike Gruber  
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Elucidating the mechanisms underlying longevity and healthy aging is a pivotal challenge in biomedical sciences. Bivalves represent an animal group with vast differences in maximum lifespans (MLSPs from 1<500 years). This also suggests interspecific aging studies and for bivalves exact ages can be determined via annual age-rings laid down in the animals’ shells. In the present study, the longest- and shortest-lived populations of the ocean quahog *Arctica islandica* – MLSP >500 years at the coasts of Iceland, MLSP <50 years in the Baltic Sea – were investigated with respect to their differential characteristics. The Baltic Sea population is challenged by high fluctuations in several environmental factors (e. g salinity, oxygen availability, temperature) in their habitat, whereas environmental conditions around Iceland are much more stable, so that these two populations parted in their physiological behavior and gene expression response.

For this study, ages and sexes of 160 Baltic Sea and 179 Icelandic individuals were determined and tissues (gill, foot, adductor muscle, mantle, heart, digestive gland) frozen for further investigations. Various differences in growth, sex distribution, cellular damage, and gene expression between the two populations could be observed. Telomere length and telomerase activity as well as lipid peroxidation, protein oxidation (protein carbonyls), and protein stability remained unchanged over the lifespan of the populations. Lower protein stability was found in the shorter-lived Baltic Sea population. At the same time, a remarkably faster and higher nucleotide oxidation accumulation and different levels of candidate gene expression distinguished the shorter-lived Baltic Sea from Icelandic animals.

While nucleic acid oxidation accumulates with time also in other organisms we could show that in the ocean quahog it correlates not only over chronological age but according to differential aging rates with biological age. Nucleic acid oxidation must therefore be linked to mechanistic primary aging whose causes, however, still remain to be investigated.
Naked mole-rats appear to have unusual biological traits that protect against both the vagaries of aging and cancer. These mouse size rodents exhibit extreme longevity with maximum lifespan exceeding 30 years and unlike most mammals show a markedly attenuated age associated change in physiological and molecular features. Moreover, in contrast to the case of laboratory mice which are notoriously short-lived and highly susceptible to spontaneous neoplasia, cancer is exceedingly rare in naked mole-rats. Astonishingly, we have observed only one incidence of spontaneous neoplasia within our large (>2800), long-maintained (35+ year) captive colony, despite careful assessment of >2500 individual necropsies. Further evidence of the marked resistance of naked mole-rats to cancer is indicated by attempts at oncogenic transformation of naked mole-rat cells. Unlike cells from other mammals, in which transformation by oncogenes RAS and SV40 T antigen causes invasive xenograft tumors, naked mole-rat fibroblasts transformed with these oncogenes have no tumorigenic effects. Similarly, topical treatment with chemical carcinogens as well as prolonged exposure to UV did not induce skin cancers in the naked mole-rat. Pronounced differences in epidermal hyperplasia, cell senescence and molecular responses to these experimental treatments were evident between mice and naked mole-rats. Although both experimental treatments induced considerable DNA damage within 24h, pronounced species differences in the upregulation of several molecular pathways including Nrf2 signaling, DNA repair and apoptosis were evident. Data acquired to date confirm our hypothesis that naked mole-rats have mechanisms in place that protect against experimental induction of cancer by both chemical and physical stressors and highlight several cytoprotective mechanisms in the naked mole-rat and that are likely to play a key role in preventing cancer initiation and progression.
Understanding the ageing process and longevity has clear medical implications, however this is also true in the environmental field. Many species, particularly those inhabiting very cold habitats such as the polar seas, develop and grow much more slowly when compared with temperate species; they have deferred maturity and can live a long time, up to 100’s of years in some cases. It is the larger, older animals that produce most larvae and therefore are the main contributors to the recruitment of subsequent generations. Thus it is important to understand how long these species live, how they respond to environmental change at different stages in their life history and the consequences for future sustainability. In this respect, molluscs are ideal models, as they have annual growth rings and are one of the few marine phylum that can be accurately aged.

The Antarctic clam, Laternula elliptica (which lives up to 36 years old) is becoming increasingly used as an ageing model for Antarctic ecosystem studies. It has been shown that, at the whole animal level, the thermal resilience and immune functioning of these clams decreases with age (Peck et al. (2009) Functional Ecol. 23: 248; Husmann et al. (2011) Journal of Experimental Marine Biology and Ecology 398: 83), also that older animals are more affected by sedimentation and burrow less rapidly (Peck et al. (2007) Oecologia 154: 479; Philipp et al. (2011) Antarctic Science 23: 127). Curiously, the rate of radical oxygen species (ROS) production as a proportion of mitochondrial oxygen consumption remains constant with age (Philipp et al (2005) Mechanisms of Ageing and Development 126: 610). Molecular analyses of responses to environmental manipulation via hypoxia and heat, are expanding our knowledge on the effect of ageing at the cellular level. Heat shock proteins have critical roles to play in resilience. The current state of the art with regard to these molecular responses will be reviewed in this talk.
Session 1: Comparative Biology of Cancer and Longevity

Type of Presentation: Poster

**Preventative Medicine: Tursiops truncatus**

Mercy Manley

Siegfried & Roy’s Secret Garden and Dolphin Habitat, The Mirage, Las Vegas, NV 89109; mmanley@mirage.com

Dolphins and other cetaceans have been in human care since the 1800’s. The first commercial dolphinarium opened to the public in 1938. Since then, humans have been captivated by these charming creatures. With hundreds of dolphins currently in human care, and increasing numbers of stranding events world-wide, the focus of marine mammal medicine turns to the prevention of disease and the social and psychological welfare of these captivating animals in our charge. Preventative medicine strategies ensure the animals’ continued well-being. By evaluating multiple facets, such as nutritional support, social grouping, medicine, diagnostics and training, we gain a complete picture of the animals’ health. We have a responsibility to continue using comparative scientific methods and collaborating with biologists, physiologists and other disciplines to increase our understanding of marine mammal diseases and to advance diagnostic and therapeutic procedures that ultimately improve the overall healthcare of cetaceans both in zoological settings and, ultimately, their wild counterparts.
SUNDAY OCTOBER 30

SESSION 2: Hsps AND PROTEOSTASIS

SPEAKERS

Yatrik Shah
Kazue Hashimoto-Torii
Brian C. Freeman
Yihong Ye

POSTER

Abbey D. Zuehlke
Session 2: Hsps and Proteostasis

Type of Presentation: Oral

**Interfering with tumor proteostasis as a therapeutic target in colon cancer.**

Yatrik M Shah

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Highly proliferative tumors are energetically stressed and require altered metabolism, scavenging mechanisms, autophagy and proteasomal protein degradation for key metabolic substrates to maintain and sustain growth. We demonstrate that porphyrin-based molecules induce a robust growth inhibition in cell lines, mouse models and patient-derived enteroid models of colon cancer. Porphyrin drugs showed marked in vivo selectivity for tumor cells, compared to normal cells. To understand the mechanism of tumor killing a quantitative proteomic approach was assessed in a panel of cancer-derived cell lines. Unexpectedly we show that intracellular porphyrins can cross-link proteins, leading to formation of high-molecular weight oligomers of proteins. Porphyrin drugs induce tumor-specific apoptosis, in part due to impaired clearance of high molecular weight oligomerized protein. We show that porphyrin mediated re-routing of autophagy and proteasomal pathways to promote clearance of oligomers results in nutrient deprivation of cancer cells. Also the failure of cancer cells to clear the protein oligomers results in persistent accumulation of toxic protein oligomers. Thus, the compromised cellular function both energetically and in the clearance of protein oligomers results in tumor-specific increase in apoptotic cell death. We clearly demonstrate the potential anticancer approach of modulating tumor proteostasis.
Session 2: Hsps and Proteostasis

Type of Presentation: Oral

**Cell-to-cell variability in HSF1 activation and neurodevelopmental disorders**

Kazue Hashimoto-Torii

Center for Neuroscience Research, Children's National Medical Center, Washington, DC, USA; Department of Neurobiology and Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, CT, USA; Department of Pediatrics, Pharmacology and Physiology, School of Medicine and Health Sciences, George Washington University, Washington, DC, USA; KHTorii@childrensnational.org

Repetitive exposure to identical or similar doses of harmful agents results in highly variable and unpredictable negative effects on fetal brain development ranging in severity from high to little or none. However, the mechanisms underlying such variability remain unclear. We report that the activation of heat shock factor 1 (Hsf1) upon exposure to harmful chemicals similarly shows highly variable and stochastic nature in mouse and human embryonic brain tissues. While Hsf1 is essential for maintaining cellular homeostasis to protect the embryonic brain from environmental stress, we found that excessive activation of Hsf1 impairs critical neurodevelopmental events such as neuronal migration. These results suggest that the mosaic activation of Hsf1 in the embryonic brain contributes to the generation of phenotypic variations in complex congenital brain disorders elicited by prenatal environmental stress. We also report the epigenetic changes, which sustain for long time after the stress exposure, in the brain according to the differential levels of earlier Hsf1 activation.
Molecular chaperones govern protein homeostasis being allied to the beginning (folding) and ending (degradation) of the protein life cycle. Yet, the Hsp90 system primarily associates with native factors including fully assembled complexes. The significance of these connections is poorly understood. To delineate why Hsp90 and its cochaperone p23 interact with a mature structure we focused on the RSC chromatin remodeler. Both Hsp90 and p23 triggered the release of RSC from DNA or a nucleosome. While Hsp90 only freed bound RSC, p23 enhanced nucleosome remodeling prior to discharging the complex. In vivo, RSC mobility and remodeling function were chaperone-dependent. Our results suggest Hsp90 and p23 contribute to proteostasis by chaperoning mature factors through energetically unfavorable events thereby maintaining the cellular pool of active native proteins. In the case of RSC, p23 and Hsp90 promote a dynamic action allowing a limited number of remodelers to effectively maintain chromatin in a pliable state.
A HECT domain ubiquitin ligase targets unassembled soluble proteins for degradation in the cytoplasm

Yue Xu¹, D. Eric Anderson², and Yihong Ye¹*

¹Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; yihongy@niddk.nih.gov
²Advanced Mass Spectrometry Core Facility, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

Most eukaryotic proteins function in multi-subunit complexes that require proper assembly. To maintain complex stoichiometry, cells use the endoplasmic reticulum-associated degradation (ERAD) system to degrade unassembled membrane subunits, but how unassembled soluble proteins are eliminated is not well defined. Here we show that degradation of unassembled soluble proteins (referred to as Unassembled Soluble Protein Degradation or USPD) requires the ubiquitin selective chaperone p97, its cofactor Npl4, and the proteasome. At the ubiquitin ligase level, the previously identified protein quality control (PQC) ligase UBR1 and the related enzymes only process a subset of USPD substrates. Instead, we identify the HECT (homologous to the E6AP carboxyl terminus) domain-containing enzyme HUWE1 as a new ubiquitin ligase for substrates bearing unshielded, hydrophobic residue-containing segments. We used a SILAC-based proteomic approach to identify endogenous HUWE1 substrates. Interestingly, most HUWE1 substrates form multi-protein complexes that function in the nucleus although HUWE1 itself is cytoplasmically localized. Inhibition of nuclear entry enhances HUWE1-mediated ubiquitination and degradation, suggesting that USPD occurs in the cytoplasm. Altogether, these findings establish a new facet of the cytosolic PQC network that removes surplus subunits to control protein homeostasis and nuclear complex assembly.
Heat shock protein 90 (Hsp90) is an essential eukaryotic molecular chaperone required for the folding and activation of proteins, termed clients. In order for Hsp90 to properly chaperone its clientele, it proceeds through an ATP-influenced cycle assisted by helper co-chaperone proteins. Although Hsp90 ATP-induced conformational changes have been well studied, the mechanism of client interaction and release is not. Hch1 is a co-chaperone in Saccharomyces cerevisiae, which is not conserved in mammalian cells. Although Hch1 shares similar amino acid sequence identity with a portion of the co-chaperone Aha1, they perform different cellular functions. We show Hch1 exacerbates the growth defect caused by the client interaction mutant Hsp82-W585T. The presence of Hch1 also reduces client function and stability. Higher eukaryotes express the Yes kinase, which is needed for the phosphorylation of position Y627 in human Hsp90. This phosphorylation results in loss of client and co-chaperone interaction. Our data demonstrate that the phosphorylation site Y627 in human Hsp90 may serve as an evolutionary substitution for HCH1.
SUNDAY OCTOBER 30

