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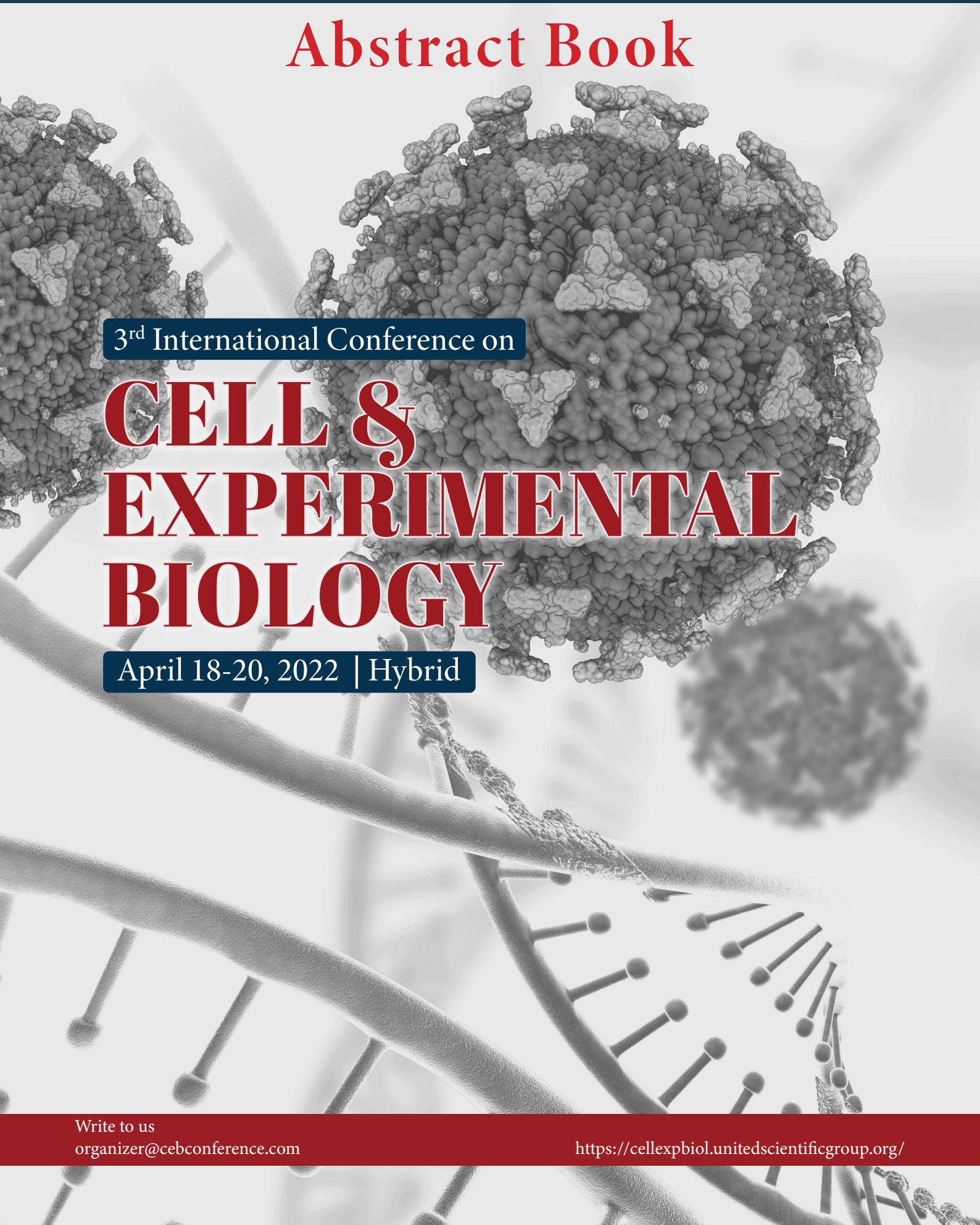

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Dynamic Cell Interactions with Extracellular Matrix in Cell Migration, Invasion, and Morphogenesis

Kenneth M. Yamada

Cell Biology Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA

Abstract

Real-time microscopy of the dynamics of cells and tissues in 3D environments is opening new windows to understanding the mechanisms of complex biological processes and diseases. Direct visualization of the dynamic movements of cells and their surrounding extracellular matrix microenvironment is allowing us to explore fundamental questions in more depth that include: How do cells migrate in 3D? How do cancer cells invade? How are organs formed? Cells can use varying combinations of cell adhesion to adjacent cells and to the surrounding extracellular matrix with localized cellular contractility to migrate, invade, and produce the complex tissue architecture needed for organ formation. Visualizing how cells move and organize into tissues is not only providing descriptive insights, but is also leading to the identification of novel, unexpected mechanisms relevant to tissue engineering.

