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FRONTIERS
MEETINGS

June 10th – 12th , 2024
Venue: CNIO Auditorium — Madrid • Spain

Molecular chaperones in cancer and protein quality control

Organising committee

Gabriela Chiosis
Memorial Sloan Kettering Institute, US

Nabil Djouder
Spanish National Cancer Research Centre-CNIO, Spain

Judith Frydman
Stanford University, US

Óscar Llorca
Spanish National Cancer Research Centre-CNIO, Spain

Paul Workman
The Institute of Cancer Research, London, UK

Speakers

Udai Banerji
The Institute of Cancer Research;
The Royal Marsden NHS Foundation Trust, UK

Jeffrey L. Brodsky
University of Pittsburgh, Kenneth P. Dietrich School of Arts and Sciences, US

Johannes Buchner
Technical University of Munich, Germany

Eugenia Clerico
University of Massachusetts Amherst, US

Benoit Coulombe
Institut de Recherches Cliniques de Montréal, Canada

Chengkai Dai
Center for Cancer Research, National Cancer Institute, US

Elke Deuerling
Konstanz University, Germany

Walid A. Houry
Biochemistry, University of Toronto, Canada

Ursula Jakob
University of Michigan, US

Matthias Mayer
Center for Molecular Biology Heidelberg – ZMBH, Germany

Rahul Samant
Babraham Institute, UK

Ritwick Sawarkar
University of Cambridge, UK

Lea Sistonen
Turku Centre for Biotechnology, Finland

EXCELENCIA SEVERO OCHOA 2012 - 2024
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES
Instituto de Salud Carlos III
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Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

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Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

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Molecular chaperones in cancer and protein quality control

Summary

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Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

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Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Organisers and Speakers

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Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Venue:

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid.

Organisers & Speakers

Gabriela Chiosis
Memorial Sloan Kettering Institute, US

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Spanish National Cancer Research Centre-CNIO, Spain

Paul Workman
Centre for Cancer Drug Discovery, The Institute of Cancer Research, UK

ORGANISERS AND SPEAKERS

CNIO - CaixaResearch Frontiers Meeting

Speakers

Udai Banerji
The Institute of Cancer Research;
The Royal Marsden NHS Foundation Trust, UK

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University of Pittsburgh, Kenneth P. Dietrich School of Arts and Sciences, US

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Turku Centre for Biotechnology, Finland

Molecular chaperones in cancer and protein quality control

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MEETINGS

Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Programme

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Monday June 10th, 2024

13:00-14:45 Registration - welcome coffee

14:45-15:00 Welcome address: Óscar Llorca

15:00-16:00 Keynote Lecture

The TRiCky business of folding proteins in the cell ***Judith Frydman****, Stanford University, Stanford, US*

16:00-16:30 Coffee break

16:30-18:45 Protein Quality Control I

Chair: Óscar Llorca

16:30 – 17:00 *Regulation of the heat shock transcription factor Hsf1 by the Hsp70 chaperone network* ***Matthias Mayer****, Center for Molecular Biology Heidelberg – ZMBH, Germany*

17:00 – 17:15 *short talk 1 The Critical Properties of the Chaperonins for Their Function in Mitochondria.* ***Lingling Chen****, Indiana University, Bloomington, US*

17:15 – 17:45 *Co-Translational Protein Modification Principles and Their Potential Implications in Cancer* ***Elke Deuerling****, Konstanz University, Konstanz, Germany*

17:45 – 18:00 *short talk 2 Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation Pathway.* ***Jorge Cuéllar,*** *National Centre for Biotechnology, Madrid, Spain*

18:00 – 18:15 *short talk 3 Protein phosphatase-2A regulates the formation of cytotoxic protein aggregates through HSP70.* ***Oliver Krämer,*** *University Medical Center Mainz, Nackenheim, Germany*

18:15 – 18:45 *Regulation of chaperone machineries* ***Johannes Buchner,*** *Technical University of Munich (TUM), Garching, Germany*

18:45-20:00 Welcome cocktail for all participants

Tuesday June 11th, 2024

09:00-12:30 Protein Quality Control II

Chair: Lea Sistonen

09:00 – 09:30 *The intersection between cellular stress response pathways, proteostasis, and cancer cell survival* ***Jeffrey L. Brodsky****, University of Pittsburgh, Kenneth P. Dietrich School of Arts and Sciences, Pittsburgh, US*

09:30 – 09:45 *short talk 4 Cotranslational folding of the eukaryotic proteome is mediated by inter-chaperone dynamics.* ***Mauricio Aguilar Rangel****, Stanford University, US*

09:45 – 10:00 *short talk 5 Prefoldin associates to human chromatin where it interacts with the FACT histone chaperon and regulates nucleosome dynamics during transcription elongation.* ***Sebastián Chávez Canonical****, Institute of Biomedicine of Seville (IBiS), Spain*

10:00 – 10:30 *Selectivity versus Promiscuity in Client Binding by Hsp70s* ***Eugenia Clerico****, University of Massachusetts Amherst, US*

10:30-11:30 Coffee break and group picture

11:30 – 12:00 *Proteomic Instability of Cancer and Non-oncogene Addiction: Heat Shock Factor 1 (HSF1) as an Oncogenic Enabler* ***Chengkai Dai****, Center for Cancer Research, National Cancer Institute, Bethesda, US*

12:00 – 12:30 *Function of Heat Shock Transcription Factors in Epithelial-Mesenchymal Plasticity* ***Lea Sistonen****, Faculty of Science and Engineering Åbo Akademi University Turku Centre for Biotechnology, Turku, Finland*

12:30-14:00 Lunch at the cafeteria

Tuesday June 11th, 2024

14:00-17:30 Chaperones, Molecular Mechanisms and Structure

Chair: Gabriela Chiosis

14:00 – 14:30 *The PAQosome, a HSP90 co-chaperone for protein complex assembly and maturation; implication in disease* ***Benoit Coulombe****, Montreal Clinical Research Institute (IRCM), University of Montreal, Québec, Canada*

14:30 – 15:00 *CryoEM studies of the R2TP cochaperone* ***Óscar Llorca****, Spanish National Cancer Research Centre (CNIO), Madrid, Spain*

15:00 – 15:15 *short talk 6 Regulation of oncogenic kinases by HSP90 molecular chaperone complexes.* ***Jasmeen Oberoi****, University of Sussex, Brighton, UK*

15:15 – 15:30 *short talk 7 Structural recognition and stabilization of tyrosine hydroxylase by the J- domain protein DNAJC12.* ***Jimena Muntaner Pérez-Urria****, Spanish National Centre for Biotechnology (CNB-CSIC), Madrid, Spain*

15:30-16:00 Coffee break

16:00 – 16:30 *Towards understanding the functions of the PAQosome and its subcomplexes* ***Walid A. Houry****, Biochemistry, University of Toronto, Ontario, Canada*

16:30 – 16:45 *short talk 8 Structures of the Tubulin cofactors as GTP-dependent multi-subunit chaperone for alpha/beta-tubulin biogenesis.* ***Jawdat Al-Bassam****, University of California – Davis, US*

16:45 – 17:00 *short talk 9 J-domain proteins: the role of their dimeric state in the Hsp70 Chaperone Machinery.* ***Veronika Lashkul****, Center for Molecular Biology of Heidelberg university (ZMBH), Germany*

17:00 – 17:30 *Elucidating the structural basis of the URI prefoldin-like complex* ***Nabil Djouder / Rayan Naser****, Spanish National Cancer Research Centre, Madrid, Spain*

17:30-19:00 Poster session - Refreshments

Wednesday June 12th, 2024

09:00-11:15 Chaperones in disease and chaperonotherapy I

Chair: Walid Houry

09:00 – 09:30 *Epichaperomes in Cancer: Unraveling Molecular Complexity for Therapeutic Innovation and Diagnostic Advancements* ***Gabriela Chiosis****, Memorial Sloan Kettering Cancer Center, New York, US*

09:30 – 10:00 *Polyphosphate - An Ancient Player in Proteostasis and Cancer* ***Ursula Jakob****, University of Michigan; Molecular Chaperones in Metabolism, Ann Arbor, US*

10:00 – 10:15 *short talk 10 Nucleolar stress as a driver of aging and neurodegeneration: A ribosomal perspective.* ***Óscar Fernandez Capetillo****, Spanish National Cancer Research Centre, Madrid, Spain*

10:15-10:45 Coffee break

10:45 – 11:00 *short talk 11 p53 protein degradation redefines the initiation mechanisms and transitional mutations in colorectal cancer.* ***Irene Herranz****, Spanish National Cancer Research Centre, Madrid, Spain*

11:00 – 11:15 *short talk 12 Point mutations of the mitochondrial chaperone TRAP1 affect its functions and pro-neoplastic activity.* ***Claudio Laquatra****, University of Padua, Italy*

Wednesday June 12th, 2024

11:15-15:30 Chaperones in disease and chaperonotherapy II

Chair: Nabil Djouder

11:15 – 11:45 *How HSP90 helps cancer proliferation* ***Ritwick Sawarkar****, University of Cambridge, UK*

11:45 – 12:00 *short talk 13 The chaperone AGR2 contributes to the complex interplay between endoplasmic reticulum stress and inflammation in pancreatic cancer.* ***Irene Felipe Abrio****, Spanish National Cancer Research Centre, Madrid, Spain*

12:00 – 12:15 *short talk 14 Expression of mitochondrial Hsp40 chaperone is beneficial for mitochondrial biogenesis.* ***Grzegorz Ciesielski****, University of North Florida, US*

12:15 – 12:45 *Clinical applications of targeting HSP90 and other molecular chaperones* ***Udai Banerji****, The Institute of Cancer Research; The Royal Marsden NHS Foundation Trust, London, UK*

12:45-14:15 Lunch at the cafeteria

14:15 – 14:30 *short talk 15* ***[online]*** *From Chaperones to Epichaperomes: Phosphorylation Triggered Shape and Function Shifting of HSP90.* ***Tanaya Roychowdhury****, Memorial Sloan Kettering Cancer Centre, NY, US*

14:30– 15:00 *Exploring chaperone vulnerabilities in (cancer) senescence* ***Rahul Samant****, Babraham Institute, Cambridge, UK*

Wednesday June 12th, 2024

15:00-15:30 Closing Lecture

Targeting the Cell's Stress Pathways for Therapeutic Benefit in Cancer ***Paul Workman****, Centre for Cancer Drug Discovery, The Institute of Cancer Research, London, UK*

15:30 – 15:45 *Wrap up:* ***Nabil Djouder***

15:45 – 16:00 Poster/short talk prizes

chedule may change due to unforeseen circumstances.

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MEETINGS

Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Monday June 10th 2024

Keynote Lecture

Chairperson: ***Óscar Llorca***

cnïo stop cancer

The TRiCky business of folding proteins in the cell

Dr. Judith Frydman
Stanford University
US

The eukaryotic chaperonin TRiC/CCT is a large hetero-oligomeric complex that plays an essential role assisting cellular protein folding and suppressing protein aggregation. It consists of two rings, each composed of eight different subunits; non-native polypeptides bind and fold in an ATP-dependent manner within their central chamber. I will discuss recent advances in our understanding of TRiC structure and mechanism which reveal how the different TRiC/CCT subunits create asymmetry in its ATP-driven conformational cycle and its interaction with non-native polypeptides, which ultimately underlie its unique ability to fold proteins that cannot be folded by other chaperones.

Molecular chaperones in cancer and protein quality control

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MEETINGS

Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Monday June 10th 2024

Session #1
Protein Quality Control

Chairperson: ***Óscar Llorca***

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Regulation of the heat shock transcription factor Hsf1 by the Hsp70 chaperone network

Dr. Matthias Mayer

Center for Molecular Biology Heidelberg University (ZMBH), DKFZ-ZMBH-Alliance
Heidelberg, Germany

Homeostasis of the proteome is essential for life. All organisms encounter repeatedly extrinsic or intrinsic conditions that lead to an imbalance of the proteome and thus to stress that impacts protein folding. To cope with such stressful conditions, a transcriptional program evolved, generally termed the heat shock response (HSR). In eukaryotic organisms, one major regulator of the HSR is the heat shock transcription factor Hsf1 that is activated under stress conditions, binds to heat shock elements in promoters and enhances of heat shock genes and releases paused RNA-Polymerase II for transcription of the various heat shock genes, including genes encoding molecular chaperones such as Hsp70. Hsf1 integrates a large variety of signals influencing its transition from the monomeric, inactive to the trimeric DNA binding competent state. The full extent of this signal integration process by Hsf1 is still not understood at a molecular level. One part of this signal integration process is the negative feedback regulation of Hsf1 by molecular chaperones most notably the Hsp70 network that we have analyzed in some detail. There are 5 bona fide Hsp70s in the nuclear-cytoplasmic compartments. We investigated the question whether they are all involved in the regulation of Hsf1 or only some of them and if there are differences in their role in this process. Hsp70s are targeted to their clients by cochaperones of the J-domain protein (JDP) family. Of 10 JDPs that are considered general JDPs in the nuclear-cytoplasmic compartment only three were able to support the regulation of Hsf1. We are currently analyzing what are the properties that empower Hsp70s and JDPs to regulate Hsf1.

SHORT TALK

The Critical Properties of the Chaperonins for Their Function in Mitochondria

Lingling Chen
Indiana University,
Bloomington, US

Human mitochondrial chaperonin hmHsp60 plays a significant role in various human diseases, including cancers, through its chaperone activity and interaction with substrate proteins. In facilitating protein folding, the heptameric hmHsp60, along with its cochaperonin hmHsp10 and ATP, creates a favorable compartment for proper protein folding. Unlike the well-characterized bacterial chaperonin represented by E. coli GroEL, hmHsp60 exhibits lower stability as the heptamer is prone to dissociation into lower oligomeric states and displays slower ATPase activities. The significance of the reduced heptamer stability or ATPase activity to the chaperone function in mitochondria remains unclear. Through our cryo-EM studies, we have identified unique sequences in hmHsp60 associated with reduced heptameric stability, and importantly, they are conserved among mitochondrial chaperonins of higher eukaryotes. This suggests the functional significance of reduced heptameric stability. Furthermore, we have observed that a pathological hmHsp60 mutant exhibits enhanced heptameric stability and ATPase activity, further supporting the functional significance of both properties. Through a combination of cryo-EM studies and molecular dynamic simulations analysis, we have elucidated the structural and computational basis for the mutant's enhanced stability and ATPase activity. Collectively, our studies demonstrate that the unique properties of hmHsp60, namely reduced heptameric stability and ATPase activity, are crucial for its function in mitochondria.