SESSION 3: EXTRACELLULAR HSPs

SPEAKERS

Jennifer Isaacs
Ayesha Murshid and Thiago J. Borges
Ed O'Brien
Michael W. Graner
Although Heat Shock Protein 90 (Hsp90) is an abundantly expressed intracellular chaperone, a relatively uncharacterized extracellular population is observed, particularly in tumor cells. To explore the function of this extracellular Hsp90 (eHsp90), we engineered cancer cells to constitutively secrete Hsp90. We demonstrate that eHsp90 is capable of disrupting epithelial polarity and facilitating a mesenchymal phenotype (EMT) in models of prostate and breast cancer. Remarkably, enforced eHsp90 expression initiates invasive tumor growth in vivo in a prostate cancer model, indicating that eHsp90 may be a clinically relevant trigger of indolent to aggressive disease. Further, eHsp90 alters the stromal microenvironment by converting normal fibroblasts to a phenotype resembling tumor-supportive cancer-associated fibroblasts (CAFs). Hence, eHsp90 appears to play a complex role in directing tumor-stromal interactions. Our most recent findings indicate that eHsp90 may be a novel facilitator of cancer stemness. Intriguingly, tumor cells with elevated surface Hsp90 exhibited a marked increase in stem-like targets, indicating that surface eHsp90 may enrich for a unique cancer stem cell (CSC) population. Taken together, our results highlight a paradigm whereby eHsp90 orchestrates myriad molecular events to promote tumor cell plasticity and to support tumor progression. Within this context, our findings complement work from other labs implicating eHsp90 as a facilitator of tissue repair and regeneration, and support a premise whereby cancer cells have hijacked this pro-plasticity function to evade chemotherapy.
Alzheimer's disease (AD) is pathologically characterized by neuritic plaques consisting of extracellular amyloid plaques (Aβ) and intracellular neurofibrillary tangles made of Tau protein which results in an inflammation, a critical pathological feature of AD, as well as other pathological effects. Our studies showed that microglia cells are capable of engulfing fibrillary Aβ peptide (fAβ) and that engulfment is increased in the presence Hsp90α in the growth medium (extracellular Hsp90α). We also observed an increased clearance of internalized fAβ by microglia, an effect which may involve activation of Nrf2 (NF-E2-related factor 2) mediated autophagy when eHsp90α is present (we observed strong activation of Nrf2 by eHsp90α in microglia). Here we also found eHsp90α to be capable of mitigating neuronal toxicity induced by fAβ activated microglia. We are now analyzing the differentially expressed genes in microglia cells in the presence of eHsp90α, by RNA next generation sequencing. These sequencing data and gene ontology analysis will provide a comprehensive representation of the microglial responses to eHsp90α treatment in the presence of fAβ. RNA sequencing analysis is aimed at confirming whether eHsp90α indeed decreases the Aβ-induced proinflammatory response, and importantly, this analysis may identify negative responses to eHsp90α treatment that would reduce its effectiveness in treatment of AD. Gene expression profiling analysis may further indicate the connection between eHsp90α, Nrf2 and an antiinflammatory response in microglia cells exposed to Aβ. Furthermore, this approach may allow the potential identification of novel biological functions of eHsp90α in AD mice.
Extracellular Hsp70s interact with Siglec-E/LOX-1 complexes and trigger differential immune responses

Thiago J. Borges*¹²#, Ayesha Murshid¹#, Cristina Bonorino², Stuart K. Calderwood¹

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# Co-first author

Heat shock proteins (Hsps) are conventionally thought of as intracellular and exert chaperone functions. However, these proteins can also be released to the extracellular milieu and play roles in immunological responses. Historically, the immunological properties of Hsp70 family members have been demonstrated to be ambiguous for still unknown reasons. For example, mouse Hsp72 can trigger inflammatory responses, while its counterpart - mycobacterial DnaK – has potent anti-inflammatory properties. Scavenger receptor LOX-1 was originally found to bind modified LDL (oxidized, acetylated). Later, it was demonstrated that it is a mouse and human Hsp70 receptor. Here, we have described for the first time that DnaK can also interact with LOX-1. Siglec-E is a potent inhibitory receptor which binds sialic acid domains and is expressed by mouse macrophages and dendritic cells (DCs). Here, we report that mouse Hsp70 and DnaK can each bind and be internalized by a receptor complex formed by LOX-1 and Siglec-E which is present in plasma membrane lipid microdomains. Mouse Hsp70 had a higher affinity to LOX-1 and triggered an inflammatory profile on DCs which was LOX-1-dependent. DnaK, however, presented a higher affinity to Siglec-E and had immune modulatory effects on DCs. In conclusion therefore, different Hsp70 family members can bind to a similar combination of innate receptors and induce opposite immune responses dependent on precise interactions with individual receptors.
Session 3: Extracellular HSPs

Type of Presentation: Oral

**Extracellular Role of HSP27 & anti-HSP27 Autoantibodies in Atherogenesis**

Ed O’Brien

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**Background:** The development of atherosclerosis is a highly inflammatory process that can be attenuated when Heat Shock Protein 27 (HSP27) levels are augmented (e.g., via over-expression, bone marrow transplantation or administration of recombinant HSP27). Recently we noted that HSP27 lowers both plaque and serum cholesterol levels, as well as mediators of inflammation. Moreover, in a small patient cohort serum (auto) antibodies (AAbs) to HSP27 are higher in healthy controls (HC) compared to patients with CAD. Interestingly, these AAbs obfuscate HSP27 ELISA measurements and prompted our laboratory to develop a novel mass spectrometry (MS) quantification method for the (true) quantification of Hsp27 serum levels.

**Purpose:**

i) Determine if elevated serum HSP27 (as measured by MS) and anti-HSP27 AAbs levels correlate with the freedom from CAD.

ii) Explore the mechanisms by which these AAbs facilitate HSP27 signaling.

iii) Determine if (and how) augmentation of AAbs in vivo reduce atherogenesis.

**Methods/Results:**

i) Quantification of serum HSP27 (using MS) and AAb levels were assessed in 242 patients with CAD or 92 subjects without (HC). Matching for sex and age, CAD patients had lower HSP27 and anti-HSP27 levels compared to HC. These results parallel our previous report on the predictive value of ELISA measurements of HSP27 levels but are two orders of magnitude higher.

ii) *In vitro*, AAbs promoted the anti-atherogenic effect of HSP27 in macrophages by enhancing NF-kB activation via TLR-4, resulting in increased secretion of anti-inflammatory interleukin-10 (IL-10) and altering key cholesterol metabolism regulatory pathways.

iii) To determine the *in vivo* role of AAbs in experimental atherogenesis, atherosclerosis-prone apoE−/− mice were fed a high fat diet and immunized using rHSP27 plus an alum adjuvant. This treatment protocol resulted in an increase in AAbs and a marked reduction in atherosclerotic plaque burden as well as serum cholesterol levels.

**Conclusions:** HSP27 autoantibodies are elevated in health compared to CAD, and appear to play an important role in augmenting the bioavailability and/or signaling function of HSP27 in the context of inflammation and atherosclerosis. Moreover, immunization to augment AAbs (and HSP27) represents a novel therapeutic strategy for addressing not only the inflammatory component of atherosclerosis, but also lowering cholesterol levels.
Session 3: Extracellular HSPs

Type of Presentation: Oral Presentation

**Stressed exosomes (“sexosomes”): stress balls or care packages in passaging stress phenotypes to recipient cells?**

Michael W Graner*, Jasmina Redzic, Thomas J Anchordoquy

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Cancer cells undergo a number of stresses, many of them self-inflicted, but often do not appear to suffer the consequences of those stresses. In some cases, the stress responses may actually prove beneficial to the tumor cells, providing them with potent resilience to their less-than-hospitable environments. Consistent tumor stresses include the Heat Shock Response and the Unfolded Protein Response (UPR), which combined encompass cytoplasmic, nuclear, and endoplasmic reticulum organellar activities in response to a wide variety of stressors. These stress-management systems may be incorporated by tumors into their stress portfolios to survive or even thrive amidst their environmental insults. We propose that exosomes from stressed cells (stressed exosomes, or “sexosomes”) are able to induce stress response phenotypes in recipient, unstressed cells, thus enabling stress responses without having to experience the actual stress. Our analyses in this report go to molecular levels, monitoring proteome changes in glioma cells when those cells are exposed to exosomes released from UPR-stressed cells. We find high overlap in the proteomes of stressed cells and unstressed cells that receive “sexosomes”, suggesting that tumors may unify their overall stress responses despite their inherent heterogeneity. The implications for general tumor biology, and in particular, therapeutic resistance, are highlighted.
SUNDAY OCTOBER 30 POSTER SESSION

TARGETING HSPs - NEW DRUGS

SPEAKERS

Jill Akkerman
Heather D. Durham
Shelli R. McAlpine
Michael Moses
Aaron Petersen
Pia Roos-Mattjus
Mohammad Shahid
Ben Lang
Mauricio Menegatti Rigo
Breast cancer is one of the leading causes of death among women and thus, finding new and effective treatments continues to be a high priority. Heat shock protein 90 (Hsp90) is a member of the stress protein family that is responsible for chaperoning proteins involved in cell signaling, proliferation and survival. Geldanamycin or 17-allylamino-17-demethoxygeldanamycin (17-AAG) represents a class of drugs capable of binding and disrupting the function of Hsp90, leading to the depletion of multiple client proteins resulting in cell cycle arrest and death. However, wide effects of 17-AAG have seen amongst cell lines and tumor types. Other inducible stress proteins, namely Hsp70, are found to be highly expressed in numerous cancers and have been linked to cell proliferation and resistance to chemotherapeutic agents. Given the protective effects of Hsp70 and elevated levels in cancer, the objective of this study was to determine if breast cancer cells expressing high levels of Hsp70 respond differently to the toxic effects of 17-AAG. Human MCF-7 breast cancer cell lines that have been stably transfected with the inducible Hsp70 gene under the control of doxycycline (On vs. Off) were used for the studies. Hsp70 On (overexpressing) and Off (control) cells were treated with 0, 50 and 100 nM of 17-AAG and several end-points were evaluated. Flow cytometry was used to determine cell cycle analysis. Cell counts were used to assess viability and Western analysis was performed to determine expression of Hsp90, Hsp70 and the oncogenic protein, AKT. Flow cytometry indicated G2/M cell cycle arrest in cells expressing normal levels of Hsp70 following 17-AAG treatment. However, no arrest was observed in cell overexpressing Hsp70. Results from cell viability studies demonstrate that Hsp70 overexpressing cells had significantly higher cell counts following treatment than cells expressing normal levels of Hsp70. Hsp70 and 90 protein levels were significantly induced in cells expressing control levels of Hsp70 in response to 17-AAG. In contrast, Hsp70 overexpressing cells showed only slight induction of Hsps. As predicted, AKT protein levels were reduced by treatment with 17-AAG. However, cells expressing elevated Hsp70 showed less degradation than controls. Taken together, these data suggest that tumors expressing high levels of Hsp70 may not respond as effectively to the effects of 17-AAG, thus, providing a rationale for targeting Hsp70 along with Hsp90 for maximal therapeutic response. Further studies will be aimed at elucidating the mechanisms of this resistant effect.
Combining epigenetic and HSP-inducing drugs to maintain neuronal gene expression and protein quality control in neurodegenerative disorders

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Introduction: This project builds on work showing that aberrant chromatin remodeling is a feature of neurodegenerative disorders including amyotrophic laterals sclerosis (ALS), repressing gene expression crucial for maintaining neuronal processes and cellular defence. Histone acetylation and the chromatin landscape also influence expression of stress-response pathways including heat shock genes. Deficiency in protein quality control is associated with ALS, and motor neurons have a high threshold for inducing expression of HSPs to chaperone misfolded proteins. Neurons are relatively resistant to HSP-inducing drugs, and even when effective (e.g., HSP90 inhibitors), the drug can lose efficacy through transcriptional repression. Histone acetylation regulates gene expression and activity of chromatin remodeling complexes, including neuron-specific complexes (nBAF) important for extension, maintenance and remodeling of dendrites. H3K9/14 acetylation was reduced and nuclear nBAF complexes were lost in cultured motor neurons expressing mutant proteins linked to familial ALS (FUS and TDP-43). The histone deacetylase (HDAC) inhibitor, SAHA (Vorinostat), maintained histone acetylation and dendritic architecture. SAHA also greatly enhanced stress-inducible Hsp70 (HSPA1A) in neurons and glia induced by the Hsp90 inhibitor, NXD30001, at concentrations below those effective alone. SAHA is a pan class I and II HDAC inhibitor and thus there is toxicity with long term use.