Dynamins Mediating Membrane Fission and Fusion

John R. Jimah, Nidhi Kundu, Sarah B. Nyenhuis, Melissa R. Mikolaj, Jonathan T. Harrison and **Jenny E. Hinshaw**

Laboratory of Cell and Molecular Biology, NIDDK, NIH, 8 Center Dr, Bethesda, MD, USA

Abstract

Dynamin superfamily proteins (DSPs) are present in all organisms and are broadly implicated in membrane fission and fusion events including endocytosis, and mitochondria division and scission. DSPs are mechanochemical GTPases whose function is dependent on oligomerization of the protein and conformational changes that occur during the GTP hydrolysis cycle. Dynamin, the founding member, is crucial for endocytosis, synaptic membrane recycling, budding vesicles from the Golgi. The emerging model entails dynamin assembling around the necks of budding vesicles as a helical polymer and upon GTP hydrolysis, undergoes a significant constriction that ultimately leads to membrane fission. Recently, we have determined high-resolution structures of assembled membrane-bound dynamin in two nucleotide states, GTP-bound and post-hydrolysis GDP bound, by cryoEM methods. Our data reveals conformational changes that allow for the constriction of dynamin tubes from >50 nm to 36 nm with an inner lumen of only 3.4 nm. In collaboration with Dr. Elizabeth Chen (UT Southwestern), we have also characterized the interaction of dynamin with actin by cryo-electron tomography. This work revealed the role of dynamin in bundling actin-based protrusions that are necessary for myoblast fusion. In addition to dynamin, we are also determining the structure of DSPs involved in mitochondrial fusion, MFN1/2 and Opa1. For Opa1, we have a preliminary helical reconstruction of a proteolytically processed short form, s-Opa1, by cryo-EM on a lipid surface and are examining the role nucleotide states play in structural rearrangements.

Epigenetics and Noncoding RNAs in Diabetic Complications and Metabolic Memory

Rama Natarajan

Beckman Research Institute of City of Hope, Arthur Riggs Diabetes & Metabolism Research Institute, CA, USA

Abstract

Diabetes is associated with significantly accelerated rates of inflammation and multiple macro- and micro-vascular complications such as hypertension, atherosclerosis and nephropathy. Moreover, in some patients with diabetes, prior episodes of hyperglycemia are associated with continued development of complications despite subsequent glycemic control, a phenomenon termed metabolic memory. Abnormal activation of vascular cells, circulating monocytes and renal cells triggered by inflammatory genes has been implicated in the pathology of diabetic vascular complications, but the underlying molecular mechanisms are not fully understood. We have examined the role of epigenetic mechanisms, including chromatin histone modifications, DNA methylation and non-coding RNAs in regulating the expression of genes associated with the pathology of diabetic vascular complications and metabolic memory. We also perform epigenome profiling and implement systems biology integrative approaches to identify diabetes- and diabetes complication-specific epigenetic signatures genome-wide in mice, and in patients with diabetes, including patients experiencing metabolic memory. We have adopted translational approaches with small molecule drugs or chemically modified antisense nucleotide inhibitors to interfere with diabetes induced changes in epigenetic marks and non-coding RNAs. Together, our studies suggest that epigenetic factors play significant roles in the development of metabolic memory and diabetic complications. These factors could be examined for biomarker and therapeutic potential.

Speakers Abstracts

Propagation of Neuronal Micronuclei Regulates Microglial Maturation

Fuminori Tsuruta[1] and Sarasa Yano[2]

[1]*Faculty of Life and Environmental Sciences, University of Tsukuba, Japan*

[2]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan*

Abstract

Microglia, resident macrophages in the central nervous system, acquire various characteristics in a region-specific manner and change their state in response to signals from the local environment. However, niche signals underlying regional microglial maturation remain largely unknown. In this study, neurons passing through a dense region close to the pial surface of the developing neocortex give rise to micronuclei and release them into the extracellular space. Moreover, neuron-derived micronuclei were found to be incorporated into microglia residing at Layer 1. Importantly, micronucleus incorporation resulted in activation of the cGAS-STING pathway, which delayed postnatal maturation of microglia from the amoeboid to ramified state. cGAS deletion indeed accelerated postnatal ramification of micronucleus-harboring microglia. Although micronuclei have been considered to emerge in cancers, these results demonstrate their physiological role as a novel mediator of intercellular communication that regulates microglial maturation. Our findings thus provide a potential possibility for acquiring microglial diversity in the early-postnatal neocortex.