Co-Translational Protein Modification Principles and Their Potential Implications in Cancer

Dr. Elke Deuerling
University of Konstanz, Department of Biology, Germany

The specific pathways of biogenesis for newly synthesized proteins depend on the factors that bind to the ribosome exit site. Accurate selection of nascent chains by the appropriate protein biogenesis factors, for example, for N-terminal protein modification, is crucial for proper protein function within cells. Unsolicited binding of nascent chain processing factors to translating ribosomes can lead to protein misfolding and mislocalization, adversely affecting cell function and viability. The mechanism controlling the access of protein biogenesis factors to ribosomes has long been a mystery. The latest data on regulating the access and interaction of protein biogenesis factors with nascent proteins will be presented, and the potential implications in cancer will be discussed.

SHORT TALK

Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation pathway

Jorge Cuéllar
National Centre for Biotechnology,
Madrid, Spain

In the ubiquitin-proteasome system (UPS), the degradation process is initiated by the recognition of ubiquitinated substrates by the 19S regulatory particle (RP), followed by unfolding and translocation of substrates into the 20S catalytic particle (CP), which executes protein degradation. In addition to the canonical subunits, proteasome function is finely tuned by cofactors containing ubiquitin-like (UBL) domains that are involved in the recruitment of substrates to the 26S proteasome.
On the other hand, Bag1 has been shown to interact with Hsc70/Hsp70 to modulate chaperone activities. Through an ATP-driven conformational cycle, Hsp70 can recognize misfolded proteins, promote refolding, prevent protein aggregation, and resolubilize protein aggregates. Despite their diverse roles, all members of the Hsp70 family contain two highly conserved structural domains: the substratebinding domain (SBD) and the nucleotide-binding domain (NBD). The ADP/ATP switch is catalyzed by a group of cochaperones called nucleotide exchange factors (NEFs), which bind to the NBD and favor ADP release from the active site and ATP re-uptake. Bag1 is one such NEF, containing both UBL and BAG domains, and interacts with the 26S proteasome via the UBL domain to degrade unfolded proteins. However, how the cochaperone Bag1 bridges the protein folding and degradation systems and how Bag1 enhances the degradation of unfolded proteins remains unknown.
In this paper, using cryoelectron microscopy (cryoEM) and various biochemical and biophysical techniques, we have shown that Bag1 plays a key role in Hsp70-mediated proteasome-dependent protein degradation, not only by physically linking Hsp70 to the proteasome (through its subunit Rpn1), thereby promoting protein delivery to the proteasome but also by inducing a series of conformational changes in the 19S that facilitate degradation of client proteins.

SHORT TALK

Protein phosphatase-2A regulates the formation of cytotoxic protein aggregates through HSP70

Oliver Krämer
University Medical Center Mainz,
Nackenheim, Germany

As a major source of cellular serine and threonine phosphatase activity, protein phosphatase-2A (PP2A) modulates signaling pathways in health and disease. PP2A complexes consist of catalytic, scaffolding, and B-type subunits. Seventeen protein phosphatase-2A (PP2A) B-type subunits direct PP2A complexes to selected substrates. It is ill-defined how PP2A B-type subunits determine the growth and drug responsiveness of tumor cells. Pancreatic ductal adenocarcinoma (PDAC) is a disease with poor prognosis. We analyzed the responses of murine and human mesenchymal and epithelial PDAC cells to the specific PP2A inhibitor phendione.
We assessed protein levels by immunoblot and proteomics and cell fate by flow cytometry, confocal microscopy, and genetic manipulation. We show that murine mesenchymal PDAC cells express significantly higher levels of the PP2A B-type subunit PR130 than epithelial PDAC cells. This overexpression of PR130 is associated with a dependency of such metastasis-prone cells on the catalytic activity of PP2A. Phendione induces apoptosis and an accumulation of cytotoxic protein aggregates in murine mesenchymal and human PDAC cells. These processes occur independently of the frequently mutated tumor suppressor p53. Proteomic analyses reveal that phendione upregulates the chaperone HSP70 in mesenchymal PDAC cells. Inhibition of HSP70 promotes phendione-induced apoptosis and phendione promotes a proteasomal degradation of PR130. Genetic elimination of PR130 sensitizes murine and human PDAC cells to phendione-induced apoptosis and protein aggregate formation. These data suggest that the PP2A-PR130 complex dephosphorylates and thereby prevents the aggregation of proteins in tumor cells.

Regulation of chaperone machineries

Dr. Johannes Buchner
Technical University of Munich
Germany

A chaperone-based quality control system ensures that proteins folds correctly, keep their native conformations and that some of them can be regulated in their activities on the conformational level. Furthermore under physiological and stress conditions unspecific aggregation is prevented. This is especially important also in the context of diseases such as cancer when the cellular protein homeostasis is out of control. Molecular chaperones are cellular machines of protein folding which share the remarkable ability of specifically recognizing non-native proteins and assisting their folding to the native state. There are several classes of molecular chaperones, which evolved independently such as the small heat shock proteins, Hsp70 and Hsp90. Progress in recent years, combining *in vitro* reconstitution and cellular assays, allows us now to define mechanisms and reaction cycles of molecular chaperones as well as the principles of their regulation.

Molecular chaperones in cancer and protein quality control

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Molecular chaperones in cancer and protein quality control

Tuesday June 11th 2024

Session #2
Protein Quality Control II

Chairperson: ***Lea Sistonen***

cnio stop cancer

The intersection between cellular stress response pathways, proteostasis, and cancer cell survival

Jeffrey L. Brodsky
University of Pittsburgh, Kenneth P. Dietrich School of Arts and Sciences, Pittsburgh, US

Protein homeostasis (proteostasis) regulates tumor growth and proliferation when cells are exposed to proteotoxic stress, such as during treatment with certain chemotherapeutics. Consequently, cancer cells depend to a greater extent on stress signaling, and—amongst other pathways—require the integrated stress response (ISR), amino acid metabolism, and efficient protein folding and degradation pathways to survive. To define how these interconnected pathways are wired when cancer cells are challenged with proteotoxic stress, we developed specific inhibitors of the Hsp70 chaperone, which oversees cellular proteostasis. We then found that the parent compound, as well as second and third generation compounds, show efficacy in various disease models. Next, we discovered that Rhabdomyosarcomas and some breast cancer lines are especially sensitive to the most potent Hsp70 inhibitor, one that compromises the activity of Hsp70 by an Hsp40 co-chaperone. Amongst a range of the breast cancer cells, their relative resistance to Hsp70 inhibition/proteostasis collapse correlated with endogenous levels of autophagy. Moreover, autophagy activation was downstream of the ISR, and ISR induction occurred via the action of two upstream sensors, GCN2 and PERK, in stress-resistant and stress-sensitive breast cancer cells, respectively. In our most recent work, we found that amino acid supplementation, and in particular arginine, was sufficient to initiate cancer cell death and limit autophagy. Consistent with the importance of amino acid availability, which when depleted activates GCN2 and autophagy, resistant cancer cells underwent apoptosis when the Hsp70 inhibitor was combined with an mTORC1 activator, which also suppresses autophagy. These data position amino acid abundance, GCN2, mTORC1, and autophagy as integrated therapeutic targets whose coordinated modulation regulates the survival of proteotoxic-resistant breast cancer cells.

SHORT TALK

Cotranslational folding of the eukaryotic proteome is mediated by inter-chaperone dynamics

Mauricio Aguilar Rangel
Stanford University, US

As polypeptides emerge from the ribosome during translation, they encounter a crowded and aqueous environment not propitious for the correct spontaneous folding of proteins. Although some small polypeptides can independently acquire their native states in the cytosol, most proteins precise the aid of molecular chaperones to avoid kinetically unfavourable intermediates that could lead to their aggregation or misfolding. Chaperones can interact with the nascent polypeptide even before it starts emerging from the exit tunnel of the ribosome and continue to do so as the nascent chain grows in size and topological complexity, demanding more specialized systems, such as the chaperonin TRiC in eukaryotes. Therefore, understanding the synergistic recruitment of the different protein chaperones to the nascent chain is paramount for understanding protein biogenesis. Here we use selective ribosome profiling sequencing on the main cotranslationally-acting chaperone systems in S. cerevisiae, allowing us to map the synergistic mode of binding of SRP, NAC, SSB2 (HSP70), Prefoldin, and TRiC to the nascent translatome. Our analysis reveals how this network of chaperones orchestrates the cotranslational folding of proteins and how this process is coupled to the speed of translation elongation.

Molecular chaperones in cancer and protein quality control

SHORT TALK

Canonical prefoldin associates to human chromatin where it interacts with the FACT histone chaperon and regulates nucleosome dynamics during transcription elongation

Sebastián Chávez Canonical

Institute of Biomedicine of Seville (IBiS),
Spain

The primary function of prefoldin is well-established as a cytoplasmic co-chaperone that favours the co-translational folding of actin and tubulin monomers and transfers them to the ATP-dependent class II chaperonin CCT. The canonical form of prefoldin comprises six subunits known as PFDN1- 6. This complex is ubiquitous across archaea and all eukaryotic organisms, and exhibits a highly conserved structure, consisting of two α and four β subunits.

While prefoldin's role in the cytoplasm is widely recognized, it is also found in the nucleus, where it has been associated with transcription regulation and pre-mRNA splicing. However, the precise molecular mechanisms connecting human prefoldin to the control of gene expression remain elusive. To unravel these mechanisms, we followed different proteomic and genetic approaches. We employed mass spectrometry and genome wide sequencing techniques to identify prefoldin interactors in chromatin, and to delineate the changes that occur in chromatin under prefoldin perturbation conditions. We also analyzed the composition of elongating RNA polymerase-associated factors to visualize how prefoldin contributes to the gene transcription.

Our findings reveal that the whole prefoldin complex is indeed present in human chromatin, localizes to active genes, exhibiting a distribution pattern similar to RNA polymerase II, and locally influences the expression of these genes. We found that this chromatinic prefoldin interacts with the FACT complex, enhancing the presence of this H2A/H2B histone chaperone at transcriptionally active genes.

Accordingly, we found that prefoldin was required for the optimal cotranscriptional dynamic of histones, and more specifically, allowing the removal of non-canonical H2A-H2B dimers. In agreement with FACT´s role in preserving chromatin integrity, we found that lack of prefoldin provoked abnormal accessibility to intragenic regions of transcribed genes and activation of repressed repetitive elements.

Molecular chaperones in cancer and protein quality control

Selectivity versus Promiscuity in Client Binding by Hsp70s

Dr. Eugenia Clerico
Department of Biochemistry and Molecular Biology, University of Massachusetts-Amherst
Amherst, US

Authors: Eugenia M. Clerico, Lea M. Doerries and Lila M. Gierasch
The family of Hsp70 cellular chaperones play a pivotal role in proteostasis, engaging in a myriad of cellular functions by leveraging their ability to bind to diverse sequences. While the consensus is that Hsp70s bind short hydrophobic segments on their client proteins, the detailed mechanism of client recognition is more complex as some Hsp70s can bind proteins selectively, even independently of J proteins, and there are variations in the degree of client selectivity observed among Hsp70 isoforms. In past work (PNAS, 2021), we found that the E. coli Hsp70, DnaK, binds various sequences both in crystals and in solution in a C- to N-, non-canonical orientation, prompting us to explore more deeply the determinants and functional implications of substrate binding orientation. In our investigation into binding orientation, we rely on 13C NMR resonance signatures and disulfide-mediated crosslinking assays. The results have revealed the key role played by compatibility of substrate sidechains with pockets on the chaperone and the dominance of this driver in determination of preferred binding orientation. This finding was particularly clear when palindromic peptides were examined, as these substrate models negated the influence of the backbone on orientation. Through the systematic use of peptide variants as model Hsp70 substrates, we conclude that optimal side-chain accommodation within binding pockets is the primary factor that governs orientation, although backbone involvement emerges under certain conditions. Notably, there are side chains that have stringent requirements for Hsp70 substrate-binding pockets, and their fit overrides other factors. Taken together, our findings elucidate a hierarchy whereby sequence information governs both orientation and side-chain fit to pockets in the Hsp70 binding site. The enhanced understanding this work provides sheds light on mechanisms of substrate binding by Hsp70s and how this enables them to carry out their diverse cellular functions.

Proteomic Instability of Cancer and Non-oncogene Addiction: Heat Shock Factor 1 (HSF1) as an Oncogenic Enabler

Dr. Chengkai Dai

Center for Cancer Research, National Cancer Institute
Frederick, USA

Unlike genomic instability, the implications of proteomic instability in cancer remain ambiguous. By governing the heat shock response or proteotoxic stress response, heat shock factor 1 (HSF1) sustains proteomic stability upon environmental insults. Apart from its importance to stress resistance and survival, HSF1 acts as a powerful, generic oncogenic enabler. Sharply contrasting with its dispensability for non-transformed cells, HSF1 becomes essential to malignancy, a phenomenon referred to as "HSF1/non-oncogene addiction of cancer". In human cancers, HSF1 is frequently overexpressed and correlated with poor clinical outcomes. Accordingly, HSF1 is emerging as an attractive therapeutic target. To elucidate the key mechanism underlying the universal pro-oncogenic role of HSF1, we employed the Neurofibromatosis type I (NF1) model system. In NF1-deficient malignant peripheral nerve sheath tumor (MPNST) cells, HSF1 depletion prompted protein polyubiquitination, aggregation, and even tumor-suppressive amyloidogenesis. In stark contrast, HSF1 is dispensable for the proteome of non-transformed human Schwann cells, the cell-of-origin of MPNSTs. Mechanistically, in MPNST cells HSF1 defends the essential mitochondrial chaperone HSP60 against the direct assault from soluble amyloid oligomers. To survive and adapt to impaired protein quality, owing to HSF1 deficiency, MPNST cells mobilized JNK to repress mTORC1 and protein translation, thereby attenuating protein quantity to alleviate proteomic imbalance. mTORC1 stimulation, via either pharmacological JNK blockade, genetic TSC2 depletion, or supplement with the leucine analog NV-5138, markedly aggravated the proteomic imbalance elicited by HSF1 deficiency. Importantly, this severe proteomic imbalance instigated necrotic cell death partly through unimpeded amyloidogenesis. Thus, HSF1 safeguards the cancer proteome to enable the oncogenic potential of mTORC1. Notably, this proof-of-concept study suggests driving proteomic imbalance as a counterintuitive yet promising therapeutic strategy to combat malignancies.