Purpose: In this study, more class-specific HDAC inhibitors were tested in the ALS culture models to search for CNS-permeant agents that promote HDAC acetylation, improve a measure of neuroprotection against ALS-linked mutant proteins and potentiate an HSP-inducing drug.

Methods: Dissociated murine spinal cord cultures were exposed to inhibitors of different HDACs or an Hsp90 inhibitor that constitutively induces HSPs (NXD30001), either alone or in combinations. HDAC inhibitors tested: SAHA (pan HDAC inhibitor as the positive control), RGFP109 (HDAC1 and 3), RGFP966 (HDAC3), CI994 (HDAC1) and Tubastatin A and ACY738 (HDAC6). Whole cultures were assessed by Western blotting and specific effects on motor neurons and other cell types by immunocytochemistry, using antibodies to acetylated histones (H3K9/K14), acetylated and total tubulin, Hsp70 and GAPDH.

Results: NXD30001 induced Hsp70 expression preferentially in neurons, but to some extent in background cells including astrocytes. SAHA was the most potent in increasing NXD30001-induced Hsp70 expression (both level and percentage of neurons expressing). RGFP109 and to some extent RGFP966 had a similar, although less robust effect. Tubastatin A potentiated NXD30001 particularly in motor neurons, but minimally in background cells. HDAC inhibitors alone at these concentrations did not induce Hsp70. RGFP109 also exhibited a profile similar to
SAHA in increasing histone 3 acetylation and nuclear retention of FUS in a culture model of ALS6 (expression of mutant FUS in cultured motor neurons), whereas Tubastatin A did not preserve mutant FUS in the nucleus.

Conclusion: New, more specific HDAC inhibitors are being developed for cancer therapy, including CNS penetrant drugs with the renewed interest in applying this approach to treat neurological disorders. As in cancer, combination therapies will likely be required to have significant impact on the disease. The data are encouraging to pursue testing of HDAC inhibitors in combination with drugs to boost expression of endogenous protective pathways including HSPs.
Molecules that bind directly to the MEEVD region on Hsp90 and block co-chaperone interactions

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Small molecules were designed from the TPR2A domain of Heat shock organizing protein (HOP) to bind directly to the C-terminus MEEVD region of Hsp90. We identified a sequence specific molecule that was optimized to bind to the MEEVD region, where these are the first small molecules reported to bind directly to Hsp90’s MEEVD region. Described are the design, structure-activity relationships, and biological outcomes produced when you bind directly to Hsp90’s MEEVD domain and inhibit the binding events of TPR-containing co-chaperones. We also discuss their mechanism by which they form binding interactions with the MEEVD region, and prodrug strategies to ensure these molecules effectively enter cells.
Targeting the HSP40/HSP70 axis as a novel strategy to treat castration-resistant prostate cancer

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Castration-resistant prostate cancer (CRPC) is frequently characterized by elevated expression of nuclear receptors able to at least partially maintain the androgen receptor (AR) transcriptional program. Elevated expression of a number of constitutively active AR splice variants lacking the ligand binding domain (LBD) (e.g., ARv7, which is ligand-independent and correlates with poor prognosis, reduced survival, and resistance to existing LBD-targeted standard of care therapy) is a frequent occurrence in CRPC. Thus, alternative approaches to disrupt AR signaling in CRPC are of great clinical importance, and a single strategy able to target AR and ARv7 remains a critical unmet need.

As a steroid hormone nuclear receptor, the AR exists in an interactive and dynamic cycle with the molecular chaperones (heat shock proteins, HSPs) HSP40/HSP70/HSP90 for proper folding and remodeling of the AR LBD to bind ligand. Notably, HSP90 inhibitors promote AR degradation and display efficacy in prostate cancer xenograft models. Although it has been shown that ARv7 functions independently of HSP90, additional chaperone requirements of LBD-deficient ARv7 are not known. Thus, we tested the hypothesis that both AR and ARv7 are dependent on HSP40/HSP70 and that targeting these chaperones with specific inhibitors (C86 and JG98, respectively) will lead to AR/ARv7 destabilization and loss of transcriptional activity in models of CRPC.

To determine if AR proteins associate with HSP40/HSP70, 22Rv1 CRPC cells (expressing endogenous AR and ARv7) were first transfected with FLAG-HSP40 or FLAG-HSP70. Immunoprecipitation with FLAG beads revealed AR and ARv7 associated with both chaperones, indicating potential functional dependence of these nuclear receptors on HSP40/HSP70. To further characterize these interactions, 22Rv1 lysate was probed with biotinylated-C86 and subjected to IP with streptavidin beads. C86 bound a significant fraction of HSP40 complexed with HSP70, AR, and ARv7. Excess unlabeled C86 or JG98 effectively competed away binding of HSP40/HSP70 to biotinylated-C86 with concomitant loss of associated AR and ARv7.

Treatment of 22Rv1 cells with C86 or JG98 led to a time and dose-dependent decrease in AR and ARv7 protein, concomitant with a significant loss of viability. We also observed that HSP40/HSP70 inhibition markedly reduced AR and ARv7 transcriptional activity, as indicated by decreased AR (PSA, TMPRSS2) and ARv7 (UBE2C) target gene expression. Finally,
treatment of mice bearing 22Rv1 xenografts with JG231 (an analog of JG98 with enhanced PK properties) led to significantly smaller tumors relative to vehicle treated mice. Together, these data confirm the continued dependence of AR and ARv7 on HSP40/HSP70 molecular chaperones and they demonstrate the feasibility of targeting the HSP40/HSP70 axis to abrogate sustained AR-mediated signaling in CRPC.
The small molecule BGP-15 alleviates chemotherapy-induced skeletal muscle impairments

Chemotherapy is an effective and commonly used treatment for many cancers. Unfortunately, chemotherapy causes debilitating side effects to numerous organs, including skeletal muscle, greatly impacting on overall health, quality of life, and mortality. We tested whether the small molecule BGP-15, could alleviate oxaliplatin-induced muscle impairments.

Balb/c mice were treated with vehicle (VEH, 0.1% DMSO), oxaliplatin (OXA, 3mg/kg) and/or BGP-15 (15mg/kg) thrice weekly for 2 weeks. Thereafter, mice were anaesthetised and the flexor digitorum brevis (FDB) and tibialis anterior (TA) muscles removed. FDB fibres were isolated and used to assess fibre diameter. Muscle mass, histology, protein content, and signalling pathways controlling protein synthesis and breakdown were assessed in TA.

OXA induced a ~25% reduction in FDB muscle fibre diameter, but no change to TA mass. OXA had no effect on TA protein concentration, however collagen, lipid and calcium staining intensity were increased by 33%, 195%, and 19% respectively, indicating impaired muscle quality. Markers of ubiquitin proteasome mediated proteolysis, Murf1 and protein ubiquitination were not affected by OXA treatment. p70S6K and rpS6, key kinases involved in protein synthesis, were decreased in OXA compared to VEH. BGP-15 co-treatment reduced TA mass by 8% compared to VEH, however it had no effect on protein content. Impairments in muscle quality were completely prevented by BGP-15. BGP-15 rescued the OXA-induced decrease in p70S6K and rpS6 but had no effect on markers of proteolysis.

These findings suggest that BGP-15 can prevent deleterious skeletal muscle side effects caused by oxaliplatin.
Structural in silico analysis of post-translational modifications present on DnaK protein

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Molecular chaperones known as Heat Shock Proteins (HSPs) are key proteins involved in a wide range of functionalities such as protein synthesis, folding, disaggregation, and degradation. One of the first chaperones to be described was the Hsp70 protein. The Hsp70 has an important role assisting substrate folding and regulating the activities of other proteins. It has been shown that post-translational modifications (PTMs) are often present in Hsp70 of several organisms, which can influence its behavior in respect to the environment and the interaction with molecular partners. Until now, a wide range of PTMs were already described for these chaperones, such as acetylation, phosphorylation, glycosilation, sumoylation and others. In this way, a PTM works as a key mechanism to add variability to what is generated from genomic information. Data on the extracellular effects of heat shock proteins are still controversial. We hypothesized that at least some of the inconsistencies observed in different studies on extracellular functions of Hsp70 could be due to PTMs. Here, we aimed to model DnaK protein, the Hsp70 chaperone from Mycobacterium tuberculosis (UniProt Entry: P9WMJ9), and analyze a set of PTMs present on the protein surface through an in silico approach. We produced DnaK protein in two different expression systems (E. coli and P. pastoris), retrieving the data from common PTMs (acetylation and phosphorylation) through mass spectrometry analysis. Since the 3D structure of DnaK was not resolved, we performed a homology modeling of the protein using Modeller software. The C-terminal region of the protein, also known as “lid” region, was modeled using an ab initio approach using the Robetta server. The final structure was energy minimized with GROMACS and validated through several programs (Verify 3D, ERRAT, ModEval and ProQ). The final model was modified with the information of PTMs using PyTMs plugin from PyMol program. The models with or without PTMs were qualitatively compared regarding topography and electrostatic potential. The PTMs occurred on different residues such as lysine, tyrosine, serine and threonine. These residues were mainly exposed on the protein surface and the electrostatic map shows important charge differences in the PTMs added to the protein when produced in different expression systems. The introduction of acetylation promoted a more negative state of the DnaK, and this negative effect was more pronounced on P. pastoris expression system. It is interesting to note that some of the PTMs were conserved between both E. coli and P. pastoris expression systems, mainly on the nucleotide (NBD) and substrate binding domains (SBD), suggesting a possible conserved role for these PTMs for proper DnaK function. Overall, each PTM could influence the dynamics of the protein, as well as the interaction affinity with other molecules, affecting extracellular properties.
Structural modeling of Hsp70-binding protein 1 (HspBP1) N-terminal region from murine and humans