Intestinal Region-Dependent Alterations of Toll-like Receptor 4 Expression in Myenteric Neurons of Type 1 Diabetic Rats

Mária Bagyánszki, Bence Pál Barta, Benita Onhausz, Afnan Al-Doghmi, János Balázs, Zita Szalai and Nikolett Bódi

Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Hungary

Abstract

The importance of the gut microbiota has been recently highlighted in gut region-specific diabetic enteric neuropathy. Toll-like receptor 4 (TLR4), a receptor for lipopolysaccharides, has a pivotal role in the initiation of inflammatory and immune responses.

Our aim was to determine the proportion of the TLR4-immunoreactive myenteric neurons and the expression of TLR4 in myenteric neurons in the different gut regions using a type 1 diabetic model.

Ten weeks after the onset of hyperglycemia segments were taken from the duodenum, ileum and colon of streptozotocin-induced diabetic, insulin-treated diabetic and control rats. Myenteric whole-mount preparations were prepared for TLR4/peripherin double-labelling fluorescent immunohistochemistry. Post-embedding immunogold electron microscopy was applied to evaluate the TLR4 expression in the perikaryon and neuropil of myenteric neurons. Tissue TLR4 levels were measured by enzyme-linked immunosorbent assay.

In controls, the number and proportion of the TLR4-immunoreactive myenteric neurons showed an increasing tendency to aboral direction. These parameters were significantly higher in the diabetics compare to the controls in the duodenum and ileum, but it was significantly lower in the colon. In diabetics, the distribution of TLR4-labelling gold particles changed regionally different way between the perikaryon and neuropil of myenteric neurons. TLR4 tissue concentration changed only in the diabetic duodenum, it decreased in muscle/myenteric plexus-containing homogenates, while it increased in mucosa/submucosa/submucous plexus-containing samples relative to controls. Insulin had beneficial effects on TLR4 expression.

These findings support that chronic hyperglycemia has segment-specific effects on TLR4 expression, contributing to the regional damage of myenteric neurons and gastrointestinal disorders in diabetic patients.

Role of Mitochondrial Epigenetic Mechanisms in Neurodegenerative Diseases

Andrea Stoccoro, Lucia Migliore and Fabio Coppedè

Department of Translational Research & of New Surgical & Medical Technologies, University of Pisa, Via Roma 55, Pisa, 56126, Italy

Abstract

Increasing evidence suggests that DNA methylation occurs in mitochondrial DNA (mtDNA), likely regulating both mtDNA replication and gene expression levels (mitoepigenetics), and that altered mtDNA methylation could contribute to the etiology of several human complex diseases, including neurodegenerative ones. The majority of the studies investigated the DNA methylation levels of the mtDNA regulatory region (D-loop), which regulates mtDNA replication and transcription, revealing that D-loop methylation levels were dysregulated in central nervous system tissues of Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) animal models, and were altered in human postmortem AD and Parkinson's disease (PD) brains.

In our laboratory we investigated D-loop methylation levels in peripheral blood of patients with late-onset AD, PD and ALS, observing altered methylation levels in AD patients and in both familial and sporadic ALS patients. Our results also suggested that D-loop methylation levels were able to regulate mtDNA replication. Moreover, we identified polymorphisms of genes related to DNA methylation reactions that are significantly associated with D-loop methylation levels. Overall our results suggest that D-loop methylation and mitochondrial replication are

strictly related to each other and their evaluation could provide useful information for a better understanding of the etiopathology underlying neurodegenerative diseases, as well as useful disease biomarkers.

I will provide an overview of mitoepigenetics studies in neurodegenerative diseases and discuss the results of our investigation of mtDNA methylation levels in patients with late-onset AD, ALS, PD, as well as preliminary results in AD individuals at the early stages of the disease.