Function of Heat Shock Transcription Factors in Epithelial-Mesenchymal Plasticity

Dr. Lea Sistonen

Cell and Molecular Biology at Åbo Akademi University; Group Leader at Turku Bioscience Center, University of Turku and Åbo Akademi University
Turku, Finland

Heat shock factors (HSFs) are the main transcriptional regulators of the evolutionarily conserved heat shock response. Among the members of the HSF family, HSF1 is the major stress-responsive factor and its contribution to malignant transformation has been widely studied. In contrast, although HSF2 has been associated with both physiological and pathological processes, the underlying regulatory mechanisms have remained unknown. Here, we analyzed the expression and subcellular localization pattern of HSF2 in a comprehensive selection of human tissues and explored the functional consequences of HSF2 regulation in a breast cancer model. We demonstrate that in benign human tissues, HSF2 displays a strong cytoplasmic localization pattern in all studied smooth muscle and endothelial cells, while its nuclear expression was limited to only a few cell types. In contrast, at the primary stages of breast cancer, ductal carcinoma in situ, HSF2 localized to the nucleus of rapidly proliferating cells in the breast duct. To characterize the function of HSF2 in malignant transformation, we treated human breast cancer cells with transforming growth factor-beta (TGF-beta) to mimic activation of invasiveness involved in epithelial-mesenchymal plasticity. TGF-beta stimuli dramatically downregulated HSF2 levels and activated target genes crucial for the acquisition of pro-metastatic behavior. Intriguingly, forced expression of HSF2 disrupted the TGF-beta-mediated gene program by dysregulating the expression of cell cycle regulators, extracellular matrix, and adhesion-related genes. Accordingly, cells expressing ectopic HSF2 displayed induced cell proliferation and reduced migration both *in vitro* and *in vivo*. Altogether, our findings expand the physiological and pathological landscape of HSF2, demonstrating that HSF2 expression is strictly controlled in benign tissues, whereas its dysregulation is connected to induced proliferation and invasiveness in different stages of breast cancer.

Molecular chaperones in cancer and protein quality control

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Madrid 10th – 12th June 2024

Molecular chaperones in cancer and protein quality control

Tuesday June 11th 2024

Session #3
Chaperones, Molecular Mechanisms and Structure

Chairperson: ***Gabriela Chiosis***

cnio stop cancer

The PAQosome, a HSP90 co-chaperone for protein complex assembly and maturation; implication in disease

Dr. Benoit Coulombe
Montreal Clinical Research Institute (IRCM), University of Montreal Québec, Canada

Biogenesis of the human interactome is a poorly understood process and the machinery involved in protein complex assembly and maturation remains mostly elusive. The Particle for Arrangement of Quaternary structure (PAQosome) is a 12-subunit co-factor of the HSP90 molecular chaperone, initially described for its involvement in assembly of RNA polymerase (pol) II and sno-RNPs. Subsequent studies performed by different groups revealed that the PAQosome participates in the assembly and maintenance of several additional complexes including U4 and U5 snRNPs, telomerase, selenoprotein mRNPs, RNA pol I and III, all six members of the PIKK family, the ribosome and others. Three mechanisms for differential client selection by the PAQosome have been identified so far, including the use of alternative i) molecular adaptors, ii) core subunit homologues, and iii) posttranslational modifications (PTMs). These three mechanisms of client selection, if used in combination, support the idea that the PAQosome regulates the assembly of several additional complexes. Data aimed at building a complete repertoire of PAQosome clients is lacking, but essential to fully understand the PAQosome function. Most recently, publications targeting molecular mechanisms of disease formation, including some cancers and neurodegenerative diseases, have unraveled implications of the PAQosome. Our group identified mutations in the genes encoding RNA pol III subunits as causative of a neurodegenerative disorder termed leukodystrophy. Interestingly, several leukodystrophy-causing pol III subunit variants were found defective in assembly of a complete pol III complex. Strikingly, interactions with the PAQosome are affected. The drug riluzole can restore normal assembly for one specific variant, and the effect of riluzole and other drugs on PAQosome function is currently being investigated.

CryoEM studies of the R2TP cochaperone

Dr. Óscar Llorca
Macromolecular Complexes in DNA Damage Response
Structural Biology Programme
Spanish National Cancer Research Centre - CNIO - Madrid, Spain

The RUVBL1-RUVBL2 complex is an AAA-ATPase essential in multiple cellular processes and pathways. It serves as an integral component of chromatin remodeling complexes implicated in DNA replication, transcription and DNA repair such as INO80 and SRCA. Additionally, it is part of R2TP, a cochaperone complex required for the assembly and activation of RNA Polymerase II, mTORC1, mTORC2 and several other large complexes, in concert with the HSP90 chaperone. The ATPase activity of RUVBL1-RUVBL2 has been linked to cancer progression and poor prognosis, leading to a search for compounds that target RUVBL1-RUVBL2 as a potential therapeutic strategy in cancer.
We have used single-particle cryo-electron microscopy (cryo-EM) to elucidate the structure and mechanisms of R2TP (1,2), and during my talk I will review our current understanding about the structure of RUVBL1 and RUVBL2 and how these ATPases work in the context of the R2TP complex, including our latest work on CB-6644. CB-6644 was described as a potent small-molecule allosteric inhibitor of RUVBL1-RUVBL2 with activity against cancer cells (3), but for which, its binding site and mechanism of action are unknown. We have determined the cryo-EM structure of the complex between RUVBL1-RUVBL2 and this compound. This along with additional cryo-EM, biochemical and mutational studies, reveal not only how CB-6644 works but also provides important mechanistic insights into RUVBL1-RUVBL2 function that were previously known.

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SHORT TALK

Regulation of oncogenic kinases by HSP90 molecular chaperone complexes

Jasmeen Oberoi
University of Sussex, Brighton,
UK

Regulation of client protein kinases by the HSP90 chaperone system is affected by phosphorylation at multiple sites on HSP90, on the kinase specific co-chaperone CDC37, and the kinase client itself, generating a complex network of posttranslational regulation of HSP90-chaperone complexes. We have determined the cryoEM structure of the oncogenic kinase BRAF(V600E) bound to HSP90-CDC37, showing how the V600E mutation favours BRAF association with HSP90-CDC37.
Further CryoEM structures of HSP90-CDC37-BRAF(V600E) complexes with the Ser/Thr phosphatase PP5, together with proteomic analysis, reveal how PP5 is activated by recruitment to HSP90 complexes, to comprehensively dephosphorylate HSP90-dependant RAF kinases, consequently disrupting interactions with important regulatory proteins. Further understanding this cycle of kinase regulation, presents opportunities to identify new ways of disrupting the cellular stabilisation of oncogenic kinases by the HSP90 system.

SHORT TALK

Structural recognition and stabilization of tyrosine hydroxylase by the J-domain protein DNAJC12

Jimena Muntaner Pérez-Urria
Spanish National Centre for Biotechnology (CNB-CSIC),
Madrid, Spain

Pathogenic variants of the J-domain protein and Hsp70 cochaperone DNAJC12 cause parkinsonism, which seems associated with a defective interaction of DNAJC12 with tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis. Here, we report the characterization of TH:DNAJC12 complex formation, showing that DNAJC12 binding stabilizes TH and delays its timedependent aggregation in an Hsp70-independent manner, while maintaining TH activity and regulatory inhibition by dopamine. Interestingly, although DNAJC12 alone is less efficient than other DNAJs to activate Hsc70, the TH:DNAJC12 complex efficiently stimulates its ATPase activity. Cryoelectron microscopy reveals two DNAJC12 monomers bound per TH tetramer, each embracing one of the two regulatory domain dimers, leaving all active sites available for substrate and dopamine interaction. Biochemical data confirm the key role of the DNAJC12 last eight residues in TH binding, explaining the molecular disease mechanism of C-terminal truncated DNAJC12 variants.

Molecular chaperones in cancer and protein quality control

Towards understanding the functions of the PAQosome and its subcomplexes

Dr. Walid Houry
Biochemistry, University of Toronto
Ontario, Canada

R2TP is a chaperone complex consisting of the AAA+ ATPases RUVBL1 and RUVBL2, as well as RPAP3 and PIH1D1 proteins. My group discovered R2TP in a screen for Hsp90 interactors. R2TP is responsible for assembly of macromolecular complexes mainly acting through different adaptors. R2TP also functions as part of a larger chaperone complex that we termed the PAQosome. Using proximity labeling mass spectrometry, we identified DPCD as an adaptor of R2TP. Here, we demonstrate that R2TP-DPCD influence ciliogenesis initiation through a unique mechanism by interaction with Akt kinase to regulate its phosphorylation levels rather than its stability. We further show that DPCD is a heart-shaped monomeric protein with two domains. A highly conserved region in the CHORD-containing proteins and SGT1 (CS) domain of DPCD interacts with RUVBL2 DII domain with high affinity to form a stable R2TP-DPCD complex both in cellulo and *in vitro*. Considering that DPCD is one among several CS-domain containing proteins found to associate with RUVBL1/2, we propose that RUVBL1/2 are CS-domain binding proteins to regulate complex assembly and downstream signaling. The function of R2TP as part of the PAQosome will also be discussed.

SHORT TALK

Structures of the Tubulin cofactors as GTP-dependent multi-subunit chaperone for alpha/beta-tubulin biogenesis

Jawdat Al-Bassam
University of California – Davis,
US

Microtubule polarity and dynamic polymerization originate from the self-association properties of the a-tubulin heterodimer. For decades, it has remained poorly understood how the tubulin cofactors, TBCD, TBCE, TBCC, and the Arl2 GTPase mediate a-tubulin biogenesis from α- and β-tubulins. Here, we use cryogenic electron microscopy to determine structures of tubulin cofactors bound to αβ- tubulin. These structures show that TBCD, TBCE, and Arl2 form a heterotrimeric cage-like TBC-DEG assembly around the a-tubulin heterodimer. TBCD wraps around Arl2 and almost entirely encircles -tubulin, while TBCE forms a lever arm that anchors along the other end of TBCD and rotates α-tubulin. Structures of the TBC-DEG-αβ-tubulin assemblies bound to TBCC reveal the clockwise rotation of the TBCE lever that twists a-tubulin by pulling its C-terminal tail while TBCD holds -tubulin in place. Altogether, these structures uncover transition states in αβ- tubulin biogenesis, suggesting a vise-like mechanism for the GTP-hydrolysis dependent a-tubulin biogenesis mediated by TBC-DEG and TBCC. These structures provide the first evidence of the critical functions of the tubulin cofactors as enzymes that regulate the invariant organization of αβ-tubulin, by catalyzing α- and β-tubulin assembly, disassembly, and subunit exchange which are crucial for regulating the polymerization capacities of αβ-tubulins into microtubules.

SHORT TALK

J-domain proteins: the role of their dimeric state in the Hsp70 Chaperone Machinery

Veronika Lashkul
Center for Molecular Biology of Heidelberg university (ZMBH), Germany

J-domain proteins (JDPs) are multi-domain proteins, characterized by an α-helical hairpin J-domain. JDPs are essential co-chaperones of Hsp70s, and together with nucleotide exchange factors (NEFs) they form the tripartite chaperone machinery involved in a wide variety of protein folding processes. As a part of this machinery, JDPs perform several functions: they recognize, bind and present non-native but also a select set of native proteins to Hsp70s; and, with their J-domain, they stimulate the ATPase activity of Hsp70s allowing Hsp70s to trap the substrates.
The most ubiquitous class A and B JDPs are present in cells in a dimeric state. However, it is still enigmatic as to why they are dimeric, and how this state may be favorable for the cell. Here, we used two approaches: we designed monomeric variants of DnaJ (E. coli class A JDP) as well as heterodimers comprising both wildtype and mutant protomers of DnaJ. Using a wide variety of methods *in vitro* (luciferase refolding, prevention of aggregation and single turn-over ATPase assays) and *in vivo* (complementation and swimming assays), we are determining for which particular process of cellular proteostasis the dimeric state of DnaJ is essential and which of the domains of DnaJ is needed in the dimeric state.
This work was funded by the Deutsche Forschungsgemeinschaft (DGF, German Research Foundation), Project-ID 462625623-MA 1278/10-1.

Elucidating the structural basis of the URI prefoldin-like complex

Nabil Djouder / Rayan Naser

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Prefoldins, highly conserved across evolution, form a heterohexameric chaperone complex in archeabacteria, comprising two α and four β subunits (α2β4). This complex adopts a jellyfish-like structure, with β-hairpin interactions at the top and coiled coils tentacles protruding from it. One known function of this complex is the assessment in the correct folding of actin and tubulin. About twenty years ago, the unconventional prefoldin RPB5 interactor (URI) prefoldin-like complex was discovered, consisting of 2 α subunits (URI and STAP1) and 3 β subunits (PFDN2, PFDN6 and PFDN4r). Despite numerous in vivo findings underscoring URI's essential role in cell homeostasis, its precise function remains elusive.