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Heat shock proteins (HSPs) are important molecular chaperones that perform a series of intracellular and extracellular functions related to protein folding, signaling, and transport. One of the first HSPs to be described was the Hsp70. In order to perform most of its functions, Hsp70 is often assisted by other proteins, known as co-chaperones. One important co-chaperone first described in 1998 that had been seen interacting with Hsp70 is the Hsp70-binding protein 1 (HspBP1). The HspBP1 is a protein with 362 amino acids and approximately 40 kDa. Recently, our group evaluated the HspBP1 levels on primary breast cancer patients and showed that (i) the HspBP1 level in sera was significantly higher compared to healthy patients and that (ii) these levels were higher in tumor tissue compared to normal adjacent tissue. Also, low expression of HspBP1 was associated with poor prognosis. These data were in agreement with previous work from other groups in murine tumors models. We hypothesized that structural features of the HspBP1 protein are important to modulate the tumoral response, influencing protein dynamics and its capability to interact with other proteins. Since the HspBP1 protein from humans and murines was not fully resolved through crystallography, we aimed to model the unsolved part (N-terminal region) using an ab initio approach. First, the sequences were obtained from UniProt (Entries Q9NZL4, for human, and Q99P31, for murine). We then used ROBETTA server to model the proteins, which returned five models with the best score over all the sampled conformations. The best model was chosen based on validation programs as follows: ModFold, QMEAN, ANOLEA, Ramachandran plot, Verify3D and ERRAT. The models from murine (mHspBP1) and human (hHspBP1) were very similar considering the structural aspect. Most of the differences between human and mouse protein were found between the residues 1 to 84, due to formation of loops as main secondary structure. Loops normally present more flexibility, hence, more variability, which is in accordance to sequence predictors that shown that this region is highly disordered and hydrophilic. The best model of hHspBP1 was also submitted to a short molecular dynamics in silico study to evaluate the behavior of this region. The generation of reliable structure protein models is an important step to look into the protein function and, subsequently, provide insights about its interaction with other molecules.
A small molecular compound modulating Heat Shock Factor activity

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Cellular stress responses enable cells to sense and respond to changes in the cellular environment. The heat shock response to various proteotoxic stresses is regulated by a family of heat shock transcription factors (HSF1-4), with HSF1 being the master regulator. HSFs are also implicated in development, aging and in many pathophysiological conditions including cancer and neurodegenerative diseases. Therefore, there is significant interest in the discovery and development of small molecules that modulate the activity of HSFs. We have studied a natural product used in Chinese medicine. This compound induces a stress response in a HSF1-dependent manner. Pretreatment of the compound with excess free thiols inhibits the response, suggesting that the compound is thiol-responsive. We will present our work in progress at the meeting.
Epigenetic changes associated with prenatal activation of heat shock signaling as novel therapeutic targets for cognitive deficits in Fetal Alcohol Spectrum Disorder

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We have recently reported that acute activation of heat shock signaling protects the embryonic brain against immediate cellular damages elicited by prenatal alcohol exposure, using the mouse model of Fetal Alcohol Spectrum Disorder (FASD). However, the long-term consequence of prenatal alcohol exposure in these protected cells remains unknown. To define epigenetic changes linked to prenatal activation of heat shock signaling in the FASD mice, we developed a novel reporter system that enables lineage tracking of the brain cells in which the heat shock signaling had been activated. Using this reporter system, we identified differentially expressed genes (DEGs) between reporter-positive and -negative neurons in the adult brain. These DEGs include the genes whose functions are critically involved in the long-term potentiation of neurons, suggesting that the epigenetic changes linked to the prenatal activation of heat shock signaling contribute to cognitive impairments in FASD. Remarkably, pharmacological treatment to antagonize one of such epigenetic changes significantly improved cognitive impairments in FASD mice. These results indicate that treatment of key epigenetic changes elicited by heat shock activation may become a new therapeutic approach for the cognitive impairments in patients with FASD.
MONDAY OCTOBER 31

PLENARY LECTURE

Michael Sherman
Type of Presentation: Plenary Lecture

**Role of Hsp70 in tumor initiation and progression**

Michael Sherman

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The heat shock protein Hsp70 has emerged as a major player in cancer and potential drug target. Indeed, knockout of the Hsp70 gene severely delayed tumor emergence in animal models, and thus established that Hsp70 plays a critical role in cancer development. Series of genetic studies established that Hsp70 is required for tumorigenesis at several steps, including tumor initiation and metastasis. These findings were corroborated by retrospective studies of human patients, showing close correlation between high levels of Hsp70 and poor prognosis of breast and other cancers. Based on these studies that established the role of Hsp70 in cancer, efforts have been undertaken to develop Hsp70 inhibitors for cancer treatment. To rationally target Hsp70, it is essential to dissect specific mechanisms by which Hsp70 promotes cancer in order to focus drug development strategy on cancer-relevant activities of the Hsp70 molecule avoiding potential side effects.

Using genetic methods, we established that specific effects of Hsp70 on cancer result from its direct interaction with a co-chaperone Bag3. This novel Hsp70-Bag3 module controls multiple signaling pathways that regulate cancer and normal cell physiology. Our collaborator Dr. Gestwicki (UCSF) identified a molecular scaffold that inhibits the Hsp70-Bag3 interaction, and these molecules mimic effects of Hsp70 depletion on signaling and tumor development. Currently, based on genetic and computational analysis we are rationally designing potent and safe combinations of inhibitors of Hsp70 with other anti-cancer drugs.
MONDAY OCTOBER 31

SESSION 4: STRESS, METABOLISM AND IMMUNOSUPPRESSION

SPEAKERS

Elizabeth Repasky
Connie J. Rogers
Caroline Le Poole
Sharon S. Evans
Recent work from our laboratory has shown that mild cold stress caused by standard housing temperatures (ST; 22°C) is sufficient to significantly accelerate the growth rate and metastasis of tumors in murine models while also suppressing their responsiveness to chemotherapy. These effects could be reversed by housing mice at warmer thermoneutral temperatures (TT; 30°C) [1-3]. Thermal stress is mediated by activation of the sympathetic nervous system and the release of norepinephrine (NE), which can be highly suppressive when signaling through β-adrenergic receptors (β-ARs) on immune cells and pro-tumorigenic when acting on tumor cells. We found that NE levels are significantly elevated in tumor-bearing mice housed at ST compared to TT, which led us to hypothesize that chronic stress induced by cool housing temperatures increases β-AR signaling that dampens the anti-tumor immune response and the efficacy of both immune modulating and radiation therapies. To explore this hypothesis, we used both physiologic (housing temperature; ST and TT) and pharmacologic blockade (using β-blockers) to modulate β-AR signaling levels in immune-competent and SCID mice bearing 4T1 or B16-OVA tumors. We found that the addition of β-blockade significantly delayed 4T1 and B16-OVA tumor growth in mice housed at ST, recapitulating the slower tumor growth observed in mice housed at TT. However, β-blockade had no impact on tumor growth in SCID mice at ST or TT indicating dependence on the adaptive immune system. Analysis of 4T1 and B16-OVA tumors from immune-competent mice showed increased IFN-γ expression in both CD4+ and CD8+ T cells in mice treated with β-blockade indicating a more robust anti-tumor immune response. Lastly, we investigated the impact of β-AR signaling on anti-PD-1 checkpoint blockade efficacy and found that reducing β-AR signaling by both physiologic (TT) and pharmacologic (β-blockade) strategies improved responses in both tumor models. Further analysis of 4T1 tumors from mice treated with β-blockade and anti-PD-1 showed an increase in IFN-γ producing CD8+ T cells compared to either β-blockade or anti-PD-1 alone. In other new data, we have found that increased adrenergic stress signaling significantly reduces the efficacy of radiation therapy in murine tumor models which appears to also be mediated by suppressing anti-tumor immunity while increasing the intrinsic radioresistance of tumor cells.

To conclude, these data indicate that elevated β-AR stress signaling caused by increased adrenergic receptor signaling (e.g., as induced by even mildly cool housing temperatures) impairs anti-tumor immunity and the response of tumors to anti-PD-1 checkpoint blockade and radiation therapies. These data also suggest a new strategy by which β-blockers could be used in combination with checkpoint inhibitors and radiation to improve clinical responsiveness in patients.
References
4T1.2 tumor-bearing mice that remain in energy balance via diet and exercise have reduced tumor growth and metastases, in conjunction with better anti-tumor responses

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Changes in energy balance (e.g., energy restriction, physical activity and obesity) can impact breast cancer risk, recurrence and survival. Changes in metabolic, inflammatory, and immune mediators are possible factors underlying this relationship. We have previously demonstrated that moderate exercise achieved via access to running wheels and mild energy (10% kcal restriction) can enhance antigen-specific T cell responses in tumor free-mice. Thus, the goal of the current study was to determine if preventing weight gain (via exercise and diet) could delay primary tumor growth and metastatic progression in tumor-bearing mice, and determine if there were any additive effects of weight maintenance and the dual administration of an allogeneic whole tumor cell vaccine on the aforementioned outcomes. Female BALB/c mice were randomized to sedentary, ad libitum fed, weight gain (WG) or exercising, energy restricted (10%), weight maintenance (WM) groups (n=20-24/group). After 8 weeks, all mice were orthotopically injected with 5x10^4 luciferase-transfected 4T1.2 cells into the fourth mammary fat pad and continued on their intervention for 35 days. After injection, mice were further randomized into vaccination (n=9-12/group) or vehicle control (n=11-12/group) groups and administered irradiated 4T1.2 cells (VAX) or vehicle (VEH) control at day 7, 14, 21, and 28 post-tumor injection.

All WM groups weighed significantly less than WG groups over the course of the study (p<0.0001). There was a significant effect of both WM and VAX alone on primary tumor growth (p<0.0001) and splenic IFN-gamma production (p=0.010); and an additive effect of WM+VAX on primary tumor growth (p<0.05), metastatic burden in lung (p=0.0267) and heart (p=0.0492); and the accumulation of splenic, pro-tumorigenic myeloid derived suppressor cells (MDSCs) (p=0.021). These results demonstrate that preventing weight gain via voluntary running wheel activity and mild dietary restriction is highly effective at delaying primary tumor growth and metastases, enhancing anti-tumor responses, and reducing splenic MDSC levels in this metastatic model. Furthermore, preventing weight gain via exercise and diet enhances the effectiveness of an allogeneic whole tumor cell vaccine. We conclude that lifestyle interventions that include diet and exercise may yield significant benefit in secondary prevention and may yield an additive benefit in combination with emerging immunotherapy treatment strategies.
Stress to the skin can serve as a precipitating factor for the autoimmune disease vitiligo. Disease development is accompanied by overexpression of HSP70A1A or inducible HSP70 (HSP70i). The heat shock protein partially co-localizes to melanosomes and can be secreted by melanocytes under stress. Indeed in T cell receptor transgenic mice that serve as a model of the disease, overexpression of HSP70i was sufficient to establish depigmentation. Moreover, we were not able to induce vitiligo in mice knockout for inducible HSP70. To block HSP70i from activating autoimmunity, we newly identified a peptide within the human molecule that is necessary for activation of human DCs by introducing modifications using site-directed mutagenesis. Specifically, we identified the HSP70iQ435A mutation, which affects the charge on a peripheral moiety protruding from the naturally folded molecule, within the substrate binding domain. The resulting molecule proved incapable of initiating an immune response towards melanocytes. Remarkably, mice prone to develop vitiligo were protected from depigmentation by HSP70iQ435A not only in prophylactic, but also in a therapeutic setting. HSP70iQ435A treatment tolerized DCs, thereby preventing skin infiltration by T cells and supporting melanocyte maintenance. These findings first suggested the therapeutic potential of HSP70iQ435A that can override the HSP70i present in the extracellular milieu, thereby essentially preventing DC activation and T cell recruitment to the site of stress. As the physiology of mouse skin is quite different from that of humans, we next wanted to test our DNA-based treatment in Sinclair swine, which develop gradually regressing melanomas and concurrent, gradual depigmentation of the skin. This allows for monitoring of drug efficacy while following its effects on melanoma growth as well. Vitiligo lesions were perilesionally treated weekly for 4 weeks, using a jet injector to introduce the DNA. We monitored the expansion of treated and untreated lesions over a period of 6 months, and found significant repigmentation of treated lesions and a trend towards improvement of distant lesions as well. Our findings hold promise for the use of modified HSP70i to tolerize autoimmune reactivity in vitiligo.
Session 4: Stress, Immunity and Metabolism