Scarless CRISPR/Cas9 Edited Cells Based on Co-editing Selection

Nina Reuven, Julia Adler and Yosef Shaul

Weizmann Institute of Science, Israel

Abstract

CRISPR/Cas9 "knock-in" editing of cell lines can be challenging, since many commonly used methods rely on homology-directed repair, which is restricted to the S-G2 portions of the cell cycle. To improve the efficiency of knock-in editing, we developed a "co-editing" protocol to select for cells active in knock-in editing. We edit two (or more) genes simultaneously, with one of them functioning as a selectable marker. By using a previously engineered temperature-sensitive (ts) mutation in an essential gene as the selectable marker, we can select for edited cells by growing the cells at the restrictive temperature. Since editing restores the ts mutation to the wild-type sequence, the selection method is scarless. To validate this method, we used HEK293 and HeLa cells with a ts mutation in the essential TAF1 gene. CRISPR co-editing of TAF1ts together with a gene of interest (GOI) resulted in up to 90% of the temperature-selected cells bearing the desired mutation in the GOI. The system was effective for inserting large cassettes encoded by plasmid donors, as well as smaller changes encoded by singlestranded oligonucleotide donors (ssODN). A notable example was the engineering of a T35A mutation in the proteasome subunit PSMB6, which eliminates its caspase-like activity. The edited cells showed a specific reduction in this activity, demonstrating the capability of this system to easily generate cell lines with biologically relevant mutations in endogenous genes. This approach offers a rapid, efficient, and scarless method for selecting genome-edited cells requiring HDR.

Piezo1 Channels Regulate Atrial Fibroblast Mechanics and Matrix Stiffness Sensing

Remi Peyronnet

Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg · Bad Krozingen, and Faculty of Medicine, University of Freiburg, Freiburg, Germany

Abstract

Many diseases are associated with fibrosis, the excess deposition of extracellular matrix, which is also a hallmark of atrial fibrillation, one of the most prevalent cardiac arrhythmias. Fibroblasts are the main drivers of extracellular matrix deposition. They are activated in response to injury or pathologically increased mechanical load. Fibroblasts sense matrix stiffness creating a regulatory loop allowing continuous adaptation of tissue stiffness to the mechanical demand. The aim of this work was to characterize mechanosensing and -transduction in human atrial fibroblasts, which is fundamental for the improvement of treatment strategies in the context of cardiac fibrosis.

We identified the stretch-activated ion channel Piezo1, which is upregulated in primary atrial fibroblasts from patients in atrial fibrillations, as a novel matrix stiffness sensor in these cells. Using light-tunable hydrogels, we found that adaptation of fibroblast stiffness to matrix stiffness is strongly altered upon Piezo1 knock-down.

Further, we demonstrate that Piezo1 expression levels correlated with human atrial fibroblast stiffness and the abundance, organization, and thickness of actin bundles. This is mediated by a signaling pathway tightly connected to canonical integrin-initiated mechanosignaling pathways. Importantly, knock-down of Piezo1 in human atrial fibroblasts reduced the expression level of a variety of pro-inflammatory cytokines and chemokines,

one of which communicates Piezo1-induced cell stiffening to neighboring cells. In this context, Piezo1 might represent an interesting target for pharmacological intervention to interfere with fibrotic remodeling in atrial fibrillation.

Taken together our results provide evidence for a paracrine mechanism of communication between fibroblasts, and they suggest Piezo1 as a mediator between mechanical and biochemical stimuli. Future experiments will characterize the role of Piezo1 in mechanically induced atrial tissue remodeling.

Uncovering the Roles of BET Family Proteins in Transcription Regulation

Mirjam Arnold[1], Annkatrin Bressin[1], Olga Jasnovidova[1], David Meierhofer[2] and Andreas Mayer[1]

[1]*Max Planck Institute for Molecular Genetics, Otto-Warburg-Laboratory, Berlin, Germany*

[2]*Max Planck Institute for Molecular Genetics, Mass Spectrometry Facility, Berlin, Germany*

Abstract

Transcription by RNA polymerase II (Pol II) is tightly regulated and its dysregulation can cause human disease. Recently, transcription elongation has emerged as a regulatory hub in metazoan gene expression. The bromodomain and extra-terminal (BET) family proteins consisting of ubiquitously expressed BRD2, BRD3, BRD4 and of testis-specific BRDT have been implicated in the regulation of transcription elongation. Despite their implication in elongation, direct BET protein-specific functions in transcription and transcription-coupled processes have largely remained unclear. Here, we combined rapid and selective protein degradation with quantitative genome-, transcriptome- and proteome-wide approaches, to reveal direct functions of BRD4 in transcription control. From our studies BET proteins and particularly BRD4 emerge as key regulators of Pol II transcription and of co-transcriptional 3'-RNA processing. Given the role of BRD4 in a broad range of human diseases our findings have potential implications for the understanding of disease mechanisms.