Using advanced protein engineering techniques, state-of-the-art biochemistry methods, and cutting-edge imaging techniques such as cryo-electron microscopy (cryo-EM) and crystallography, we aim to elucidate the structural basis and functional mechanisms of the URI prefoldin-like complex. Our approach involves expressing the URI prefoldin-like complex members in a MultiBac system, followed by production and reconstitution. Preliminary findings suggest that the newly discovered prefoldin ASDURF may serve as the potential missing β subunit, contributing to the formation of the heterohexameric URI prefoldin-like complex. Additionally, our experiments demonstrate that the URI prefoldin-like complex remains stable under various experimental conditions.

Furthermore, negative staining EM has provided initial insights into the overall structure of the URI prefoldin-like complex, laying the groundwork for further investigations. These results mark significant progress in our ongoing efforts to uncover the cellular implications and molecular behavior of the URI prefoldin-like complex.

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Molecular chaperones in cancer and protein quality control

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Session #4
Chaperones in disease and chaperonotherapy I

Chairperson: ***Walid Houry***

Molecular chaperones in cancer and protein quality control

Epichaperomes in Cancer: Unraveling Molecular Complexity for Therapeutic Innovation and Diagnostic Advancements

Dr. Gabriela Chiosis
Memorial Sloan Kettering Institute
US

HSP90 is not merely a single protein; in disease states such as cancer and neurodegenerative disorders, it metamorphosizes both structurally and functionally giving rise to epichaperomes—distinctive hetero-oligomeric formations of tightly bound chaperones, co-chaperones, and other factors. This transition is not merely a biochemical curiosity; it represents a fundamental cellular mechanism for responding to various disease-associated stressors, whether genetic, proteotoxic, or environmental. Unlike standard chaperones that aid in protein folding, epichaperomes exert a maladaptive influence, sequestering proteins and reshaping the assembly and connectivity of critical proteins that sustain pathological states. The discovery of epichaperomes has profound implications for therapeutic intervention: by selectively targeting the pathological conformations of chaperones within epichaperomes—without interfering with their normal cellular functions—we can significantly enhance the precision and effectiveness of anti-chaperone therapies. This talk will delve into the latest breakthroughs in our understanding of epichaperomes, from their structure and mechanisms to their strategic targeting in therapy.

Molecular chaperones in cancer and protein quality control

Polyphosphate - An Ancient Player in Proteostasis and Cancer

Dr. Ursula Jakob
University of Michigan
Ann Arbor, US

Inorganic polyphosphate (polyP), one of the first high-energy compounds on earth, defies its extreme compositional and structural simplicity with an astoundingly wide array of biological activities across all domains of life. Recent studies demonstrated that this simple polyanion stabilizes protein folding intermediates and scaffolds select native proteins, allowing it to act as molecular chaperone that protects cells against protein aggregation, pro-amyloidogenic factor that accelerates both physiological and disease-associated amyloid formation, and as a modulator of liquid-liquid phase separation processes. These activities help to explain polyP's known roles in stress responses and pathogenicity, provide the mechanistic foundation for its potential role in human neurodegenerative diseases and cancer, and open a new direction regarding its influence on cell migration through condensate formation. The talk will highlight critical unanswered questions and point out potential directions that will help to further understand the pleiotropic functions of this ancient and ubiquitous biopolymer.

SHORT TALK

Nucleolar stress as a driver of aging and neurodegeneration: A ribosomal perspective

Óscar Fernández Capetillo
Spanish National Cancer Research Centre,
Madrid, Spain

For reasons that remain poorly understood, alterations in ribosome biogenesis, translation and nucleoli have a particular impact on neurons and hematopoietic cells. Accordingly, mutations associated to neurodegenerative diseases are frequently related to nucleolar biology. A specific example of this is ALS (amyotrophic lateral sclerosis), a fatal neurodegenerative disease lacking a cure. The most frequent mutation in ALS patients is an intronic repeat expansion in C9ORF72, which leads to the production of 2 types of arginine-rich dipeptide repeats (poly(PR) and poly(GR)) that cause nucleolar stress and cell death. We recently provided a mechanism that explains the toxicity of these peptides. By additional work, we have now discovered that these peptides trigger a widespread accumulation of orphan ribosomal proteins and mTOR hyperactivation, a hallmark of ribosomopathies. In mice, we see that the systemic expression of these peptides causes nucleolar stress and accelerates ageing, which can be substantially alleviated by mTOR inhibition. Our work suggests that ALS might be a motor-neuron specific ribosomopathy. Our current ideas on this project will be discussed.

Molecular chaperones in cancer and protein quality control

SHORT TALK

p53 protein degradation redefines the initiation mechanisms and transitional mutations in colorectal cancer

Irene Herranz

Spanish National Cancer Research Centre,
Madrid, Spain

Incidence of colorectal cancer (CRC) is increasing likely due to unknown mechanisms driving initiation and progression. The initial model proposed by Fearon and Volgestein posits it as a multi-hit neoplasia, originating from adenomatous-polyps induced by WNT activation, ultimately progressing to aggressiveness through p53 loss. Integrating human data with mouse genetics, we redefine this paradigm, highlighting pivotal roles of MYC, oncogenic URI and p53 degradation to initiate CRC. At early stages, APC loss activates MYC to transcriptionally upregulate URI, triggering p53 proteasomal degradation, essential for tumor initiation and mutation burden accrual in CRC mice. Remarkably, reinstating p53 levels via genetic URI depletion or p53 super-expression in intestine of CRC mice with APC loss or β-catenin activation prevents tumour initiation and extends lifespan. Our data reveal a "two-hit" genetic model central to CRC initiation, wherein MYC/URI axis intricately controls p53 degradation, offering mechanistic insights into transitional mutation acquisition essential for CRC progression.

Molecular chaperones in cancer and protein quality control

SHORT TALK

Point mutations of the mitochondrial chaperone TRAP1 affect its functions and pro-neoplastic activity

Claudio Laquatra
University of Padua,
Italy

The mitochondrial chaperone TRAP1 is a key regulator of cellular homeostasis and has important implications in neurodegeneration, ischemia and cancer. Recent evidence has indicated that TRAP1 mutations are involved in several disorders, even though the structural basis for the impact of point mutations on TRAP1 functions has never been studied.
By exploiting a modular structure-based framework and molecular dynamics simulations, we investigated the effect of five high-occurrence mutations within TRAP1 on its structure and stability. Each mutation differentially impacts on longrange interactions, intra and inter-protomer dynamics and ATPase activity. Changes in these parameters reflect on functions as the expression of TRAP1 mutant forms reveal diverse ability of modulating the activity of its interactor succinate dehydrogenase (SDH). In keeping with this, TRAP1 point mutations affect the growth and the migration of aggressive sarcoma cells, and alter the sensitivity to the selective TRAP1 inhibitor. Our work provides new insights about the structure activity relationship of TRAP1 identifying crucial amino acid residues that regulate TRAP1 proteostatic functions and pro-neoplastic activity

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Session #5
Chaperones in disease and chaperonotherapy II

Chairperson: ***Nabil Djouder***

Molecular chaperones in cancer and protein quality control

How HSP90 helps cancer proliferation

Dr. Ritwick Sawarkar
University of Cambridge
Cambridge, UK

The role of molecular chaperones in folding proteins in the cytosol has been intensely investigated. Chaperones such as HSP70 and HSP90 are also present in the nucleus, where they interact with key oncogenic drivers, likely facilitating proliferative transcriptional program. Data showing specific molecular actions of the chaperones HSP70 and HSP90 at sites of gene expression will be discussed in the context of oncogenic transcriptional program.

Molecular chaperones in cancer and protein quality control

SHORT TALK

The chaperone AGR2 contributes to the complex interplay between endoplasmic reticulum stress and inflammation in pancreatic cancer

Irene Felipe Abrio

Spanish National Cancer Research Centre,
Madrid, Spain

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease. The chaperone Anterior gradient protein 2 (AGR2), a member of the protein disulfide isomerase family, is predominantly localized in the endoplasmic reticulum (ER). AGR2 is a specific interactor of the unfolded protein response sensor IRE1β in colon epithelial cells. Dysregulation of AGR2 has been linked to cancer progression and other diseases, including PDAC.

GWAS have identified a CTRB2 exon 6 deletion variant associated with increased PDAC risk. It has been proposed that this variant results in a truncated protein that accumulates in the ER, leading to ER stress, inflammation, and cancer. We developed a new mouse strain recapitulating this variant on its mouse ortholog, Ctrb1 (Ctrb1 exon6-del). We have profiled the pancreas of Ctrb1 exon6-del mice leveraging histology, transmission electron microscopy, protein analysis, RNA-seq, and spatial transcriptomics. Ctrb1 exon6-del mice express a truncated protein that accumulates in the ER, associated with reduced chymotrypsin activity and total protein synthesis. While the histological aspect of the pancreas is inconspicuous, ultrastructural studies show evidence of dramatic ER stress. Transcriptomic analyses show that the pancreas of Ctrb1 exon6-del mice displays elevated ER stress, acinar program downregulation and low inflammation, Agr2 being one of the most upregulated genes. AGR2 is expressed at high levels in Ctrb1 exon6-del acinar cells, whereas it is almost completely absence in WT acinar cells. In mutant pancreas, AGR2 displays a mosaic distribution, suggesting heterogeneous acinar cell responses to stress. AGR2 is also detected in the nucleus of acinar cells together with other chaperones, such as BiP. Cells with high Agr2 expression show an enrichment of a reprogramming signature.

Our results indicate a complex interplay between ER stress and inflammation in pancreatic disease. The upregulation of AGR2 suggests a potential role in pancreatic dysfunction and its relevance as a therapeutic target in diseases involving aberrant protein folding. These studies should contribute to translate the knowledge to humans, offering insights into the pathogenesis of pancreatitis and PDAC, towards potential preventive approaches.

SHORT TALK

Expression of mitochondrial Hsp40 chaperone is beneficial for mitochondrial biogenesis

Grzegorz Ciesielski
University of North Florida,
US

Tid1 is a human mitochondrial Hsp40 chaperone that cooperates with mitochondrial Hsp70, Mortalin. Studies on Tid1 function reported in recent years suggest its somewhat conflicting physiological roles; i.e. on the one hand, Tid1 is widely reported to be necessary for the maintenance of the mitochondrial genome and function of the electron transport chain, and on the other hand, it is reported to serve as a cancer suppressor via the mitochondrial p53 apoptotic pathway. To investigate its function, we have obtained a stable HeLa cell line constitutively overexpressing Tid1. The cell line was healthy, and no apoptosis was observed.
Notably, we observed enhancement in mitochondrial biogenesis as inferred from a three-fold increase in the mitochondrial DNA copy number. This is likely due to an increase in the replication initiation rate as 7S DNA levels were significantly elevated. Notably, the increase of Tid1 levels did not affect levels of the mitochondrial replication factors, Pol γ and TWNK, suggesting that its role in mtDNA replication does not result from facilitating their stability. Investigating further the putative direct role of Tid1 in mitochondrial DNA replication, we observed that *in vitro* Tid1 stimulates DNA synthesis by Pol γ, seemingly by increasing its DNA substrate turnover but not its processivity. This effect was further potentiated in the presence of its chaperone partner, mitochondrial Hsp70, Mortalin. Notably, our biolayer interferometry analysis did not confirm Tid1's affinity for Pol γ. Instead, we found that Tid1 binds DNA directly. Upon these results we propose that Tid1 and Mortalin serve as the mtDNA replication factors.

Clinical applications of targeting HSP90 and other molecular chaperones

Dr. Udai Banerji

NIHR Professor of Molecular Cancer Pharmacology
The Institute of Cancer Research - The Royal Marsden NHS Foundation Trust
London, UK

Heat shock protein 90 (HSP90) inhibitors are an exemplar of drug discovery efforts involving molecular chaperones focusing on vulnerabilities of cancer cells to protein encoded by driver oncogenes. Multiple HSP90 inhibitors have been tested in clinical trials and an oral HSP90 inhibitor pimitespib is licensed for use in pretreated gastrointestinal stromal tumors in Japan. The narrow therapeutic index of this class of anticancer drugs due to degradation of wildtype counterparts of the mutated proteins and other proteins key to survival of normal tissue has led to the use limited use of HSP90 inhibitors. There are ongoing efforts to improve therapeutic indices of HSP90 inhibitors by selectively delivering HSP90 to cancer cells using antibody drug conjugate technology. Further, HSP90 binding drugs have been researched for a range of medical uses including functional imaging modalities like positron emission tomography. More recently, research into the wider field of chaperones, have led to use of drugs binding chaperones such as cyclophilin A to cause functional inhibition of mutant specific KRAS signaling and highlights the potential of current and future chaperone research in oncology.

Molecular chaperones in cancer and protein quality control

SHORT TALK

ONLINE

From Chaperones to Epichaperomes: Phosphorylation Triggered Shape and Function Shifting of HSP90

Tanaya Roychowdhury
Memorial Sloan Kettering Cancer Centre,
NY, US

While the intricate network of client-chaperone interactions is vital for cellular homeostasis, cells respond to stress by forming specialized, long-lived multimeric higher molecular weight chaperone complexes known as 'epichaperomes'. These complexes play a pivotal role in rewiring protein-protein interaction networks, ultimately influencing cellular adaptability and proliferation, and potentially contributing to disease development.
This presentation highlights recent findings on the structural and regulatory aspects of epichaperomes, with special emphasis on the significance of post-translational modifications (PTMs) in shaping their structure and function. A key finding is the identification of specific PTMs on HSP90 at serine residues situated within an intrinsically disordered region encompassing the charged linker, as critical determinants in epichaperome assembly. Our data demonstrate that the phosphorylation of these serine residues promotes HSP90's interaction with other chaperones and co-chaperones, leading to the formation of the long-lived multimeric epichaperome complexes. Furthermore, our study establishes a direct link between epichaperome function and cellular physiology, especially in contexts where robust proliferation and adaptive behavior are essential, such as stem cell maintenance and cancer. These findings not only provide mechanistic insights but also hold promise for the development of novel therapeutic strategies targeting epichaperomes, thus aiding the transition from bench to bedside.