Type of Presentation: Oral

Myeloid-derived suppressor cells provide novel mechanism of resistance at thermally-sensitive vascular checkpoints during cancer immunotherapy

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Cancer immunotherapy has emerged as a promising approach to achieve durable responses in primary and metastatic cancer patients that are refractory to standard chemotherapy and radiotherapy. However, evidence that only a subfraction of cancer patients benefit from immunotherapy raises important questions about the underlying mechanisms of resistance. We have previously identified vascular beds within lymph nodes and tumor sites as a thermally-sensitive checkpoint controlling the efficacy of adoptive T cell immunotherapy in preclinical murine tumor models. Specialized blood vessels within lymph nodes are the lynchpin governing expansion of cytotoxic CD8+ effector cells while the intrinsic adhesive properties of tumor vessels dictate whether these cytotoxic antitumor immune cells gain access to tumors which is a necessary step for contact-dependent lysis of malignant cellular targets. We found that vessels within lymph nodes and tumors can be converted to sites of high trafficking for CD8+ T cells using thermal therapy regimens spanning a wide temperature range; i.e., systemic thermal therapy in which core body temperature is elevated to 39.5°C for 6 hours, or radiofrequency ablation whereby local temperatures within tumor tissue are increased to 90°C for 1 minute. We determined that a unifying feature of thermally-sensitive tumor systems was the minimal expansion of myeloid-derived suppressor cells (MDSC). In contrast, preclinical tumor models with a high MDSC burden were refractory to adjuvant thermal therapy in the context of adoptive T cell transfer immunotherapy, as determined by impaired L-selectin-dependent trafficking of CD8+ T cells across lymph node vessels and the failure of tumor vessels to support E/P-selectin, CXCR3, and ICAM-1–dependent trafficking of cytotoxic CD8+ T cells. Taken together, these studies identify novel mechanisms of immune evasion executed by tumor-induced MDSC that subverts the immune-boosting activity of thermal therapy by preventing T cell access to lymph nodes as well as tumor targets within the complex tumor microenvironment.

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MONDAY OCTOBER 31

SESSION 5: MOLECULAR CHAPERONES AND CANCER

SPEAKERS

Len Neckers
Julia Yaglom
Jane Trepel
Irina Guzhova
Boris Margulis

POSTERS

Barbara Lipinska
Toshihiko Torigoe
Xiu-Hua Liu
Tomasz Wenta

Note: This session will finish at 3:30 instead of 3:00.
Session 5: Molecular Chaperones and Cancer

Type of Presentation: Oral

**Targeting the HSP40/HSP70 molecular chaperone axis as a novel treatment strategy for castrate-resistant prostate cancer**

Michael Moses, Yeong Sang Kim, Sunmin Lee, Sue Wickner, Jason Gestwicki, Jane Trepel and Len Neckers*

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Heat shock proteins HSP40, HSP70 and HSP90 are molecular chaperones required for conformational stabilization/activation of many nuclear receptors, including androgen receptor (AR) and glucocorticoid receptor (GR). Although AR antagonists (e.g., enzalutamide) and blockade of androgen synthesis (e.g., with abiraterone) initially block AR function and improve patient survival, these treatments almost invariably lead to emergence of castration-resistant prostate cancer (CRPC). CRPC is frequently characterized by elevated expression of AR splice variants (ARv) and/or alternative expression of GR. ARv lack the ligand binding domain, making these proteins androgen-independent and insensitive to anti-androgen therapy. Likewise, CRPC can upregulate GR to activate a cohort of AR-dependent genes and thus bypass the need for AR-driven transcription. While GR and AR depend on HSP40/HSP70/HSP90 chaperone machinery for activity, the chaperone requirements of ARv are not known. Because HSP90 interacts with the ligand binding domain, AR splice variants are insensitive to HSP90 inhibitors. We have examined whether ARv remain dependent on HSP40/HSP70 and we have explored the sensitivity of ARv and GR proteins to pharmacologic disruption of the HSP40/HSP70 axis.
Rational design of potent combinations of Hsp70 inhibitors with other anti-cancer drugs

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The heat shock protein Hsp70 has emerged as a major player in cancer and potential drug target. In collaboration with Dr. Jason Gestwicki (UCSF) our lab has developed a series of novel allosteric inhibitors of Hsp70 that mimic effects of Hsp70 depletion on cell signaling and show potent anti-cancer activity in several xenograft models. To facilitate the path to full preclinical and clinical studies, we have developed an approach to rationally design synergistic and safe combinations of our Hsp70 inhibitors with other anti-cancer drugs. This approach involves rapid screen for genes that sensitize and protect from our inhibitors, followed by pathway and gene expression analysis. Further computational analysis allows predicting which drugs can synergize with Hsp70 inhibitors. This approach was validated and identified several potent drug combinations.
Session 5: Molecular Chaperones, Cancer and Apoptosis

Type of Presentation: Oral

**Innovations in Hsp90 and Cancer Therapy**

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In this talk I will briefly review the history and current landscape of Hsp90 inhibitors in monotherapy and combination therapy clinical trials, and then introduce some innovative ways in which the unique biology of Hsp90 is being leveraged in patients for cancer chemoprevention and for pharmacodynamic monitoring of target engagement in immunotherapy trials in solid tumor patients.
Role of J domain-containing Hsp70 co-chaperones in its function in highly malignant glioma cells.

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One of major molecular chaperones, Hsp70, possesses a high protective activity and the increase of its content makes cancer cells more resistant to a variety of anti-tumor therapies; conversely, the induction of the chaperone release from cancer cells render the latter sensitive to cytotoxic lymphocytes. In both functions, pro- and anti-tumorogenic Hsp70 is assisted by its co-chaperones, Hdj1 and Hdj2, and we studied the role of the three proteins in C6 rat glioblastoma by reducing their content by using lentiviral infection of the appropriate shRNA. It was shown that knock-down of Hsp70 caused significant reduction of tumorigenicity in both in vitro and in vivo simulations. Moreover, the activity of cytotoxic lymphocytes towards Hsp70- cells was also diminished probably due to the weakening of the chaperone transport characteristics. Knock-down of Hdj1 had not critical effect on C6 cell physiology while the shRNA-mediated reduction of Hdj2 caused multiple changes in the cell behavior. Among these effects in vitro were the elevation of aggressiveness as found by increased growth rate, colony formation, mobility and metalloproteinase activity. Importantly, the enhanced tumorigenicity of Hdj2-deficient cells retained in vivo: the tumor size grew 1.5-fold over cells with normal content of the co-chaperone and the animal death score was much higher in case of Hdj2-depleted cells. This is the first demonstration of anti-carcinogenic function of Hdj2 co-chaperone. Studying the behavior of C6 cell depleted of the Hdj1/2 co-chaperones we found that Hdj2 negatively affects the extracellular transport of the endogenous Hsp70 that pulled out by its exogenous analog. It is concluded that Hdj2 is the meaningful target for anti-cancer therapy based both on substances targeting endogenous Hsp70 and on immunomodulatory proteins/peptides focused on the extracellular transport of the endogenous Hsp70.
Small molecules inhibiting Hsp70 function as potential anti-tumor drugs

Vladimir Lazarev, Dmitryi Sverchinskyi, Sergey Niskanen, Elena Mikhaylova, Irina Guzhova, Boris Margulis*

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Hsp70 chaperone plays a protective role in cancer cells becoming an important target for therapeutic compounds. A part of such substances may reduce the expression of heat shock protein genes, while the other target chaperonic activity of Hsp70, its ability to recognize denatured protein substrate and convert it to active conformation. The cells in which chaperonic function is suppressed demonstrate reduction of growth rate, decrease of mobility and as general attribute loss of tumorigenic phenotype. In a search for chaperonic inhibitors we created two assays and using them performed screening of small molecules from the collection of InterBioScreen (Chernogolovka, Moscow.). We found N-amino-ethyl amino derivative of colchicine among the inhibitors (AEAC) whose toxicity was three orders lower than that of original compound. The library of Hsp70 peptide fragments (91 of 15-mers, BioSynthesis, USA) was also subjected to screening and one peptide Incyt2 was found to reduce chaperonic activity of Hsp70. According to data of DARTS and differential calorimetry both AEAC and Incyt2 bind Hsp70 and reduce its chaperonic capacity in vitro. In combination with doxorubicin AEAC and Incyt2 increase the anti-tumor efficiency of the drug in cell model of B16 mouse melanoma. Thus the novel method of high-throughput screening of Hsp70 chaperone modulators was established allowing to screen chemical libraries. Two compounds discovered, AEAE colchicine derivative and Hsp70 peptide fragment, demonstrated pronounced anti-tumor activity in combination with the established drug, doxorubicin, and may become novel medicines with the broad spectrum of effects.
A role of the unique interactions between the LB loop of protease domain with the PDZ domain of the human pro-apoptotic HtrA3 protease

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Human HtrA3 protein belongs to the evolutionarily conserved HtrA family of serine proteases/chaperones, which are responsible for protein quality control and regulation of various physiological processes in a cell. Human HtrA proteins (HtrA1-4) are involved in oncogenesis and neurodegenerative disorders, and are considered as promising targets in treatment of these diseases. The HtrA3 protease is connected with oncogenesis via its proapoptotic activity. Upon cellular stress (e.g. anticancer drugs) HtrA3 is released to cytoplasm where it stimulates apoptosis. The HtrA3 release is accompanied by autocleavage of its N-terminal domain [1]. The truncated HtrA3 protein (ΔN-HtrA3) is a homotrimer composed of the protease (PD) and PDZ domains. The PD domain is of a chymotrypsin type, composed mainly of beta strands which are connected by loops; some of the latter have a catalytic and/or regulatory function. The LB loop carries histidine of the Ser-His-Asp catalytic triad and is six amino acid residues longer compared to the LB loops of other human HtrAs. This unique amino acid sequence is conserved among HtrA3 proteins of various species. The LB loop interacts closely with the PDZ domain of the same subunit (LB - PDZ interactions) in a way not found in other HtrAs, forming a ring-like structure [2]. The influence of this unique interaction on HtrA3 structure and enzymatic function is not known. The aim of this study was to investigate the role of the LB - PDZ interactions in sustenance of quaternary structure and proteolytic activity of the ΔN-HtrA3 protease. We constructed a set of ΔN-HtrA3 variants with substitutions of amino acid residues in the PDZ domain and a deletion of the unique six residues in the LB loop. Analysis of the mutated proteins by size exclusion chromatography showed that the LB – PDZ interactions stabilize the trimeric form of the ΔN-HtrA3. Enzymatic activity studies of the variants revealed that the decrease of the LB- PDZ interactions caused a significant decrease of the ΔN-HtrA3 protease substrate affinity and catalytic efficiency. The results presented in this work indicate the importance of the intra-subunit LB - PDZ interactions for maintaining quaternary structure and proper proteolytic activity of the ΔN-HtrA3 protein.
Acknowledgments: This work was supported by project grant no. UMO-2013/09/B/NZ1/01068 from the National Centre for Science (Poland) to BL and University of Gdansk grant no. 583-L130-B921-15 to PG.

Literature cited
The PERK/Nrf2 pathway is involved in endoplasmic reticulum stress-related apoptosis in H9c2 rat myoblastic cells

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**Background:** Endoplasmic reticulum is a principal site for protein synthesis and folding, calcium storage, and signaling. Alterations in the endoplasmic reticulum environment, such as perturbation of Ca2+ homeostasis, elevated protein synthesis, deprivation of glucose, altered glycosylation, and the accumulation of misfolded proteins, cause endoplasmic reticulum stress (ERS). The signaling mediating ERS includes three pathways as follows: (1) Protein kinase R-like ER kinase (PERK), (2) inositol- requiring enzyme-1(IRE1), and activating transcription factor 6 (ATF6. Among the three pathways, PERK regulates protein synthesis, folding, and apoptosis. The present study aimed to investigate the role of PERK / nuclear factor erythroid-related factor 2 (Nrf2) pathway-mediated endoplasmic reticulum stress (ERS) in thapsigargin (TG) and tunicamycin (TM) - induced H9c2 cell apoptosis.