Erogorgiaene Analog Inhibits (p)ppGpp Synthetases Preventing Persister Formation in Mycobacteria

Roman Sidorov[1,2] and Alexander Tkachenko[1,2]

[1]*Institute of Ecology and Genetics of Microorganisms, Perm Federal Research Center, the Ural Branch of Russian Academy of Sciences, Russian Federation*

[2]*Perm State University, Russian Federation*

Abstract

Bacterial persistence associated with biofilms is directly linked with recalcitrant TB infections and antibiotics treatment failure. A novel antibacterial DMNP is capable to inhibit mycobacterial (p)ppGpp synthetases, the enzymes that promote bacterial persistence and biofilm formation. DMNP is a derivative of erogorgiaene, which is a natural compound that was initially identified in marine coral species *Antillogorgia elisabethae*.

We discovered that DMNP has antipersister activity. Unlike other clinically used antibiotics which are more active against exponential phase cells, DMNP is more active against stationary phase *Mycolicibacterium smegmatis* cells. We used mycobacterial expression plasmids to demonstrate that the DMNP effect is decreased when either *M. smegmatis* (p)ppGpp synthetases Rel_{Msm} or RelZ are overexpressed. Both rel_{Msm} and *relZ* genes knockout mutations enhanced the DMNP antibacterial properties, and this effect was complemented with expression plasmids. In vitro, DMNP inhibited (p)ppGpp-synthesizing activity of purified Rel_{Msm} in a concentration-dependent manner. DMNP is similarly active towards Rel_{Mtb} from *Mycobacterium tuberculosis*. Molecular docking analysis revealed a possible binding site on Rel_{Mtb} protein. These data suggest that DMNP targets mycobacterial (p)ppGpp synthetases thus blocking persister formation.

Furthermore, DMNP is capable to eradicate biofilms and decrease cell motility in *M. smegmatis*. When added in combination, DMNP substantially enhanced the antibacterial action of streptomycin or rifampicin. To sum up the findings, DMNP may serve as a promising lead compound for the development of new approaches to antimycobacterial therapies aimed at persister cells formation.

Versatile and Tunable Selenocysteine Reporters Using an Intein-based System

Natalie Krahn[1], Christina Z Chung[1], Ana Crnković[1] and Dieter Söll[1, 2]

[1]*Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA*

[2]*Department of Chemistry, Yale University, New Haven, CT, USA*

Abstract

Selenoproteins possess unique properties that are conferred by the presence of selenocysteine (Sec). These attributes make the production of various recombinant selenoproteins desirable. One methodology to site-specifically insert Sec into proteins utilizes the natural elongation factor (EF-Tu) to recode UAG stop codons. However, improvements to this technology are limited due to the lack of screening and selection markers that can discriminate against nonspecific UAG decoding and enable high-throughput screening. Thus, we developed a strategy to adapt established reporters to recognize insertion of Sec. This strategy involves using inteins which rely exclusively on Sec for splicing. Inteins are auto-catalytic enzymes that splice themselves out of a precursor protein. We show that the M86 mini-intein cassette can be placed in-frame within any selection and screening marker, generating an active reporter only upon successful intein splicing. As compete splicing can only occur when a catalytic Sec is present, the amount of synthesized reporter directly measures UAG-directed Sec incorporation. Furthermore, we show that the sensitivity of these reporters can be tuned depending on the position at which the intein cassette is inserted. Importantly, we show that results obtained with intein-containing reporters are comparable to Sec incorporation levels determined by mass spectrometry of isolated recombinant selenoproteins. This result validates the use of these intein-containing reporters to screen for evolved components of a translation system yielding increased selenoprotein amounts.

Uncovering the Peculiar Evolution of Thyroid Peroxidases and their Ancestors from Non-Vertebrates Towards Mammals with Consequences for Thyroid Hormone Biosynthesis

Marcel Zamocky[1,2], Jana Harichová[1] and Paul G. Furtmüller[2]

[1]*Institute of Molecular Biology Slovak Academy of Sciences, Bratislava Slovakia*

[2]*University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Institute of Biochemistry, Muthgasse 18, 1190 Vienna, Austria*