Exploring chaperone vulnerabilities in (cancer) senescence

Rahul Samant
Babraham Institute
UK

Cellular senescence—an irreversible state of cell-cycle arrest—is a hallmark of both ageing and cancer. Senescent cells arise in response to a range of toxic stresses, including oncogene activation and DNA damage. Therefore, the senescent state is proposed to have evolved, amongst other roles, as a potent anti-tumour mechanism in multi-cellular life. However, several cytotoxic cancer therapeutic interventions themselves induce senescence in a subset of the treated tumour cells. Such therapy-induced senescence is emerging as a major driver of tumour resistance and, through acquisition of a hyper-secretory, pro-inflammatory phenotype, contributes to relapse and recurrence in a cell non-autonomous manner. Interventions to selectively eradicate senescent cancer cells are currently highly sought-after. Across a range of stress-induced senescence models in human primary and cancer cells, we have discovered differences in protein quality control systems between proliferative and senescent states—especially at the level of molecular chaperones and ubiquitin-mediated clearance systems. Given recent findings that proteostasis modulators act as senolytics, our work points towards specific vulnerabilities in protein quality control that could be exploited as part of two-hit cancer therapeutic strategies.

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Closing Lecture

Chairperson: ***Nabil Djouder***

Molecular chaperones in cancer and protein quality control

Targeting the Cell's Stress Pathways for Therapeutic Benefit in Cancer

Paul Workman

Centre for Cancer Drug Discovery, The Institute of Cancer Research, London, UK

Based on an innovative phenotypic cell-based screen targeting the Heat Shock Factor 1 (HSF1) activity, followed by multiparameter medicinal chemistry optimisation, we discovered NXP800, an orally active, potent inhibitor of cancer cell proliferation. Evaluation in a mini-panel of human cancer cell lines and tumour xenografts revealed high sensitivity in ARIDIA-deficient human ovarian cancer models, subsequently confirmed in the large Sanger panel and isogenic systems. Using RNAseq we identified overlapping gene expression modulation in human cancer cell lines exposed to NXP800, including expected changes in HSF1-regulated genes together with alterations in ATF4-regulated gene expression associated with activation of the integrated stress response (ISR). Consistent with activation of the ISR, NXP800 induces phosphorylation of EIF2a and increased expression of downstream ISR markers/effectors ATF4, ATF3, CHAC1 and CHOP – both in human ovarian cells *in vitro* and corresponding tumour xenograft models *in vivo*. Using either systematic siRNA knockdown, CRISPR knockout or inhibition by two small-molecule tool compounds from different chemotypes, we discovered that GCN2 alone is required for the phosphorylation of EIF2a, ATF4 induction and activation of the ISR by NXP800. Also, inactivation of GCN2 markedly reduced the antiproliferative activity of NXP800. Furthermore, ISR induction inhibited HSF1 activation, confirming the mechanistic link between ISR activation and inhibition of HSF1-mediated transcription. NXP800 induced regression in ARID1A-deficient human ovarian tumour xenografts and also in endometrioid tumour xenografts deficient in ARID1A or ARID1B. Studies are currently underway to determine precisely how NXP800 stimulates GCN2 activity and the role of ARID1A deficiency. NXP800 exhibits minimal PGP efflux; good oral PK/PD biomarker properties; clean safety panel profile; clear therapeutic index; consistent Pharmacological Audit Trail; and acceptable dose-to-human prediction – so was progressed into clinical development. NXP800 has received FDA Fast Track Designation in ARID1A mutant ovarian cancer; Phase 1a is complete and Phase 1b is underway (NCT05226507) in collaboration with the GOG Foundation and the European Network of Gynecological Oncological Trial Group (ENGOT). NXP800 has also received FDA Orphan Designation in bile duct cancer.

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Organisers & Speakers' Biographies

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Dr. Gabriela Chiosis

Memorial Sloan Kettering Institute
US

Dr. Gabriela Chiosis' research focuses on understanding how stressors—internal and external factors disrupting biological systems—impact human diseases. Recognizing the complexity of diseases that involve multifaceted origins and cellular responses, her work shifts away from traditional protein-centered approaches. Instead, it manipulates epichaperomes to target protein-protein interaction (PPI) networks that dictate cellular responses to stressors, affecting entire biological systems. By mapping these networks and their dysfunctions, her research aims to develop precise, effective treatments for complex diseases like cancer and neurodegenerative disorders, thereby advancing the field of network precision medicine.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Nabil Djouder

Growth Factors, Nutrients and Cancer Group Molecular Oncology Programme
Spanish National Cancer Research Centre-CNIO
Spain

Dr. Nabil Djouder, a French scientist, obtained his PhD in Molecular Pharmacology and Pharmacochemistry from the University of Strasbourg. Djouder's pioneering work includes investigating the URI prefoldin-like complex, a heterohexameric chaperone complex with implications in cellular homeostasis and disease development. Through the creation of genetically engineered mouse models, Djouder and his colleagues have shed light on URI's role in oncogenesis, its modulation of protein activity in response to environmental stressors, and its potential as a regulator of cellular homeostasis. His research indicates that URI may be an important cellular machinery which loss will lead to early embryonic development and which function might be a crucial component of a quality control system that mitigates proteotoxicity and suppresses disease development. Djouder's advocacy for this field underscores the importance of unraveling the URI prefoldin-like complex's role in various pathologies, including cancer, thus paving the way for future advancements in prefoldin biology in disease therapeutics.

Molecular chaperones in cancer and protein quality control

Dr. Judith Frydman

Stanford University
US

Judith Frydman is a Professor in the Departments of Biology and Genetics at Stanford University. She received her PhD from the University of Buenos Aires, Argentina and did postdoctoral training in Ulrich Hartl's lab, where she discovered the eukaryotic chaperonin TRiC/CCT and showed protein folding in eukaryotic cells occurs cotranslationally with the aid of molecular chaperones.
The Frydman lab aims to understand how the network of molecular chaperones and the ubiquitin-proteasome pathway maintain proteostasis in eukaryotic cells and how its disfunction leads to disease and aging. Her lab also harnesses these insights to develop therapeutic approaches to ameliorate human diseases including neurodegenerative diseases and to identify interventions that disfavor the production of toxic protein species.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Óscar Llorca

Macromolecular Complexes in DNA Damage Response Group
Structural Biology Programme
Spanish National Cancer Research Centre - CNIO
Madrid, Spain

Óscar Llorca obtained his PhD in 1996 from the Autonomous University of Madrid. Then, he performed his postdoctoral studies at the Institute of Cancer Research (ICR), London, UK, with a Marie Skłodowska-Curie fellowship. In 2002 he moved back to Spain as a Group leader at the Centre for Biological Research (CIB), CSIC, Madrid. Since June 2017, he has been leading the Macromolecular Complexes in DNA Damage Response Group at CNIO, and he is also a Director of the Structural Biology Programme. His main interest is the use of cryo-electron microscopy to study large macromolecular complexes in the DNA damage response and DNA replication, and molecular chaperones. He has published 126 articles in peer-reviewed journals, including Nature, Science, Nature SMB, Nature Communications, Molecular Cell, The EMBO Journal, Proceedings of the National Academy of Sciences (PNAS), and several others.

Molecular chaperones in cancer and protein quality control

Dr. Paul Workman

Centre for Cancer Drug Discovery, The Institute of Cancer Research,
London, UK

Paul Workman is Harrap Professor of Pharmacology and Therapeutics and Group Leader of the Signal Transduction and Molecular Pharmacology team at The Institute of Cancer Research (ICR), London. Until August 2021, he served for seven years as Chief Executive and President of ICR, and for almost twenty years was Director of ICR's CRUK Cancer Therapeutics Unit. Paul was also Founding Director of the CRUK Convergence Science Centre at ICR/Imperial College and currently is Co-Director of the CRUK Children's Brain Tumour Centre of Excellence at ICR and Cambridge University. Paul has been instrumental in the discovery of multiple clinical candidates, acting on protein kinases, PI3 kinases, the molecular chaperone HSP90, and the Integrated Stress Response/Heat Shock Factor 1 pathway.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Udai Banerji

NIHR Professor of Molecular Cancer Pharmacology
The Institute of Cancer Research - The Royal Marsden NHS Foundation Trust
London, UK

Professor Udai Banerji is the Deputy Director of the Drug Development Unit. He plays a key role in linking the pre-clinical expertise in drug discovery at The Institute of Cancer Research to the first-in-human evaluation of these drugs at the Royal Marsden NHS Foundation Trust. His interests include the discovery and development of AKT, RAF, CHK1, MPS-1, HSF-1 and folate targeted drugs. He has been the principal or sub-investigator of over 100 first-in-human clinical trials of anti-cancer agents.

In addition to running phase I clinical trials, Professor Banerji's independent laboratory interests include biomarkers and drug resistance. He heads the Clinical PD Biomarker group that sets up, validates and runs pharmacodynamics assays to be used on normal and tumour tissue to support phase I studies. He also runs the Clinical Pharmacology Adaptive Therapy team focused on understanding mechanisms of resistance to targeted therapy and generating hypotheses for trials of combinations of targeted therapy.

Molecular chaperones in cancer and protein quality control

Dr. Jeffrey L. Brodsky

University of Pittsburgh, Kenneth P. Dietrich School of Arts and Sciences
Pittsburgh, US

Jeffrey Brodsky is the Avinoff Professor of Biological Sciences, and he directs the Center for Protein Conformational Diseases at the University of Pittsburgh. Dr. Brodsky received his Ph.D. at Harvard University and performed post-doctoral research at the University of California, Berkeley, prior to joining the faculty at Pittsburgh in 1994. His research seeks to understand: how non-native proteins are destroyed, (2) how chaperones mediate protein quality control "decisions", (3) how stress responses affect proteostasis, and, most relevant, (4) whether chaperone-targeted drugs can ameliorate diseases in which the proteostasis network is altered, most notably cancer.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Johannes Buchner

Technical University of Munich
Germany

Johannes Buchner received his PhD from the University of Regensburg, Germany, After a postdoctoral stay at the National Cancer Institute in Bethesda, USA, he was a group leader at the University of Regensburg. Since 1998 he is a professor at the Technische Universität München, Munich, Germany. He is a member of the Leopoldina, the German National Academy of Sciences. His research interests include mechanistic studies on molecular chaperones, principles of antibody structure, as well as their evolution. Johannes Buchner received several prizes, including the Hans Neurath Award, the Kossel-Award, the Max Bergmann Medal, the Schleiden Medaland the Warburg Medal.

Molecular chaperones in cancer and protein quality control

Dr. Eugenia Clerico

Department of Biochemistry and Molecular Biology, University of Massachusetts-Amherst
Amherst, US

My doctoral research in Argentina under the direction of Mario Ermacora focused on protein folding. Loving this field, I augmented my training by postdoctoral work with Susan Golden studying proteins regulating circadian rhythms. In my current position as a Research Associate Professor at UMass Amherst, my research explores the roles of Hsp70 chaperones in protein homeostasis. In collaboration with Lila Gierasch, I am delving into the mechanisms underlying Hsp70 function, including interactions with co-chaperones and substrates. My goal is to advance our understanding of Hsp70 biology, paving the way for therapeutic interventions targeting protein folding diseases and other Hsp70-related pathologies.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Benoit Coulombe

Montreal Clinical Research Institute (IRCM), University of Montreal
Québec, Canada

Benoit Coulombe is the Director of the Translational Proteomics Research Unit of the Montréal Clinical Research Institute, and Full Research Professor at the Department of Biochemistry and Molecular Medicine of the University of Montréal. He was an undergraduate in Biochemistry, and obtained his PhD from the University of Montréal. Dr. Coulombe then undertook postdoctoral work at the University of Toronto and at the Free University of Brussels. In 1993, he initiated his academic career as an assistant professor at the University of Sherbrooke. Dr. Coulombe then moved to the IRCM as a Full Research Professor in 2001.

Molecular chaperones in cancer and protein quality control

Dr. Chengkai Dai

Center for Cancer Research, National Cancer Institute
Frederick, USA

Dr. Chengkai Dai is currently a NIH Stadtman Investigator. He received his Bachelor of Medicine degree from Tianjin Medical University, China and his Ph.D. degree from University of Texas-Graduate School of Biomedical Sciences at Houston, USA. As a postdoctoral fellow, he joined Dr. Susan Lindquist's laboratory at Whitehead Institute for Biomedical Research at Boston and studied the role of heat shock factor 1 (HSF1) in tumorigenesis. Following his postdoctoral training, Dr. Dai established his own laboratory at The Jackson Laboratory, Maine, USA. In 2016, he joined the Mouse Cancer Genetics Program at Center for Cancer Research, National Cancer Institute, USA.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Elke Deuerling

University of Konstanz, Department of Biology
Germany

Elke Deuerling is a molecular biologist. She received her PhD in 1995 with distinction from the University of Bayreuth. From 2003 to 2006, she was an independent group leader and Heisenberg Fellow in Heidelberg, and since 2007, she has been a full professor at the University of Constance, Germany. She is known for her pioneering research on the functions of ribosome-associated chaperones and the molecular mechanisms of co-translational protein processing. She was elected as an EMBO member in 2023 and will receive the Walter Neupert Medal in 2024 for her outstanding scientific contributions.