**Methods:** ERS was induced by ERS inducers TG and TM in H9c2 cells pretreated with or without all-trans retinoic acid (ATRA, a Nrf2 inhibitor) or tert-butylhydroquinone (tBHQ, a Nrf2 activator). Apoptosis was detected by using Annexin V / PI double staining and terminal deoxynucleotidyl transferase mediated dUTP-biotin nick end labeling (TUNEL) assay, and CCK-8 assay was used for measuring cell viability. Western blotting was used to detect the ERS-related proteins: calreticulin (CRT), activating transcription factor 4 (ATF4), and C/EBP homologous protein (CHOP), and total and phosphorylated PERK and Nrf2. Immunofluorescence staining was used to assess the subcellular distribution of Nrf2.

**Results:** We found that TG and TM induced ERS-related apoptosis in H9C2 cells in a dose-dependent manner. TG (20 nmol/L) and TM (160 ng/mL) treatment for 48h induced apoptosis in H9C2 cell. Compared with control, TG or TM treatment upregulated expression of CRT, ATF4 and CHOP, increased PERK phosphorylation and Nrf2 nuclear translocation in H9C2 cells in a dose-dependent manner. ERS inhibitors Taurine (Tau) and taoursodeoxycholic acid (TUDCA) significantly alleviated TG and TM-induced PERK phosphorylation, Nrf2 nuclear translocation, upregulation of ATF4 and CHOP expression, and apoptosis in H9C2 cell. To further investigate the role of Nrf2 in PERK-mediated ERS-related apoptosis, we pretreated cells with All-trans retinoic acid (ATRA) to decrease, or tert-butylhydroquinone (tBHQ) to increase nuclear translation of Nrf2 for 24 hours, prior to TG or TM treatment. We found that ATRA (20nmol/L) alleviated, while tBHQ (10 umol/L) aggravated TG or TM-induced Nrf2 nuclear translocation and apoptosis in H9C2 cells. Conclusion: The results suggest that the PERK / Nrf2 pathway is involved in the TG and TM-induced ERS-related apoptosis in H9C2 cells.

This work was supported by the the National Natural Science Foundation of China (31471094) and the National Basic Research Program of China (973 Program No. 2015CB554402).
Session 5: Molecular Chaperones, Cancer and Apoptosis

Type of Presentation: Poster

**Immune responses against cancer stem cell-specific stress proteins: application to prophylactic cancer vaccine**

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Cancer stem-like cells (CSCs) are defined as the small population of cancer cells with stem-like phenotypes and high capacity for tumor initiation. These cells may have a huge impact in the field of cancer therapy since they are extremely resistant to standard chemotherapy and therefore likely to be responsible for disease recurrence. We have analyzed the immunopathological properties of CSCs of human solid cancers and identified several genes that were characteristic to CSCs. Remarkably, some of them were expressed exclusively in testis among normal adult organs and involved in the spermatogenesis and function of spermatozoa. siRNA-mediated knockdown of these genes caused decreased sphere-forming capacity and tumor-initiating capacity, therefore indicating that they might be associated with the maintenance of stem like phenotype of CSCs. Our data suggest that spermatogenesis-associated genes are aberrantly expressed in CSCs and function as key molecules for tumor initiation. In addition, we show evidence that CSC-specific cytotoxic T cells are induced from peripheral blood of cancer patients. We named these functioning antigens "Somato-Germinomics antigens" and propose an immunotherapeutic strategy targeting CSCs.
Identification of the cellular substrates of the pro-apoptotic HtrA3 protease/chaperone

Tomasz Wenta1*, Przemysław Glaza1, Dorota Żurawa-Janicka1, Andrea Lipińska2, Barbara Lipińska1

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The proteins of the HtrA (High temperature requirement A) family are very well conserved in evolution and they function as protein quality controllers and as regulators of many important cellular processes. The HtrAs are serine proteases which may also act as molecular chaperones. They possess protease domains (PD) of the chymotrypsin type and PDZ domains which bind substrates or regulatory ligands. In humans there are four members (HtrA1-4) of this family. Under stressful conditions (e. g. the presence of anticancer drugs) HtrA3 acts as a pro-apoptotic protein via the intrinsic, mitochondria-mediated pathway. It is proposed that HtrA3 suppresses tumor cell invasiveness. HtrA3 also plays a significant role in the formation and function of the placenta. Molecular mechanism of HtrA3 function in a cell is not well understood. So far, the only known cellular partners of HtrA3 are HtrA4 and myosin-9. Unusually among the HtrAs, HtrA3 has two isoforms, the long (HtrA3L), encompassing the PD and PDZ domains, and short (HtrA3S), lacking the PDZ domain [1,2]. The aim of this study was to identify cellular substrates of both HtrA3 isoforms.

Using “pull down” and LC-MS techniques we identified at least seventeen cellular proteins which interact with HtrA3L and HtrAS. These HtrA3 partners belong to three main groups: (1) structural cytoskeleton proteins (e. g. tubulin, actin, vimentin) and proteins involved in cytoskeleton formation (e. g. T-complex protein-1, heat shock protein β1); (2) factors connected with biosynthesis of proteins; (3) proteins involved in response to stressful conditions. Using western blotting, immunoprecipitation and reverse immunoprecipitation we found that HtrA3 forms complexes with β-tubulin and actin both in vitro and in vivo, and with vimentin – in vitro. Furthermore, the anti-apoptotic protein, XIAP, interacted with HtrA3 in vitro. We also showed that both isoforms of HtrA3 cleaved β-tubulin, actin, vimentin and XIAP in cell lysates, and that the purified, proteolytically inactive HtrA3 stimulated tubulin polymerization in vitro. Interestingly, T-complex protein-1 interacted with HtrA3 in vivo and in vitro but was not cleaved. Collectively, our results suggest that HtrA3 is involved in modulation of cytoskeleton stability. Moreover, they suggest that HtrA3 may stimulate apoptosis via degradation of XIAP. We believe that these results provide new insights into the function of HtrA3 in the cell.

Acknowledgements
This work was supported by the National Science Center (Poland), project grant no. UMO-2013/09/B/NZ1/01068 to BL and University of Gdansk grant no. 538-L136-B265-16 to TW
Literature cited:
MONDAY OCTOBER 31

SESSION 6: HSF1 IN HEALTH AND DISEASE

SPEAKERS

M. Gabriella Santoro
Lea Sistonen
Nahid F. Mivechi
Valérie Mezger

POSTERS

Seiji Ishii
Mohan E. Tulapurkar

Note: This session will begin at 4:00 instead of 3:30.
Heat shock factor-1 (HSF1) is a central regulator of heat-induced transcriptional responses. It is recognized that HSF1, alone or through interaction with other stress-regulated factors including the critical regulator of inflammatory responses NF-κB, contributes to establish a cytoprotective state in several pathological conditions. HSF1 is generally found as an inert monomer in unstressed cells; upon exposure to proteotoxic stress, it is derepressed in a stepwise process that involves trimerization, nuclear translocation, phosphorylation/sumoylation, and binding to heat-shock elements (HSE). HSF1-binding to HSE triggers a rapid shift in the transcriptional program resulting in the expression of molecular chaperones constituting a major component of mammalian cells protection against proteotoxic stress. However, HSF1-binding sites have been described also in genes encoding proteins with non-chaperone function, whose role in proteotoxic stress is only partially understood. We have now identified the NKRF gene encoding for the silencer protein NF-κB Repressing Factor as a novel human HSF1-target gene. Human NKRF transcription is strictly dependent on HSF1 and is triggered at temperatures above 39°C in different types of human cancer and primary cells, including peripheral blood monocytes. The HSF1-binding HSE-sequence in the NKRF-promoter critical for heat-induced transcription was identified. Interestingly, NKRF protein was found to be mainly localized in the nucleoli of human cells. The nucleolus, a dynamic nuclear compartment long regarded as the cell ribosome factory, is emerging as an important player in the regulation of cell survival and recovery from stress. Our results indicate that NKRF is essential for nucleolus homeostasis and cell survival under proteotoxic stress. The mechanisms involved in NKRF-mediated surveillance of nucleolar function during stress will be discussed.
Session 6: HSF1 in Health and Disease

Type of Presentation: Oral

Transcriptional reprogramming of genes and their regulatory elements in acute stress

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Plasticity of transcriptional programs is fundamental for all biological processes from cellular growth and differentiation to coordinated functions of tissues and whole organisms as the transcriptional programs define the identities of cells and their responses to various stimuli. To investigate the mechanisms by which human cells promptly and profoundly reprogram their RNA synthesis upon acute heat stress, we profiled the genome-wide nascent transcription in K562 cells exposed to a 30-minute heat shock and compared the transcripts with those present under normal growth conditions. The results from Precision Run-On sequencing (PRO-seq) revealed an induction of hundreds of genes and a repression of thousands of genes in heat-shocked cells. In addition to protein-coding genes, many distal regulatory elements that possessed an open conformation prior to stress, were also actively transcribed and we named them as distal Transcribed Regulatory Elements (dTREs). Importantly, PRO-seq showed that the release of promoter-proximal RNA Polymerase to productive elongation is the decisive step for either stress-induced transcriptional upregulation or downregulation. To understand how RNA synthesis is regulated in the context of dynamic chromatin environment, we analyzed the chromatin architecture and how HSF1 interacts with the local chromatin as well as how the transcriptional stress response of genes and dTREs is coordinated across the human genome. Our results highlight the delicate spatial organization of chromatin that pre-wires gene expression and defines the directionality at the gene promoters upon acute stress.
Session 6: HSF1 in Health and Disease

Type of Presentation: Invited Speaker

Regulation of energy metabolism by heat shock factor Hsf1

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Metabolic energy reprogramming facilitates adaptations to a variety of stress conditions and cellular dysfunction, but how the energetic demands are monitored and met in response to physiological stimuli remains elusive. Our data support a model demonstrating that heat shock factor 1 (HSF1), a master transcriptional regulator of the chaperone response, has been co-opted from its role as a critical protein quality-control regulator to having a central role in systemic energy sensing and for metabolic adaptation to nutrient availability.
Session 6: HSF1 in Health and Disease

Type of Presentation: Invited Speaker

**Fetal Alcohol Syndrome, Neuroinflammation, and Epigenetic Regulations in the Diseased Developing Brain**

Agathe Duchateau (CNRS, USPC, UMR7216 Epigenetics & Cell Fate, Paris, France)

Anne-Laure Schang (CNRS, USPC, UMR7216 Epigenetics & Cell Fate, Paris, France)

Federico Miozzo (CNRS, USPC, UMR7216 Epigenetics & Cell Fate, Paris, France)

Pierre Gressens (INSERM 1141, Robert Debré Hospital, Paris, France)

Aurélie de Thonel (CNRS, USPC, UMR7216 Epigenetics & Cell Fate, Paris, France)

Délara Sabéran-Djoneidi (CNRS, USPC, UMR7216 Epigenetics & Cell Fate, Paris, France)

Valérie Mezger* (CNRS, USPC, UMR7216 Epigenetics & Cell Fate, Paris, France)

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We aim to unravel the transcriptional and epigenetic mechanisms by which prenatal stress can impact the developing brain, and, at temporal distance from the insult, influence the integrity of the adult brain. This is based on the following concepts: i) Brain development and integrity is exquisitely controlled by epigenetic mechanisms and alterations of the epigenome have been characterized in the brain of patients with neurospychiatric disorders, and in rodent models in response to environmental stress. ii) Prenatal environmental stress increases the risk of developing psychiatric disorders. iii) the same genes that are required for neurogenesis are also involved from the formation of axons and dendrites, synapses, and synaptic plasticity. The stress-induced deposition of aberrant epigenetic marks during development might therefore impact their expression later in life. But the mechanisms by which fetal stress leads to the deposition of abnormal epigenetic signatures on these genes that are required for adult brain performance, remain unclear.