Abstract

We are investigating heme peroxidases from Cephalochordata that are predecessors for the development of thyroid peroxidases in mammals. For this purpose, we have performed in depth phylogeny of the whole peroxidase-cyclooxygenase superfamily focused on detailed study of the divergence of heme peroxidases between main lineages of Chordata. Synteny of corresponding amphioxus heme peroxidase genes was followed in 25 representative genomes. Ancestral sequences of animal heme peroxidases were reconstructed in important nodes of a robust evolutionary tree based on 620 full length protein sequences. Structure-function implications based on the sequence alignment for selected subfamilies are discussed. In order to probe the structural integrity, conformational, and thermal stability of *Branchiostoma belcheri* peroxidase (BbePOX4) produced recombinantly in *Pichia pastoris* that may be involved in halogenation reactions, a comprehensive investigation by using complementary biophysical techniques including UV–vis and circular dichroism spectroscopy, SEC-MALS, differential scanning calorimetry (DSC) as well as a kinetic characterisation with ligand and substrates have been undertaken. Our results give important insights on the complex protein evolution that led to a peroxidase involved in thy-

roid hormone biosynthesis in mammals. Our research is supported with project P 31707 from Austrian research foundation FWF and with project APVV-20-0284 from Slovak Research and Development Agency.

Identification of Overexpressed Genes in Malignant Pleural Mesothelioma as Potential Targets for Novel Therapies

Federica Morani, Luisa Bisceglia, Lara Guerrieri, Federica Gemignani, Stefano Landi

Department of Biology, University of Pisa, Pisa, Italy

Abstract

Malignant pleural mesothelioma (MPM) is a rare aggressive chemotherapy-resistant tumor with a bad prognosis and still without effective therapies. Therefore, it is urgent to identify novel therapeutic targets for improving patients' survival and quality of life. In our previous work, we performed an integrated gene expression analysis on RNAseq data of MPM patients from TCGA dataset and reference samples from GEO. Gene lists were further refined by published literature, functional enrichment analysis and correlation with patients' overall survival. A final list of 15 genes was detected. The goal of the present study is to detect novel genes playing a role for the maintenance of the malignant phenotype, suggesting novel molecular targets for future therapies. In order to identify the MPM-driver genes we used the strategy of identifying those with an aberrant overexpression in MPM specimens, as compared to normal pleura, because inhibitors are easier to be designed as compare to gene activators. An easy way to evaluate the driver role in carcinogenesis for a given gene is to cause its functional knock-down using small interfering RNA (siRNA). We efficiently silenced the selected genes in Mero-14, Mero-41, Mero- 95, ZL-55, REN and MSTO MPM cell lines and in a non-malignant mesothelial model (Met-5A). Functional effects of gene silencing were monitored by live-cell analysis system using cellular proliferation/apoptosis/migration assays. The encouraging results showed that there is a large variability among cell lines, with the strongest responsiveness to gene-depletion observed in MSTO cells.

The Intracellular Regulator Cyclic PIP Stimulates Protein-Dephosphorylation in All Cells of a Body

Heinrich K. Wasner

BioReg Biopharm, TiLab, University of Illinois at Chicago, IL

Abstract

After the discovery of cyclic AMP, Earl Sutherland was convinced that every 'switch on' signal demands an equally strong 'switch off' signal. The task was to find and characterize this counterpart to cyclic AMP. It is prostaglandyl-inositol cyclic phosphate (cyclic PIP).

The rather stable cyclic AMP is synthesized in 1 reaction step from ATP. But, the rather labile cyclic PIP is biosynthesized in 5 reaction steps: Insulin or noradrenaline (alpha-receptor action) activate phospholipase C. This leads to release of polyunsaturated fatty acids, which can be converted to prostaglandin E. Equally activated phospholipase A releases from lipids inositol phosphates as inositol-(1:2)-cyclic, 4-bisphosphate, which is converted to GDP-inositol (1:2)-cyclic phosphate. The particular cyclic PIP synthase, activated by tyrosine phosphorylation, combines PGE and the inositol (1:2)-cyclic phosphate part of activated inositol phosphate to cyclic PIP.

Cyclic AMP activates protein kinase A. It regulates metabolism by phosphorylating 'key-enzymes'. Cyclic PIP triggers dephosphorylation of these enzymes by dose-dependently inhibiting protein kinase A up to 100% and activating phosphoprotein ser/thr phosphatase holoenzymes 7-fold. At the cellular level cyclic PIP, for instance, activates 10-fold the glucose uptake into adipocytes; in pancreatic beta-cells it inhibits the glucose stimulated secretion of insulin; it antagonizes in the slime mold Dictyostelium discoideum the cyclic AMP induced aggregation and sporulation; in hepatocytes it switches off the cyclic AMP triggered autophagy and proteolysis.