Molecular chaperones in cancer and protein quality control

Dr. Walid Houry

Biochemistry, University of Toronto
Ontario, Canada

Walid A. Houry is Professor in the Department of Biochemistry and Department of Chemistry at the University of Toronto. Dr. Houry obtained his PhD from Cornell University and then did his postdoctoral training at the Sloan-Kettering Institute in New York City and at the Max-Planck-Institute for Biochemistry in Munich, Germany. He is interested in the general area of cellular stress responses and the role of molecular chaperones and proteases in these responses. His group is also interested in the development of novel anticancers, antibiotics, and antivirals by identifying compounds that target these chaperones and proteases and result in the dysregulation of protein homeostasis in the cell. He has been recognized with national and international awards including awards.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Ursula Jakob

University of Michigan
Ann Arbor, US

Ursula Jakob received her Ph.D. in 1995 from Regensburg University, after which she was a postdoctoral research fellow at U-M from 1996 to 1998 with a fellowship from the German government. In 2000, she received the Burroughs Wellcome Fund Career Award in the Biomedical Sciences. She was chosen as a "Biological Scholar," a prestigious recognition made by the University of Michigan. She then became an assistant research scientist until 2001 when she joined the faculty in Molecular, Cellular and Developmental Biology, rising through the ranks to full professor in 2011. In 2011, she received a U-M Faculty Recognition Award, and joined the Department of Biological Chemistry in the University of Michigan Medical School as a secondary appointment. In 2014, she was elected to the Bavarian Academy of the Sciences and Humanities. In the same year, she was named the Patricia S. Yaeger Collegiate Professor. In 2020, Dr. Jakob was elected into the German National Academy of the Sciences.

Molecular chaperones in cancer and protein quality control

Dr. Matthias Mayer

Center for Molecular Biology Heidelberg University (ZMBH), DKFZ-ZMBH-Alliance
Heidelberg, Germany

Matthias P. Mayer studied biology at the University of Freiburg, Germany, and got his PhD in 1990 (supervisor Prof. H. Kleinig). He was postdoctoral research associate in the Department of Chemistry at the University of Utah, Salt Lake City, Utah, USA (PI Prof. C. D. Poulter); at the University Medical Center, Geneva, Switzerland (PI Prof. C. Georgopoulos); and at the Institute for Biochemistry and Molecular Biology of the University of Freiburg, Germany (PI Prof. B. Bukau). In 2002 he moved to the Center for Molecular Biology of Heidelberg University (ZMBH) in Heidelberg, Germany, where he became independent group leader in 2005.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Rahul Samant

Babraham Institute
UK

The Samant Lab at Babraham Institute uses multi-dimensional proteomics, live-cell imaging, and biochemistry to understand how ubiquitin-mediated signalling regulates PQC in healthy and diseased contexts. Rahul did his PhD under Paul Workman (Institute of Cancer Research, UK), studying oncoprotein kinase degradation upon HSP90 inhibitor treatment of cancer cells. His postdoc work with Judith Frydman (Stanford University, USA) probed the importance of ubiquitin chain topology in misfolded protein degradation across subcellular compartments.

A major lab focus is on how proteostasis networks are re-wired in various apoptosis-resistant cellular states—including cancer senescence—with the goal of harnessing vulnerabilities to promote lifelong health.

Molecular chaperones in cancer and protein quality control

Dr. Ritwick Sawarkar

University of Cambridge
Cambridge, UK

Ritwick Sawarkar completed his PhD in evolutionary developmental biology at the Indian Institute of Science (India) in 2010. After a postdoctoral stint at ETH-Zurich, Dr. Sawarkar started his own lab in 2014 at the Max Planck Institute of Immunobiology and Epigenetics and moved to The Medical Research Council (MRC), University of Cambridge (UK) in 2019.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Lea Sistonen

Cell and Molecular Biology at Åbo Akademi University; Group Leader at Turku Bioscience Center, University of Turku and Åbo Akademi University
Turku, Finland

Lea Sistonen is Professor of Cell and Molecular Biology at Åbo Akademi University (Turku, Finland) since 2000 and Group Leader at Turku Bioscience Center (University of Turku and Åbo Akademi University) since 1994. Her research on genome-wide transcriptional programs has contributed to our understanding of the maintenance of protein homeostasis, not only in acute stress but also during normal growth and development as well as in malignant transformation. Using ChIP-seq and PRO-seq techniques in human cells, the Sistonen laboratory has demonstrated the functional relationship between the occupancy of HSFs, changes in the chromatin architecture and de novo transcription of both genes and enhancers.

Molecular chaperones in cancer and protein quality control

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Molecular chaperones in cancer and protein quality control

Poster Session

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01

Protein markers for cancer stem cells identification

Adouda Adjiri, PhD

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Background:
Protein expression is altered in cancer cells with up-regulation of advantageous proteins and down-regulation of disadvantageous ones. While this is accomplished in astonishing coordinated manner; it allows cancer cells to adapt, survive and ensure unlimited growth. The epidermal growth factor receptor 2 (HER2) and the vascular endothelial growth factor (VEGF), as two examples, are up-regulated in different cancers. However, targeting such overly-expressed proteins has not resulted in cancer eradication.
Methodology:
Modeling cancer initiation led to stipulate a gain of new function(s) in transformed cells as compared to normal cells in which such function(s) must be absent. This will mark a clear cut difference between cancer and non-cancer cells. Identification of such new functions and their biochemical characterization will unravel the nature of cancer stem cells, which are behind resistance and recurrence phenomena suffered in clinics today.
Results:
Over expression of a given protein is not causative of transformation but a symptom of metabolic rewiring occurring in transformed cells. The protein model for cancer genesis hypothesizes a gain of function(s) through a breakup of a protein, generating entities capable of bringing new activities to the cell, thus giving rise to cancer stem cells. These gained protein markers could likely be shared with normal cells present in the tumor microenvironment via exosomes, teaching them to support malignancy, as portrayed in (CAFs) and (CAMs), etc.
Conclusion:
Because cancer is a complicated disease, innovative ideas are needed and going beyond genomics can accelerate discoveries and bring relief to cancer patients. The design of innovative cancer treatments requires a deeper understanding of the molecular mechanisms governing metabolic rewiring seen in cancer cells. This leads to unravel protein functions that are present in cancer cells but absent in normal cells. Such discoveries will lead us to put the finger on cancer stem cells, thus helping to eradicate them.

02

Identification of the R2T complex of Arabidopsis thaliana

Alberto Palacios-Abella[1,†], Andrés López-Perrote[2,†], Jasminka Boskovic[2], Sandra Fonseca[3], Cristina Úrbez[1], Vicente Rubio[3], Óscar Llorca[2,*], **David Alabadí[1,*]**

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3 Centro Nacional de Biotecnología, Madrid, Spain
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Heat shock protein 90 (HSP90) is a molecular chaperone that contributes to the maturation and activation of substrates in various cellular pathways. Its activity is supported by various co-chaperones. One of these is R2TP, a complex of RuvBL1-RuvBL2-RPAP3-PIH1D1 in humans, which is involved in the assembly of various multiprotein complexes, including mTORC1 and Box C/D and Box H/ACA snoRNPs. In addition, several R2TP-like co-chaperones have been identified in humans, such as R2T, which lacks PIH1D1, but these are less well characterized. In seed plants, there are no PIH1D1 orthologs. Here we have identified the R2T complex of Arabidopsis thaliana. R2T associates with the prefoldin-like complex *in vivo* through direct interactions between several subunits. AtRPAP3 interacts directly with AtRuvBL2a and recruits AtHSP90 to the complex, as in yeast and mammals. These interactions occur in both the cytosolic and nuclear compartments. We have determined the R2T structure using cryo-EM (see the accompanying poster by López-Perrote et al.) and identified the AtRPAP3 residues that contact AtRuvBL2a. We have demonstrated the importance of these residues for the interaction in planta. Furthermore, we have identified the AtRPAP3 residues contacting the C-terminal MEEVD peptide of AtHSP90 and have demonstrated their importance for the interaction. We also show that AtRPAP3 interacts with AtHSP90 when the chaperone is in an open state. Taken together, our results show that AtRPAP3 recruits AtRuvBL1-AtRuvBL2a and AtHSP90 via a mechanism that is also conserved in other eukaryotes.

03

Prefoldin URI controls non-homologous end joining repair and immunotherapy responses.

Mariana Angulo-Aguado[1], Irene Herranz-Montoya[1], Cristian Perna[2], Solip Park[3] and Nabil Djouder[1]

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2 Department of Pathology, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid 28034, Spain.
3 Computational Cancer Genomics group, Structural Biology Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain.

Colorectal cancer (CRC) is rapidly spreading globally and remains a significant contributor to cancer-related mortality. It is recognized as one of the most challenging cancers to treat with immunotherapy, achieving responses in less than 8% of patients. There is thus an urgent need to understand the mechanisms underlying this resistance to improve treatment strategies and enhance patient survival outcomes. Here, through the integration of *in vitro* systems, genetically engineered mouse models, and bioinformatics studies, we identify the cochaperone unconventional prefoldin RPB5 interactor (URI), as a key component of the non-homologous end joining (NHEJ) DNA repair machinery. URI loss affects the DNA-dependent protein kinase catalytic subunit complex, impairing NHEJ in various cell lines. Consequently, genetic URI ablation in mouse intestinal epithelium not only slow down the progression of APC loss-driven CRC but also increases intratumoral DNA damage, enhances DNA sensing receptor pathways and promotes immune cell infiltration, thereby sensitizing mice to immune checkpoint blockers. Finally, bioinformatics analysis across multiple cancer patient cohorts identifies URI as a potential biomarker for immunotherapy. Our findings emphasize that URI inhibition could serve as a crucial therapeutic target and neoadjuvant for enhancing cancer immunotherapy efficacy.

04

Cryo-EM structure of the c-Raf/Cdc37/Hsp90 complex

Xavi Aran-Guiu[†]; Emily Outwin[†]; Pascale Schellenberger[‡]; Laurence Pearl[†*]

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c-Raf kinase is a central component of the Ras/Raf/MEK/ERK signal transduction cascade, which can be dysregulated in tumour cells resulting in uncontrolled cell proliferation, resistance to apoptosis or resistance to chemotherapy and radiotherapy. c-Raf is stabilised through its interaction with Hsp90 and Cdc37. Previous studies show that inhibitors that either block the ATPase-coupled conformational change of Hsp90, or the interaction of the kinase with Cdc37, target clients for ubiquitination and proteasomal degradation. This highlights the potential of the Hsp90 system as a drug target. Here, we use cryo electron microscopy to elucidate the mechanism of interaction between c-Raf, Cdc37 and Hsp90 in closed conformation.

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05

Chaperoning of p53 by small heat shock proteins (sHsps)

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Department Bioscience, Technische Universität München, Germany

The tumor suppressor protein p53 serves as a guardian of genomic integrity and is crucial for the maintenance of cellular homeostasis. Mutations in the TP53 gene can cause abnormal accumulation of p53 aggregates, which have been described in several types of cancer. To counteract protein misfolding and aggregation, cells rely on a network of molecular chaperones, including the Hsp70 and Hsp90 chaperone families. While these are known to process p53 in an ATP-dependent manner, another family of molecular chaperones, the small heat shock proteins (sHsps), operate independently of ATP by binding to unfolded or misfolded proteins. They are referred to as "holdases" and prevent protein aggregation by stabilizing their substrates and holding them in a refolding-competent state. Two well-studied members of this family are Hsp27 (HspB1) and aB-Crystallin (HspB5). They are known to have diverse functions beyond their role in heat shock response and have found to be overexpressed in various types of cancer. In this study, we investigate how sHsps affect p53 structure and function. We discovered the formation of specific complexes that prevent the tumor suppressor from aggregating. Further analysis revealed an interplay with other components of the chaperone family of proteins, suggesting sHsps to be an integral component of the p53 chaperone pathway.

06

Mechanism of allosteric inhibition of RUVBL1-RUVBL2 ATPase by the smallmolecule CB-6644

Carmen García Martín, Andrés Lopez Perrote, Jasminka Boskovic, Óscar Llorca

Spanish National Cancer Research Center (CNIO)

RUVBL1/2 is a hetero-hexameric ATPase that plays a crucial role in several cellular processes, including chromatin remodeling and the assembly of mTORC1 and mTORC2 (1, 2). CB-6644, an aminopyrazolone compound, inhibits allosterically RUVBL1/2 and has demonstrated antitumor activity in animal models (3). However, its binding and mechanism of action are not yet understood. We applied cryoelectron microscopy to determine the ~2.4 Å resolution structure of the RUVBL1/2 complex bound to ATP and CB-6644, which, together with biochemical experiments, reveals the mechanism of action of CB-6644. ATP binding to RUVBL1/2 induces large conformational changes that CB-6644 recognizes by interacting at the interface between two subunits. CB-6644 traps this ATP-bound conformation and prevents ATP hydrolysis. Interestingly, our results suggest a mechanism that couples nucleotide state, the conformation of RUVBL1/2 DII domains, and the interaction of RUVBL1/2 with other proteins. Our findings reveal how ATP regulates RUVBL1/2 and how this is affected by CB-6644 binding.

References:
(1) The Role of Hsp90-R2TP in Macromolecular Complex Assembly and Stabilization. Lynham, J. and W. A. Houry. 2022. *Biomolecules* 12(8).
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07

Modifications in the Inner Surface of the Synthetic poly-CCT5 Chaperonin to Promote Nanoparticle Encapsulation

Jorge Gutiérrez[1], Sergio Pipaón[1], Jesús G Ovejero[2], María del Puerto Morales[2], Jorge Cuéllar[1] and José M Valpuesta[1].

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2 Department of Materials for Health of the Materials Science Institute of Madrid (ICMM), Sor Juana Inés de la Cruz 3, Campus of the Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain.

Chaperones assist in the de novo protein folding and prevent protein aggregation. One of the most important chaperone families are the chaperonins (Hsp60s), which are organized as two oligomeric back-two-back rings generating a cavity in each ring where the substrate is placed for its folding. The most complex and important of all chaperonins is the eukaryotic CCT (Chaperonin Containing TCP-1) whose structure and the folding mechanism are key for nanotechnological applications. The main aim of this project is to build a stable synthetic cylindrical structure capable of encapsulating chemical reagents or small proteins. It has been shown that CCT5 is able to self-oligomerize. When compared to the eukaryotic CCT, poly-CCT5 is easier to purify, can be genetically modified in all subunits and allows a more manageable image processing. These capabilities could enable poly-CCT5 to act as a nanocontainer delivering molecules to specific targets.
Our group used negative staining EM to assess the encapsulation of various nanoparticles inside synthetic poly-CCT5. VENOFER, an iron-sucrose coating NP, produced the best results overall and was chosen for Cryoelectron microscopy (CryoEM) analysis. We generated a 3.3 Å 3D reconstruction of the NP-bound poly- CCT5, with the NP presumably held by CCT5 apical domains. As part of this project, we are now focusing our efforts on the design and the structural characterization of three poly-CCT5 mutants, which rearrange the charge distribution on the cavity, to improve nanoparticle internalization and to prevent undesired interactions.