We address this question by studying Heat Shock Factors that represent a unique entry point into a link between stress, epigenetics, and brain development/integrity.

Using fetal alcohol exposure (FAE) and neuroinflammation as paradigms of prenatal stress in mouse models, as well as genome-wide approaches (DNA methylome and ATAC-Seq), we investigate how HSFs contribute to the deposition of short- and long-term epigenetic marks (DNA methylation) that modify DNA accessibility and can impact brain integrity and function.
Session 6: HSF1 in Health and Disease

Type of Presentation: Poster

**Involvement of heterogeneous activation of Heat Shock Factor 1 in the formation of focal cortical dysplasia elicited by prenatal environmental challenges**

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Mitsuaki Fujimoto, Department of Biochemistry and Molecular Biology, Yamaguchi University School of Medicine, Ube, Japan

Kristen Brennand, Department of Psychiatry and Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Salk Institute for Biological Studies, Laboratory of Genetics, La Jolla, CA, USA

Akira Nakai, Department of Biochemistry and Molecular Biology, Yamaguchi University School of Medicine, Ube, Japan

Valérie Mezger, CNRS, UMR7216 Epigenetics and Cell Fate, Paris, France; University Paris Diderot, 75013 Paris, France; Département Hospitalo-Universitaire DHU PROTECT, Paris, France

Fred Gage, Salk Institute for Biological Studies, Laboratory of Genetics, La Jolla, CA, USA

Pasko Rakic, Department of Neurobiology and Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, CT, USA
Prenatal environmental challenges, such as maternal stroke and drug use affect fetal cortical development, thereby increase the risk of intellectual disability, epilepsy and so on. Heat Shock Factor 1 (HSF1) - Heat Shock Protein (HSP) signaling (HSF1-HSP signaling) is required to protect the embryonic cortex from exposure to a variety of environmental challenges, thereby reduces the risk of the mental disorders (Hashimoto-Torii K. et al, Neuron, 2014). In contrast to general assumption that HSF1-HSP signaling is equipped equally by most types of the cells for their protection, here, we show that HSF1-HSP signaling is activated at various levels among the cells in the fetal cortices exposed to the environmental challenges and that this variable activation may be a cause of cortical dysplasia. First we tested HSF1-HSP signaling activation in human iPSCs-derived neural stem/progenitor cells (NSPCs) under exposure to ethanol, hydrogen peroxide, and methyl mercury by quantifying the number of HSP70 mRNA using single molecule Fluorescence In Situ Hybridization (smFISH). In contrast to the consistent expression of housekeeping genes including GAPDH, HSP70 expression shows significant variability among NSPCs in response to these environmental challenges. This result suggests that HSF1-HSP signaling is activated at different level in individual cells by a variety of environmental challenges. By generating in vivo mouse fluorescence reporter system for the HSF1-HSP signaling, we also confirmed the heterogeneity of the activation in fetal cortices exposed to environmental challenges. We next examined potential effects of the bursty activation of the signaling that is induced in a part of cells exposed to the challenges on cortical development. By introducing constitutively active form of HSF1 (caHSF1) into normal embryonic cortex, we found that the pyramidal neurons electroporated with the caHSF1 impaired the radial migration. Importantly, we also found that the impaired neuronal migration caused by exposure to challenges is mitigated by reducing such bursty activation of the HSF1-HSP signaling. Altogether, these results demonstrate that a part of cortical cells may activate protective signaling at excess level in response to prenatal environmental challenges, and that this excess activation adversely affects the cortical development.
Activation of heat shock response augments fibroblast growth factor-1 expression in wounded lung epithelium: a possible mechanism for progressive lung fibrosis


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We previously showed that coincident exposure to heat shock (HS; 420C for 2h) and TNFα synergistically induces apoptosis in mouse lung epithelium. We extended this work by analyzing HS effects on human lung epithelial responses to clinically relevant injury. Cotreatment with TNFα and HS induced little caspase-3 and PARP cleavage in human small airway epithelial cells (SAECs) and A549 and BEAS2B cells. Scratch wound closure rates almost doubled when A549 and BEAS2B cells and air liquid interface cultures of human bronchial epithelial (NHBE) were heat-shocked immediately post wounding. Microarray, qRT-PCR, and immunoblotting showed FGF1 to be synergistically induced by HS and wounding. Enhanced FGF1 expression in HS/wounded A549 was blocked by inhibitors of p38MAPK (SB203580) or heat shock factor (HSF)-1 (KNK-437) and in HSF1-knockout BEAS2B cells. PCR demonstrated FGF1 to be expressed from the two most distal promoters in wounded/heat-shocked cells. Wound closure in heat-shocked A549 cells was reduced by FGF receptor-1/3 inhibition (SU-5402) or FGF1 depletion. Exogenous FGF1 accelerated A549 wound closure in the absence but not presence of 41 HS. In the presence of exogenous FGF1, HS slowed wound closure, suggesting it increases FGF1 expression but impairs FGF1-stimulated wound closure. Frozen sections from normal and IPF lung were analyzed for FGF1 and HSP70 by immunofluorescence confocal microscopy and qRT-PCR. FGF1 and HSP70 mRNA levels were 7.5- and 5.9-fold higher in IPF than normal lung and the proteins co-localized to fibroblastic foci in IPF lung. We conclude that HS signaling may have an important impact on lung injury, healing, and fibrosis.
TUESDAY NOVEMBER 1

PLENARY LECTURE

Carmen Garrido
As cancer cells accumulate mutations, violate physiological laws and acquire sets of hallmarks, they require a constitutively high level of chaperones like HSP70 (heat shock protein-70) for their survival/maintenance. HSP70 is a major stress-inducible chaperone with intra- (cytoprotective) and extracellular (danger signal) functions. We have demonstrated that whereas cancer cells release exosomes with membrane-bound HSP70 (HSP70-exosomes), normal cells do not (Gobbo et al, J Natl Cancer Instit, 2016). We have developed a peptide aptamer (A8) that binds to the extracellular loop of membrane-bound HSP70 (Rerole et al, Cancer Res, 2011). Using A8 as a high affinity ligand and a technique of interference biolayer (BLI), we have developed an easy optical approach to capture HSP70-exosomes from human fluids to demonstrate that the amount of HSP70-exosomes both in blood and urines is much higher in cancer patients than in healthy individuals (patent 2015 Inserm transfert). Since one single cancer cell can release hundreds of tumour-derived exosomes, HSP70-exosomes detection must precede the apparition of circulating tumour cells and therefore may be an interesting marker in cancer. We have started a clinical trial (ExoDiag. Inclusion of patients started in September 2015) that so far confirm the interest of HSP70-exosomes for metastasis diagnosis purposes.

From a therapeutic perspective, we have demonstrated that HSP70-exosomes induce the activation of myeloid-derived suppressive cells (Chalmin et al J Clin Invest, 2010) and that this activation is blocked by the peptide aptamer A8. As a consequence, A8 induces a strong intra-tumour infiltration of immune cells (cytotoxic T-cells and macrophages) and the regression of the tumour (Gobbo et al, J Natl Cancer Institute 2015). As most anticancer drugs induce the release of HSP70-exosomes, we believe they may benefit from a combination with a molecule like A8, able to restore the development of an anti-cancer immune response.
TUESDAY NOVEMBER 1

SESSION 7: HSPs MODULATE THE IMMUNE RESPONSE

SPEAKERS

Cristina Bonorino
Xiang-Yang (Shawn) Wang and John Subjeck (jointly)
Kamal Moudgil
Michael A. Lynes
Session 7: HSPs Modulate the Immune Response

Type of Presentation: Oral

Alveolar macrophage and dendritic cell modulation by mycobacterial DnaK inhibits allergen-specific T cells preventing allergic airway inflammation

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Mycobacterial DnaK is the prokaryote counterpart of mammalian Hsp70. This protein has powerful anti-inflammatory effects in more than one animal model of inflammation. In the present study, we investigated a possible modulatory role for DnaK in an allergic asthma model, demonstrating preventive amelioration of ovalbumin (OVA)-induced allergic airway disease. Mice were sensitized with OVA receiving intranasal DnaK before challenged with OVA. Reduced OVA-induced airway hyper responsiveness was observed, as well as reduced lung-infiltrating eosinophilia. Resolution of airway pathophysiology was associated with a downregulation of MHC II and CD86 and increased IL-10 production in alveolar macrophages (AMs) as well as in lung dendritic cells (DCs). Furthermore, DnaK-treated animals presented a reduction in OVA-specific Th2 cells recruitment to inflamed lung, as well as reduced proliferation and effector function of these cells. Finally, DnaK could also modulate inflammatory markers in sputum-derived macrophages from asthmatic patients, indicating translational applicability. In conclusion, our results suggest that treatment with DnaK could constitute a novel preventive approach to the establishment of allergic airway disease.
Dynamic roles of extracellular chaperone molecule Grp170 in immune modulation and implications in cancer immunotherapy

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The molecular chaperone 170 kDa glucose-regulated protein (Grp170) exhibits diverse effects on cellular responses to stressors. In addition to holding protein substrates or antigenic polypeptides, this large stress protein also interacts with pathogen-associated molecular patterns (PAMPs), such as microbial DNA. These dynamic interactions consequently lead to enhanced cross-presentation of antigens or amplified activation of an inflammatory response by engaging distinct pattern recognition receptors (e.g., toll-like receptors). The innate immunity enhanced by Grp170-based chaperoning results in improved clearance of invading microbes. These chaperoning-facilitated immunological outcomes are mediated by distinct intracellular pathways or organelles. Given the superior antigen- and PAMP-shuttling capacity of the Grp170, we modified it by integrating an additional pathogen-derived ‘danger’ signal to strengthen immune co-stimulation that is crucial for functional activation of antigen-presenting cells and T-cell priming. This multi-component chaperone complex vaccine is highly effective in mobilizing cytotoxic T lymphocyte response and eradicating established tumors. Given the ongoing phase I trial of a Hsp110-gp100 recombinant chaperone vaccine in patients with metastatic melanoma, our results set the stage for developing a new generation of recombinant chaperone vaccines for cancer immunotherapy.
Session 7: HSPs Modulate the Immune Response

Type of Presentation: Oral

Immuno regulation in arthritis via crossreactive T cells against a pathogenic/protective epitope of HSP60

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Despite the ubiquitous expression of heat-shock proteins (Hsps) in an individual, the immune system is not fully tolerant to them. In addition, foreign Hsps are known to be highly immunogenic, and immune response to such proteins is observed in many inflammatory and autoimmune diseases. Previous studies by others and us in the rat adjuvant arthritis (AA) model of human rheumatoid arthritis (RA) as well as observations in patients with RA and juvenile idiopathic arthritis (JIA) have unraveled immunoregulatory attributes of T cells directed against self Hsp60. We have shown that immunization of Lewis rats with self (rat)-Hsp65 (Rhsp65) leads to protection from rather induction of arthritis. This protection involves T cells against Rhsp65, including the subset against the epitope region 465-479 of Rhsp65 (R465). Similarly, treatment of rats with a peptide depicting the pathogenic epitope of mycobacterial hsp65, 177-191 (B177) also affords protection against disease. Interestingly, a subset of T cells directed against R465 were found to be crossreactive against B177, thus providing one explanation for the protective effect of B177. The observation that the T cells against a regulatory epitope of self hsp60 are crossreactive with a pathogenic epitope of the homologous foreign hsp65 is somewhat paradoxical. However, this T cell crossreactivity might represent a natural way to control pathogenic inflammation. We suggest that during the course of disease, the previously pathogenic disease-inducing epitope of Bhsp65 that is still processed and presented from persisting foreign hsp60 in the body might engage the crossreactive T cell subset primed by self hsp60 that is induced during disease, leading to suppression of ongoing inflammation. We further propose that such crossreactivity might also extend to antibodies directed against foreign and self hsp60 homologs in arthritis. Information on such T/B cell epitopes might expand the choice of peptides to be explored for their therapeutic use to control arthritis.
Session 7: HSPs Modulate the Immune Response