Polymeric SUMO-2/3 Chain Modification Regulates Chromosome Alignment to the Metaphase Plate During Mitosis in Mammalian Cells

Divya Subramoniana, Te-An Chenb, Nicholas Paolinib, and **Xiang-Dong "David"** Zhang

Department of Biological Sciences, Wayne State University, Detroit, MI, USA

Department of Biology, SUNY Buffalo State, Buffalo, NY, USA

Abstract

SUMO-2/3 modification plays a critical role in targeting the kinesin protein CENP-E to kinetochores followed by CENP-E mediated chromosome alignment to the spindle equator during mitosis. However, it is still unclear about how SUMO-2/3 modification modulates CENP-E kinetochore localization in mammalian cells. Here, we first demonstrate that the known CENP-E interacting kinetochore protein Nuf2 is not only essential for CENP-E localization to kinetochore but also specifically modified by polymeric SUMO-2/3 chains. Furthermore, the mitotic defects in CENP-E kinetochore localization and chromosome congression caused by global inhibition of sumoylation are rescued by the fusion protein between Nuf2 and the SUMO-conjugating enzyme Ubc9 for stimulating Nuf2 SUMO-2/3 modification and by the fusion protein between Nuf2 and trimeric SUMO-2 chain for simulating the trimeric SUMO-2/3 chain modification of Nuf2. On the other hand, the other forms of Nuf2-SUMO fusion proteins that imitate the Nuf2 modifications by SUMO-2/3 monomer, SUMO-2/3 dimer, and SUMO-1 trimer, respectively, are unable to rescue the same mitotic defects. Compared to Nuf2, the trimeric SUMO-2 chain-modified Nuf2 displays a significantly higher binding affinity to CENP-E wild type with a functional SUMO-interacting motif (SIM) but not the CENP-E SIM mutant. In conclusion, polymeric SUMO-2/3 chain modification of Nuf2 facilitates CENP-E kinetochore localization and chromosome congression during mitosis.

Pigment Epithelium-derived Factor Induces CRX Alterations in the Mouse Retina

Ivan T. Rebustini, Susan E. Crawford and S. Patricia Becerra

Section of Protein Structure and Function, LRCMB-NEI-NIH, Bethesda, MD 20892-0608, USA

Department of Surgery, NorthShore University Research Institute, Affiliate of University of Chicago Pritzer School of Medicine, Evanston, IL 60201, USA

Abstract

The gene *Serpinf1* encodes for Pigment Epithelium-Derived Factor (PEDF), which protects photoreceptors from cell death. The *Serpinf1* null mouse exhibit's normal retinal function; however, PEDF deficiency increases retinal degeneration susceptibility. CRX is a transcription factor that regulates the expression of photoreceptor specific genes, including opsins and phosphodiesterases (PDE). This study aims to explore whether regulation of CRX is one mechanism by which PEDF exerts photoreceptor survival.

Serpinf1$^{-/-}$, *Serpinf1*$^{+/-}$ and *Serpinf1*$^{+/+}$ mice at 3 months of age were used. Mouse retinal explants were prepared and treated with recombinant human PEDF and zaprinast (a PDE inhibitor) to induce photoreceptor death. Cell death was determined using PSVue-550, a fluorescent probe for apoptosis membrane marker. Subcellular distribution of CRX, PDE6A and pan-acetylation was detected by immunofluorescence. The transcriptional levels of *Crx*, *Pde6a*, *Opn1mw* and *Opn1sw* were assessed using qPCR.

PEDF treatment induced the expression of *Crx* and its regulated genes *Pde6a*, *Opn1mw* and *Opn1sw*. PEDF also increased the CRX immunoreactivity in the nuclei of photoreceptors. In contrast, the nuclear CRX immunoreactivity was suppressed in photoreceptors from retinas of *Serpinf1*$^{-/-}$ mice. PDE6A immunoreactivity and histone acetylation also decreased in *Serpinf1*$^{-/-}$ photoreceptors when compared to those from *Serpinf1*$^{+/+}$ mice. Zaprinast-induced photoreceptor death was more pronounced in *Serpinf1*$^{-/-}$ retinal explants than in wild type controls. PEDF pre-treatment prior to the zaprinast treatment improved photoreceptor survival and enhanced CRX immunoreactivity in retinal explants.