POSTER SESSION

08

The cryo-EM structure of the R2T co-chaperone complex

Andrés López-Perrote, Alberto Palacios-Abella, Jasminka Boskovic, Sandra Fonseca, Cristina Úrbez, Vicente Rubio, David Alabadí, Óscar Llorca

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Metazoan R2TP is a complex that functions as a specialized HSP90 co-chaperone and assists the maturation of a subset of clients, including mTOR and ATM kinases, RNA polymerase II or box C/D small nucleolar ribonucleoproteins (snoRNP), among others (1). In addition, adaptor subunits such as the TELO2–TTI1–TTI2 (TTT) complex and the URI1 Prefoldin-like complex help R2TP during the assembly
and maturation of certain clients (2). R2TP is essential and evolutionarily conserved across species from yeast to metazoan, and comprises 4 subunits, RUVBL1, RUVBL2, RPAP3 and PIH1D1. However recent findings show the coexistence of non-canonical R2TP complexes, that lack either RPAP3 or PIH1D1 subunits, assembling the so-called R2P and R2T complexes, respectively (3). Similar to higher eukaryotes, plant R2TP is essential for protein homeostasis. Interestingly, vascular plants such as Arabidopsis thaliana do not have a PIH1D1 homologous gene, and the AtRPAP3 subunit is a simpler version of the human protein. Thus, a reduced version of the complex containing only AtRuvBL1-AtRuvBL2a and AtRPAP3 (R2T) is found in this model organism. Here we analyze the architecture of the Arabidopsis thaliana R2T complex using high resolution cryo-electron microscopy (cryo-EM), which also serves as a model for human R2T complexes. In contrast with yeast and human R2TP where an hexameric RUVBL1-RUVBL2 ring serves as scaffold for the rest of the complex, R2T is assembled as a double-ring dodecameric AtRuvBL1-AtRuvBL2a platform with one AtRPAP3 molecule anchored to each ring. Our results suggest that, although functionally conserved, R2T and R2TP co-chaperone complexes might have differences in their mechanism.

Molecular chaperones in cancer and protein quality control

09

The chaperonin CCT is involved in the interconnection of extracellular vesicle production and lipid metabolism with kinesin dynamics.

Amelia Rojas-Gómez[1,2], Sara G. Dosil[1,2], Francisco J. Chichón[3,4], Nieves Fernández-Gallego[1,2], Alessia Ferrarini[5], Enrique Calvo[5], Diego Calzada-Fraile[2], Silvia Requena[1], Joaquin Otón[7,8], Alvaro Serrano[9], Rocio Tarifa[5], Montserrat Arroyo[1], Andrea Sorrentino[8], Eva Pereiro[8], Jesus Vázquez[2,9], José M. Valpuesta[4], Francisco Sánchez-Madrid[1,2,9], **Noa B. Martín-Cófreces [1,2,9,10].**

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6 Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, UK
7 ALBA Synchrotron Light Source, Barcelona, Spain
8 Laboratory of B Lymphocyte Biology, Fundación Centro Nacional de Investigaciones Cardiovasculares-Carlos III, Madrid, Spain
9 CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain
10 Videomicroscopy Unit, Instituto de Investigación Sanitaria La Princesa (IIS-Princesa), Madrid, Spain

The term "cell proteostasis" encompasses a multitude of processes, including gene transcription, protein translation, the folding of de novo proteins, post-translational modifications, secretion, degradation and recycling. By profiling the proteome of extracellular vesicles (EVs) derived from T cells, we identified the chaperonin complex CCT, which plays a role in the correct folding of specific proteins. To ascertain whether CCT is involved in the biogenesis or sorting of components within EVs, we employed a strategy of limiting CCT cell content through siRNA. We observed that cells undergo alterations in their lipid composition and metabolic rewiring towards a lipid-dependent metabolism, with increased activity of peroxisomes and mitochondria. Through cryoelectron microscopy and in vivo fluorescence microscopy, we observed the dysregulation of the dynamics of interorganelle contacts between lipid droplets, mitochondria, peroxisomes and the endolysosomal system. This process accelerates the biogenesis of multivesicular bodies, leading to higher EV production. Indeed, the content in lipid droplets and peroxisomes were altered in these cells, and the mitochondria's ability to use glucose was changed, with a rewiring of cell metabolism to the use of lipids. By isolating mitochondria, we observed that the dynamic regulation of microtubule-based kinesin motors was altered, pointing to differential localisation of organelles in cells and to the disruption of mitochondrial fission-fusion balance. This effect was accompanied by a defective regulation of post-translational modification of microtubules, which were found to be more acetylated, thus facilitating kinesin binding. CCT is known to assist in the folding of de novo tubulin, the fundamental building block of microtubules. This suggests that tubulin turnover is necessary for the correct positioning of intracellular organelles. These findings demonstrate an unanticipated role for CCT in the interconnections between proteostasis, lipid metabolism and intracellular organization.

POSTER SESSION

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Interaction of high risk HPV E7 oncoprotein with P1H1D1 component of R2TP complex is important for its stability and pRBE2F dissociation in cervical cancer.

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HPV is associated with more than 95% of invasive cervical cancers. High-risk types such as HPV type 16 and type 18 are the main strains implicated in cervical carcinogenesis. HPV encodes two proteins, E6 and E7, which together promote cellular proliferation and prevent apoptosis. The HPV virus executes this job by abrogating the function of two key tumor suppressors, pRB and P53. pRB and p53 functions are abolished by E7 and E6 respectively. One of the least studied areas is how these tumor suppressors are assembled and remodelled. Recently, a newly discovered multimolecular co-chaperone R2TP complex, also called PAQosome (Particle for arrangement of quaternary structure), has been proposed to help in the assembly and remodelling of various cellular complexes. The R2TP complex is considered the master regulator of cell growth and survival. The R2TP complex is composed of four proteins: RUBL1, RUVBL2, PIH1D1, and RPAP3. PIH1D1 recognizes a particular CK2 phosphorylated motif in proteins and then, with the help of these proteins, brings other large complexes to the R2TP complex for assembly and remodelling. We analysed the human and HPV proteome for this particular motif. Notably, we found that the HPV E7 protein has the same consensus motif and has been shown to be phosphorylated by CK2. We purified PIH1D1 and performed pull down experiments with the lysate of cervical cancer cell lines followed by western blot with E7 we observed an interaction between these two proteins, this interaction is phosphorylation-dependent. Upon silencing of PIH1D1, we observed a significant decrease in E7 protein levels. Further E7 is known to disrupt the interaction between pRB and E2f. We performed a competition assay between E7, pRB, and E2F in the presence of PIH1D1. Notably, we observed the earlier disappearance of E2F in the pRB antibody pulled down coimmunoprecipitates in the presence of PIH1D1. We further performed a cell-based assay in PIH1D1-silenced cervical cancer cell lines

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Targeting the HSP90-PAQosome interaction as a potential path to new cancer therapies

Yena Asme Moursli, Pinard M., Coulombe B.

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The Particle for Arrangement of Quaternary structure also known as the PAQosome is a large and complex molecule composed of 12 subunits that form two principal groups of proteins interacting together. The first group is represented by the R2TP core complex, and it is made up of RUVBL1 (Pontin), RUVBL2 (Reptin), RPAP3 and PIH1D1. WDR92, POLR2E, and the UPC make up the prefoldin-like (PFDL) module, which is the second group of proteins. The PAQosome complex interact with HSP90 to secure the assembly and folding of multiple proteins in multi-subunit complexes. One of the survival mechanisms used by cancer cells is precisely the HSP90 system. HSP90, which is overexpressed in multiple cancer cells, maintains the proteins that enable cancer cells to grow and divide. Several of these proteins are either direct PAQosome client proteins or substrates of PAQosome client proteins such as PIKKs. Due to this existing relationship between HSP90 and PAQosome, there is increasing evidence that the PAQosome has an oncogenic role in multiple cancers. Therefore, one cancer therapy strategy that is explored is to target HSP90 interacting region with RPAP3. Another approach is to target one of the PAQosome client's selection mechanisms and specifically prevent the recruitment of proteins that promote cancer cell growth and division. Acquiring a deeper understanding of these mechanisms is crucial for achieving this. Over the past decade, the Coulombelab has investigated the role of RPAP3 in these recruitment mechanisms. Ourteam showed that RPAP3 containsserine residues within the CK2 consensus site that can be phosphorylated by CK2 *in vitro*. The association of multiple ribosomal assembly factors with ribosome subunits is affected by these phosphorylation sites. The PAQosome complex, as suggested by these data, may have a complex role in ribosome assembly, potentially acting at different stages of ribosome biogenesis. These findings indicate that the phosphorylation of RPAP3 is another mean of client specificity/ selectivity by the HSP90- PAQosome complex. Emerging trends are also suggesting that cancer cells have a specialized class of ribosomes that promotethe oncogenic translation program. Blocking these oncoribosomes assembly by targeting their recruitment mechanisms could be a potential cancer treatment target to explore

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Extracellular Nucleophosmin1 promotes breast cancer progression

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Nucleophosmin (NPM1) is a multifunctional phosphoprotein that shuttles between the nucleolus and cytoplasm with several cellular functions such as ribosome biogenesis, chromatin remodeling, cell cycle progression and genomic stability.
In tumor cells, the level of NPM is often elevated and can result in increased cell growth and proliferation via hyperactivation of the ribosome machinery and in preventing apoptosis.
We found that NPM can be also released in the extracellular culture medium of various human (MDA-MB-231, BT549, HCC1937, MDA-MB-468) and murine (E0771, 4T1, LLC, B16 and CT-26) cancer cells lines but not from non-tumor cells (such as MCF-10). We discovered that NPM is able to create supermolecular complexes in the extracellular mileu of these cell lines with the chaperone HSP90 and its co-chaperone Morgana, indicating that the formation of these complexes is a specific feature of the tumor context. While the role of extracellular HSP90 (eHSP90) as a promoter of cancer progression is well established, the involvement of its extracellular co-chaperones remains poorly understood.
We observed that extracellular NPM (eNPM) promotes cancer cell migration *in vitro* without affecting tumor cell proliferation through the binding to cell surface receptors as TLR4. Preliminary result obtained in a syngeneic mouse model indicates that eNPM promotes cancer cell migration also *in vivo*. Indeed, intratumoral injections of recombinant NPM (rNPM) promotes metastasis formation. Interestingly NPM treatments also support primary tumor growth, suggesting a possible activity of eNPM on the tumor microenvironment. Define the composition of extracellular NPM complexes and investigate the pro-tumoral activity of eNPM could lead to development of new therapeutic approaches in cancer.

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CryoEM Structure of the y-Tubulin Ring Complex while nucleating microtubules

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Microtubules are essential cytoskeletal elements that provide structural support to cells and play critical roles in processes such as cell division, motility, and intracellular transport. While the dynamic instability of the microtubules has been extensively characterized, the mechanism of microtubule nucleation, which relies on the y-tubulin ring complex (yTuRC), remains a fundamental question in cell biology.

Previous structural studies revealed an asymmetric, and seemingly inactive conformation of this complex1,2,3,4, challenging the previously suggested role of yTuRC as a microtubule template. We have solved the cryo-EM structure of the human yTuRC during the process of microtubule nucleation5. This sheds light on how this complex becomes active and adopts its functional conformation.

A major challenge during image processing has been the heterogeneity of the sample, consisting of yTuRC-nucleated microtubules in several stages during the initiation of microtubule formation. This has been solved by employing deep learning-based classification algorithms, resolving yTuRC in several stages during the nucleation process and revealing the mechanism that closes yTuRC to become a perfect template for 13-protofilament microtubules.

This work establishes a structural framework for understanding the regulation of yTuRC activity in cells, paving the way for future research on the mechanisms governing microtubule cytoskeleton organization and its implication in diseases.

References:
1. *Nature* 578, p467;
2. *Cell* 180, p165;
3. Dev. *Cell* 53, p603;
4. *Science* Adv. 6, eabe0894;
5. *Science* 383, p870.

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Development of Hsp90 C-terminal domain inhibitors for the treatment of Ewing sarcoma

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3 Laboratory for Molecular Structural Dynamics, Theory Department, National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia

Ewing sarcoma is one of the most common malignant bone and soft tissue tumors in children and adolescents. Despite advances in the treatment of Ewing sarcoma, the prognosis is dismal, and survival rates for patients with metastases or recurrence of the disease are unacceptably low (20-30%). As chemotherapy for Ewing sarcoma relies solely on cytotoxic drugs, which have many adverse effects, there is a great need for novel targeted therapies and new drug combinations for the treatment of Ewing sarcoma that offer advantages over current therapeutic options. In Ewing sarcoma, EWS::FLI1 acts as an aberrant transcription factor and is the major oncogene causing the disease in >85% of cases. EWS::FLI1 is itself a client protein of Hsp90. Therefore, inhibition of Hsp90 is one of the possible approaches to inhibit the growth of the otherwise highly aggressive Ewing sarcoma.

We have recently identified and optimized different structural classes of Hsp90 C-terminal domain (CTD) inhibitors and investigated their antiproliferative activities in different cancer cell lines. The binding of the inhibitors to Hsp90β was investigated by TR-FRET Hsp90 CTD assay, trNOESY and STD NMR as well as microscale thermophoresis. In the Ewing sarcoma cell line SK-N-MC, our C-terminal Hsp90 inhibitors induced apoptosis, caused cell cycle arrest, and reduced intracellular levels of Hsp90-dependent oncogenic client proteins, including EWS::FLI1, without inducing the heat shock response. Furthermore, our Hsp90 CTD inhibitors showed *in vivo* activity in SK-N-MC xenografted zebrafish larvae.