Type of Presentation: Oral Presentation

The many paths by which metallothioneins modulate the immune response

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Metallothioneins (MT) are small, cysteine-rich proteins that are highly conserved over large evolutionary distances. In healthy cells, their roles include the management of essential divalent metal ions such as copper and zinc, the regulation of the redox environment, and (as a consequence) influence cellular signal transduction cascades and transcription factor activity. In recent studies, we have found that the intracellular pool of MT influences the developmental programming of T cells, possibly as a consequence of its management of free zinc in the cell. The intracellular pool of MT has a very low redox potential that suggests a possible mechanism for translating redox signaling into intracellular zinc signaling. Activated CD4+ cells are better able to release intracellular zinc when the cell contains an intracellular pool of MT, and this effect is associated with increased p38 MAPK activation. These critical contributions to cellular homeostasis can be disturbed by a variety of stressors that can each serve as potent inducers of MT. MT can be induced by a broad range of chemical, biological, physical, and psychological stressors and they fit the definition of cellular stress response proteins. Under certain conditions, MT can be released from cells and, when present in the extracellular environment, can influence the progression of an inflammatory response. We have found that a monoclonal anti-MT antibody made in our laboratory can reduce the pro-inflammatory effect of the extracellular MT pool and diminish the severity of chronic and acute forms of dextran sodium sulfate (DSS)-induced colitis in mice. Taken together, these results suggest that manipulations of MT, both within and outside the cell, may represent novel opportunities for therapeutic immunomodulation and impact brain integrity and function.
WEDNESDAY NOVEMBER 2

PLENARY LECTURE

László Vigh
Type of Presentation: Plenary Lecture

**Novel HSP co-modulator drug candidates operating through membrane lipid therapy**

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Alterations in cellular stress management (stress sensing, signalling, adaptation) are well described in ageing and important disease states and are known to be coupled with characteristic “membrane defects” (Vigh et al., *TIBS*, 2007; Nagy et al., *PNAS*, 2007; Hooper et al., *CSAC*, 2014; Török et al., *BBA*, 2014). We established a relationship between specific distribution of lipid nanostructures (“rafts”) and the concomitant changes in the level, profile and cellular distribution of HSPs (*Brameshuber et al., J.Biol.Chem.*, 2010; Balogh et al., *PLoS ONE*, 2011). A comparative lipidomics study explored key lipid molecular species with the potential to activate heat shock protein response (*Balogh et al., BBA*, 2010; *Peter et al., Mol.Memb.Biol.*, 2012; *Balogh et al., FEBS L*, 2013; *Peter et al., BBA*, 2016). A subpopulation of HSPs is membrane associated mostly via specific lipid linkers. These subclasses of HSPs can modulate major attributes of the membranes, like fluidity and bilayer propensity (*Török et al., PNAS*, 1997; 2001; *Tsvetkova et al., PNAS*, 2002; *Balogi et al., JBC*, 2008; *Nakamoto and Vigh, CMLS*, 2007; *Horvath et al., BBA*, 2008), overall stability or trafficking (*Nimmervoll et al., FEBS L*. 2015). The above findings served as underlying principles for drug development strategy, called “membrane lipid therapy” (MLT). MLT concept proposes the use of small molecules - specifically designed to modify the lipid composition and/or structures and microdomains of membranes - as disease modifying agents by reverting the malfunction or altering the expression of ageing and disease-specific protein or lipid signal cascades (*Escriba et al., Progr.Lipid.Res.*, 2015). The HSP co-inducer, lipid-interacting hydroximic acid (HA) derivatives (Bimoclomol, Arimoclomol, BGP-15, etc.) are typical MLT agents. Some of those fluidize, yet stabilize membranes (*Vigh et al., Nature Med.*, 1997; *Török et al., PNAS*, 2003) and remodel lipid rafts (*Gombos et al, PloS One*, 2011). Besides being MLT drug candidates these multitarget molecules are also known as PARP-1 inhibitors, agents to block TNF induced proinflammatory pathways, to improve mitochondrial efficiency and to reduce ROS production (reviewed in: *Crul et al., Curr. Pharm. Des.* 2013). BGP-15 and Arimoclomol were tested successfully in various preclinical disease models (*Chung et al., PNAS*, 2008; *Salah et al., Sci.Transl.Med.*, 2016) and have been established in various Phase II/III clinical trials (*Kirkegaard et al., Sci.Transl.Med.*, 2016).

Our recent research goal was to identify non-proteotoxic compounds – which are more specific in their primary membrane and lipid targets than HA derivatives - and are potent/selective Hsp co-modulators. Based on favorable toxicological data and strong HSP co-modulator (inducer or silencer) activities, we selected the novel 1,4-dihydropyridine (1,4-DHPs) derivatives. We documented, that some 1,4-DHPs are broadly neuroprotective and have the ability to enhance memory in normal animals as well as to prevent memory deficits in AD transgenic mice. The neurotrophic and memory enhancing activities of selected 1,4-DHPs were clearly associated with their capacities to increase the levels of Hsp70 and Hsp25 (*Kasza et al., J.Alz.Res.*, 2016; *Hooper et al., Sci.Transl.Med.*, 2016).
et al., CSAC, 2016). We propose, that 1,4-DHPs are allo-network drugs (Szilagyi et al., Curr.Top.Med.Chem., 2013), acting indirectly, via the inter-protein propagation of changes in specific cellular stress signalling networks.
SESSION 8: SMALL HSPs AND HUMAN DISEASES

SPEAKERS

Robert M. Tanguay
Carmen Garrido
Serena Carra
Zarah Batulan
Aging is characterized by the accumulation of molecular damages that lead to organismal decline and the onset of age-associated disease. At the cellular level, aging is associated with the accumulation of dysfunctional mitochondria. Since these organelles are involved in many important cellular processes, different mechanisms exist to maintain their integrity: reactive oxygen species (ROS) scavenging, mitochondrial unfolding protein response (UPR\textsuperscript{MT}) and mitophagy. While the later aims at clearing defective mitochondria, the UPR\textsuperscript{MT} triggers the expression of a set of proteins aimed at re-establishing mitochondrial homeostasis. The induction of mitochondrial chaperones expression, particularly of Hsp60 and Hsp70, is a hallmark of the UPR\textsuperscript{MT} pathway. In \textit{Drosophila melanogaster}, Hsp22 is also localized in mitochondria and takes part in the pathway. In addition to being preferentially up-regulated during aging and in oxidative stress conditions, Hsp22 increases lifespan and resistance to stress upon over-expression. Its over-expression also changes the mitoproteomic profile and increases mitochondrial protease activity. Interestingly, Hsp22 co-migrates with components of the electron transport chain upon stress, emphasizing its role in mitochondrial homeostasis. Altogether, the results establish a role of Hsp22 in proteostasis and highlight the role of the UPR\textsuperscript{MT} in preventing the aging process.

(Supported by the Canadian Institute Health Research)
Session 8: Small HSPs and Human Diseases

Type of Presentation: Oral

**Alpha-B-crystallin: a therapeutic target in pulmonary fibrosis**

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Idiopathic pulmonary fibrosis (IPF) is a rare, lethal disease with a mean survival of three years after diagnosis. It has an unknown aetiology and there is presently no effective treatment to prevent or reverse it. IPF is characterized by the proliferation of myofibroblasts and the accumulation of extracellular-matrix (ECM) in the lungs. Transforming Growth Factor (TGF)-β1 is the major pro-fibrotic cytokine involved in IPF and is responsible for myofibroblast proliferation, ECM synthesis and trans-differentiation of alveolar epithelial cells into myofibroblasts. Among the different HSPs, we have demonstrated that small HSPs and in particular HSP27 (HSPB1) and αB-crystallin (HSPB5) are strongly overexpressed in human IPF lungs (Wettstein *et al.*, FASEB J. 2013, Bellaye *et al.*, Journal of Pathology. 2014). We have data showing that αB-crystallin knock-out mice are protected from induced pulmonary fibrosis (by exposure to bleomycin, or overexpression of IL-1β or TGF-β1. Burgy *et al.*, Science Transl Med, 2016). Furthermore, our *in vitro* data both in alveolar epithelial cells and lung fibroblasts show that αB-crystallin is essential for the mono-ubiquitination and nuclear translocation of Smad4, a key TGF-β1 signal transducer protein. αB-crystallin overexpression disrupts Smad4 mono-ubiquitination by interacting with Smad4 itself and its E3-ubiquitin ligase, TIF1γ, thus limiting Smad4 nuclear export. We confirmed the interaction between Smad4 and αB-crystallin *in vitro* by Fluorescence Lifetime Imaging-monitored Förster Resonance Energy Transfer (FLIM-FRET). Furthermore, our BioLayer Interferometry experiments demonstrate that αB-crystallin favors TIF1γ/Smad4 direct interaction. Altogether, our data allow us to state that αB-crystallin, by chaperoning Smad4, possesses a key role in the TGF-β1 pro-fibrotic signaling pathway and thus is an important regulator of fibrogenesis.
Granulostasis: A surveillance function of the HSPB8-BAG3-HSP70 chaperone complex that maintains stress granule integrity and dynamism

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Stress granules (SGs) are ribonucleoprotein complexes induced by stress. They sequester mRNAs and disassemble when the stress subsides, allowing restoration of translation. In amyotrophic lateral sclerosis (ALS) aberrant SGs cannot disassemble, accumulate and are degraded by autophagy. However, the molecular events causing the formation of aberrant SGs and the molecular players regulating this transition are largely unknown. Here, we report that defective ribosomal products (DRiPs) accumulate in SGs, affecting their dynamic behaviour and resistance to RNAse. We found that only a minor fraction of aberrant SGs is targeted by autophagy, while the vast majority disassembles in a process that requires assistance by the HSPB8-BAG3-HSPA1A chaperone complex. We propose a system of chaperone-mediated SG surveillance, or granulostasis, which regulates SG composition and dynamism, preventing SG persistency. Granulostasis operated by chaperones might be particularly important in (motor neuron) degenerative diseases, such as ALS that are characterized by aberrant SG dynamics.
Atherosclerosis is a chronic, progressive, cardiovascular condition marked by the accumulation of lipids and inflammatory markers in the arterial wall that, if left untreated, can cause obstructions in blood flow, eventually leading to heart attack, and/or death. Previous findings have shown that HSP27 serum levels are lower in patients with coronary artery disease (CAD), and are predictive of future adverse cardiovascular events. The atheroprotective effects of HSP27 were also observed in cell and murine experimental models of atherosclerosis (for ex. promoting anti-inflammatory cytokine release and reducing plaque cholesterol content) - however, the precise mechanisms underlying these processes remain unclear. Here, we demonstrate two possible ways, both involving exosomes, in which HSP27 is atheroprotective. First, we observe that HSP27 localizes to membranes of exosomes isolated from THP-1 cells, and that HSP27-exosome in vitro complexes promote the release of the anti-inflammatory cytokine, IL-10, partially through TLR-4 signaling and downstream NF-kB activation. Second, HSP27-autoantibody complexes increase cholesterol efflux from THP-1 macrophages through exosomes. Together, these findings indicate that macrophage-derived exosomes may mediate HSP27 atheroprotection by enhancing the anti-inflammatory response and cholesterol efflux.