The inhibition of tumor growth was comparable to that of the clinically evaluated Hsp90 N-terminal domain inhibitor 17-DMAG, making the Hsp90 CTD inhibitors promising candidates for further research.

References:
Zajec Ž, et al. Optimisation of pyrazolo[1,5-a]pyrimidin-7(4H)-one derivatives as novel Hsp90 C-terminal domain inhibitors against Ewing sarcoma. *Bioorganic chemistry.* 2023, 131, 1-16.
Sturtzel C. et al. Refined high-content imaging-based phenotypic drug screening in zebrafish xenografts. npj *Precision Oncology.* 2023, 7, 44.

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Unveiling the effect of extracellular HSP90 Co-chaperone Morgana in Triple Negative Breast Cancer cells

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Introduction

Heat shock proteins represent a class of molecules that are upregulated by cells in response to stressful stimuli. Since cancer cells are stressed, they are "chaperone-addicted". It has been described that many chaperones are secreted by cancer cells in the extracellular milieu where they can exert different functions.

HSP90 is the most abundant and important chaperone and it is crucial in sustaining survival and growth in cancer cells. Morgana is a highly conserved HSP90 co-chaperone; we previously found that Morgana is secreted by cancer cells in the extracellular microenvironment where, in association with HSP90, it binds to some cell surface receptors such as TLR2, TLR4 and LRP1, thus inducing cell migration.

Results and discussion

Integrins constitute a class of cell surface receptors and their recycling represents one of the most relevant player in cell migration. In this study, we discovered that extracellular Morgana treatment leads to a reduction in Integrinβ1 level on the cell surface, while increasing total and endocytosed protein level within the cell. In parallel, we observed an increase in both the quantity and size of Rab 5-positive endosomes, indicative of the activation of recycling vesicle pathway. Additionally, we identified that Integrinβ1 internalization is facilitated through its interaction with LRP1, a well-established receptor involved in integrin endocytosis. This interaction coincided with a reorganization of other Morgana-associated cell receptors that are TLR4 and TLR2.

Conclusions

Our findings reveal that extracellular Morgana promotes cancer cell migration by triggering the endocytosis of Integrinβ1 and facilitating its recycling over degradation. This phenomenon is facilitated by a reorganization of the interaction among certain cell membrane receptors.

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Previous CNIO Frontiers Meetings and CNIO Cancer Conferences

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2023

METASTASIS
06/11/2023 – 08/11/2023
Organisers: Julio Aguirre-Ghiso, Caroline Dive, Eva González-Suarez, Héctor Peinado, Manuel Valiente

GENOME ORGANISATION AND STABILITY
22/05/2023 – 23/05/2023
Organisers: Felipe Cortés, Óscar Fernández-Capetillo, Ana Losada, Andre Nussenzweig

2022

DIET, NUTRITION AND CANCER CELL METABOLISM
24/10/2022 – 26/10/2022
Organisers: Nabil Djouder, Nikla Emambokus, M. Carmen Fernández-Agüera, Valter Longo, Marcos Malumbres

MOLECULAR, CELLULAR AND ORGANISMAL DRIVERS OF AGING
09/05/2022 – 10/05/2022
Organisers: Maria A. Blasco, Alejo Efeyan, Thomas Rando

2019

HETEROGENEITY AND EVOLUTION IN CANCER
23/09/2019 – 25/09/2019
Organisers: Fátima Al-Shahrour, Arnold Levine, Solip Park, Raúl Rabadán

ESTRUCTURAL AND MOLECULAR BIOLOGY OF THE DNA DAMAGE RESPONSE
20/05/2019 – 22/05/2019
Organisers: Óscar Llorca, Rafael Fernández Leiro, Laurence H. Pearl, Titia Sixma

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2018

MOLECULAR, CELLULAR AND ORGANISMAL HALLMARKS OF AGING
07/05/2018 – 09/05/2018
Organisers: Maria A. Blasco, Alejo Efeyan, Kathleen Collins, Thomas Rando

FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY
09/07/2018 – 11/07/2018
Organisers: Victoria Aranda, Nabil Djouder, Joao Monteiro, Marisol Soengas, Laurence Zitvogel

2017

PRIMARY AND SECONDARY BRAIN TUMORS
19/02/2017 – 22/02/2017
Organisers: Massimo Squatrito, Manuel Valiente, Richard Gilbertson, Michael Weller

MOLECULAR CHAPERONES IN CANCER
02/05/2017 – 04/05/2017
Organisers: Nabil Djouder, Wilhelm Krek, Paul Workman, Xiaohong Helena Yang

2016

CANCEROMATICS III – TUMOR HETEROGENEITY
13/11/2016 – 16/11/2016
Organisers: Fátima Al-Shahrour, Núria Malats, Alfonso Valencia, Chris Sander

2015

METASTASIS INITIATION:
MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES
28/09/2015 - 30/09/2015
Organisers: David Lyden, Yibin Kang, Gemma Alderton, Victoria Aranda, Li-kuo Su, Héctor Peinado

NEW TRENDS IN ANTICANCER DRUG DEVELOPMENT
22/03/2015 - 25/03/2015
Organisers: Manuel Hidalgo, Alberto Bardelli, Lillian Siu, Josep Tabernero

2013

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER
27/05/2013 - 29/05/2013
Organisers: Robert Benezra, Ana Losada, Marcos Malumbres, René Medema

2012

ALLOSTERIC REGULATION OF CELL SIGNALLING
17/09/2012 - 19/09/2012
Organisers: Francesco Gervasio, Ermanno Gherardi, Daniel Lietha, Giulio Superti-Furga

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2011

RECAPTURING PLURIPOTENCY:
LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER
07/11/2011 - 09/11/2011
Organisers: Maria A. Blasco, Konrad Hochedlinger, Manuel Serrano, Inder Verma

CANCEROMATICS II :
MULTILEVEL INTERPRETATION OF CANCER GENOME
28/03/2011 - 30/03/2011
Organisers: Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

BREAST CANCER
07/02/2011 - 09/02/2011
Organisers: Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

2010

CANCER PHARMACOGENETICS:
PERSONALIZING MEDICINE
22/11/2010 - 24/11/2010
Organisers: Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

MOLECULAR CANCER THERAPEUTICS
08/03/2010 - 10/03/2010
Organisers: Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

2009

THE ENERGY OF CANCER
02/11/2009 - 04/11/2009
Organisers: Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

CANCER-OM-ATICS II: MULTILEVEL INTERPRETATION OF CANCER GENOME
06/07/2009 - 08/07/2009
Organisers**:** Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

STEM CELLS AND CANCER
23/02/2009 - 25/02/2009
Organisers: Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

2008

SIGNALLING UPSTREAM OF mTOR
03/11/2008 - 05/11/2008
Organisers: Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sánchez-Céspedes

STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL
23/06/2008 - 25/06/2008
Organisers: Niko Grigorieff, Eva Nogales and Jose María Valpuesta

DEVELOPMENT AND CANCER
04/02/2008 - 06/02/2008
Organisers: Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

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2007

LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY
05/11/2007 - 07/11/2007
Organisers: Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS
11/06/2007 - 13/06/2007
Organisers: Robert N. Eisenman, Martin Eilers and Javier León

MOLECULAR MECHANISMS IN LYMPHOID NEOPLASM
19/02/2007 - 21/02/2007
Organisers: Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

2006

TELOMERES AND TELOMERASE-CNIO / JOSEF STEINER CANCER CONFERENCE
13/11/2006 - 15/11/2006
Organisers: Maria A. Blasco and Jerry Shay

MEDICINAL CHEMISTRY IN ONCOLOGY
02/10/2006 - 04/10/2006
Organisers: Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

INFLAMMATION AND CANCER
22/05/2006 - 24/05/2006
Organisers: Curtis Harris, Raymand Dubois, Jorge Moscat and Manuel Serrano

PTEN AND THE AKT ROUTE
08/05/2006 - 10/05/2006
Organisers: Ana Carrera, Pier Paolo Pandolfi and Peter Vogt

2005

CANCER AND AGING
07/11/2005 - 09/11/2005
Organisers: Maria A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano

MAP KINASES AND CANCER
30/05/2005 - 01/06/2005
Organisers: Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda

ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS
07/03/2005 - 09/03/2005
Organisers: Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks

2004

CADHERINS, CATENINS AND CANCER
29/11/2004 - 01/12/2004
Organisers: Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

STRUCTURAL BIOLOGY OF CANCER TARGETS
27/09/2004 - 29/09/2004
Organisers: Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

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2003

APOPTOSIS AND CANCER
01/12/2003 - 03/12/2003
Organisers: Gabriel Nuñez, Marisol Soengas and Scott Lowe

SMALL GTPases IN HUMAN CARCINOGENESIS
16/06/2003 - 18/06/2003
Organisers: Juan Carlos Lacal, Channing Der and Shuh Narumiya

TARGETED SEARCH FOR ANTICANCER DRUGS
17/03/2003 - 19/03/2003
Organisers: Amancio Carnero and David H. Beach

2002

MECHANISMS OF INVASION AND METASTASIS
18/11/2002 - 20/11/2002
Organisers: Maria A. Blasco and Jerry Shay

THE CELL CYCLE AND CANCER
30/09/2002 - 02/10/2002
Organisers: Marcos Malumbres, Charles Sherr and Jiri Bartek

CANCER EPIGENETICS : DNA METHYLATION AND CHROMATIN
29/05/2002 - 31/05/2002
Organisers: Manel Esteller and Stephen B. Baylin

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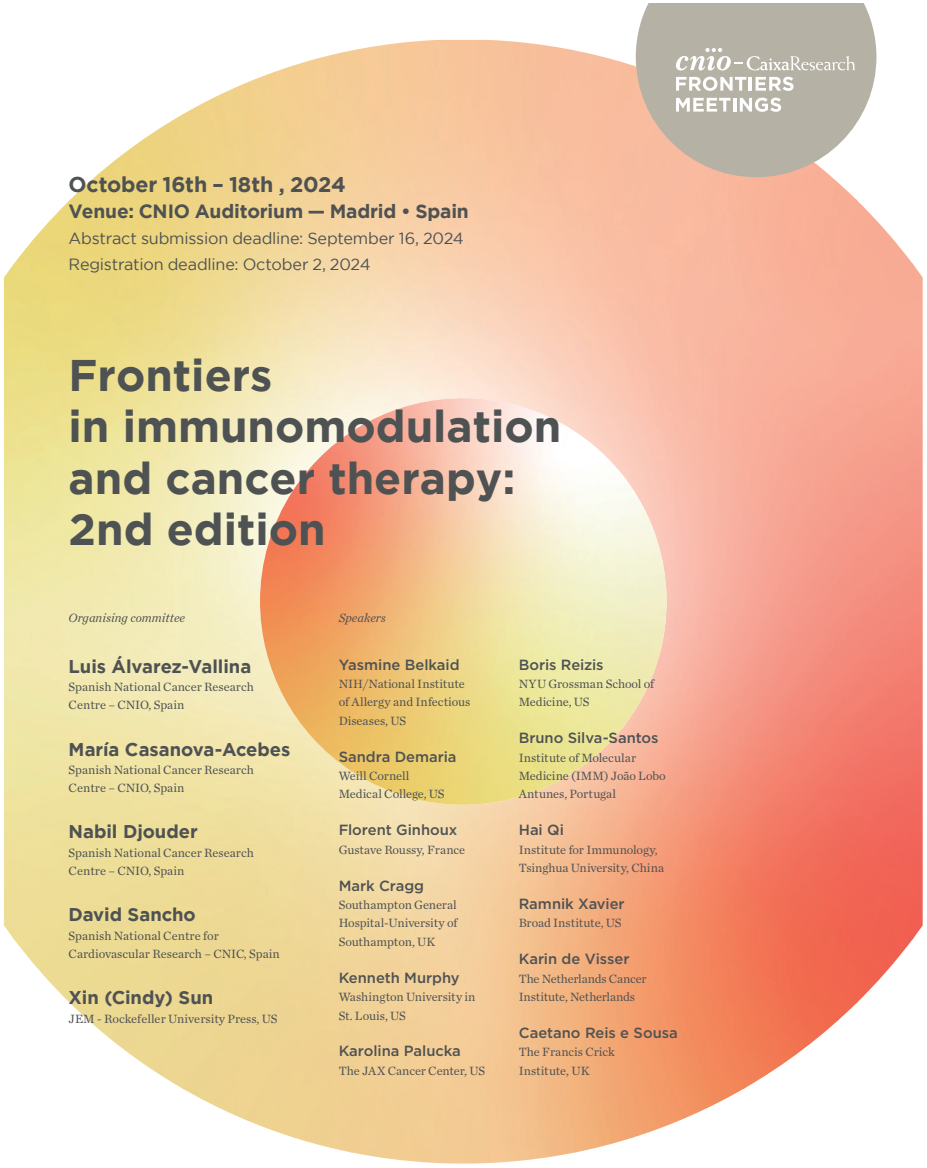

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2025

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May 28th – 30th, 2025

Venue:
Spanish National Cancer Research Centre – CNIO Auditorium, Madrid, Spain

Organizing committee:
Óscar Llorca, Spanish National Cancer Research Centre-CNIO, Spain
Rafael Fernández Leiro, Spanish National Cancer Research Centre-CNIO, Spain
Eva Nogales, Berkeley, University of California, US

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Centro Nacional de Investigaciones Oncológicas (CNIO)
Spanish National Cancer Research Centre
Melchor Fernández Almagro, 3
28029 Madrid, Spain
www.cnio.es

Coordination and edition:
Mercedes Moro and Mara Lizcano, CNIO, Madrid, Spain
Production of art and design by Gedosol, S.L.
Photographic archive CNIO

Printed in Spain

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As a non-profit organisation, we would like to thank all those who sponsored this conference. Such contribution helps us to ensure that our conferences will continue to establish the CNIO as a point of reference for the international cancer research community.

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