The findings imply that PEDF activated the CRX-associated transcription factor network. They provide a novel insight linking extracellular PEDF to nuclear CRX during photoreceptor survival.

New Role of Apurinic/apyrimidinic Endonuclease 1 Protein in the Degradation of Dysfunctional Abasic mRNA in Mitochondria

Carlo Vascotto[1], Arianna Barchiesi[1], Veronica Bazzani[1], Agata Jabczynska[2], Lukasz S. Borowski[2,3] and Roman Szczesny[2]

[1]*Department of Medicine, University of Udine, Italy*

[2]*Institute of Biochemistry and Biophysics, Polish Academy of Science, Poland*

[3]*Faculty of Biology, Institute of Genetics and Biotechnology, Poland*

Abstract

APE1 is a multifunctional protein which plays a central role in the maintenance of nuclear and mitochondrial genomes repairing DNA lesions caused by oxidative and alkylating agents. In addition, it works as a redox signaling protein regulating gene expression by interacting with many transcriptional factors. Apart from these canonical activities, recent studies have shown that APE1 is also enzymatically active on RNA molecules. We unveiled for the first time a new role of the mitochondrial form of APE1 protein in the metabolism of RNA in mitochondria. Our data demonstrate that APE1 is associated with mitochondrial messenger RNA and exerts endoribonuclease activity on abasic sites. Loss of APE1 results in the accumulation of damaged mitochondrial mRNA species, determining impairment in protein translation and reduced expression of mitochondrial-encoded proteins, finally leading to less efficient mitochondrial respiration. Altogether, our data demonstrate that APE1 plays an active role in the degradation of the mitochondrial mRNA and has a profound impact on mitochondrial well-being.

Arsenic Toxicity on Metabolism and Autophagy in Brown Adipose Tissue

Seung-Hyun Ro, Jiyoung Bae, Yura Jang and Heejeong Kim

Department of Biochemistry, University of Nebraska – Lincoln, Lincoln NE, USA

Abstract

Arsenite, a trivalent form of arsenic, is an element that occurs naturally in the environment. Humans are exposed to high dose of arsenite through consuming arsenite-contaminated drinking water and food, and the arsenite can accumulate in the human tissues. Arsenite induces oxidative stress, which is linked to metabolic disorders such as obesity and diabetes. Brown adipocytes dissipating energy as heat have emerging roles for obesity treatment and prevention. Therefore, understanding the pathophysiological role of brown adipocytes can provide effective strategies delineating the link between arsenite exposure and metabolic disorders. Our study revealed that arsenite significantly reduced differentiation of murine brown adipocytes and mitochondrial biogenesis and respiration, leading to attenuated thermogenesis via decreasing UCP1 expression. Oral administration of arsenite in mice resulted in heavy accumulation in brown adipose tissue and suppression of lipogenesis, mitochondrial biogenesis and thermogenesis. Mechanistically, arsenite exposure significantly inhibited autophagy necessary for homeostasis of brown adipose tissue through suppression of Sestrin2 and ULK1. These results clearly confirm the emerging mechanisms underlying the implications of arsenite exposure in metabolic disorders.

Expansion of the Genetic Code Through the Use of Modified Ribosomes

Sidney M. Hecht

Biodesign Center for BioEnergetics, and School of Molecular Sciences, Arizona State University Tempe, AZ, USA

Abstract

Several strategies now exist for the ribosomal synthesis of proteins containing non-proteinogenic amino acids. These enable the incorporation of one or more modified amino acids into predetermined positions in a protein. While a wide variety of amino acid side chains not found in natural proteins can be incorporated, bacterial ribosomes do not incorporate amino acid analogues such as D-amino acids or -amino acids.

In the past several years, we have developed a strategy for modifying the 23S ribosomal RNA in *E. coli* ribosomes; this is the ribosomal constituent that mediates the peptide bond formation. By the use of structurally modified puromycin analogues, libraries of clones harboring plasmids with modified 23S rRNAs can be screened to identify clones capable of incorporating modified amino acids not normally incorporated by bacterial ribosomes, including D-amino acids, α-amino acids, dipeptides and dipeptidomimetics, as well as phosphorylated amino acids.

Presently, I will discuss the incorporation into proteins of dipeptides and dipeptidomimetics, as well as phosphotyrosine. Unique properties of the derived proteins will be described, and the discovery of a putative new function of the NFkB inhibitory protein IκBα will be discussed. Finally, the incorporation of a strongly fluorescent dipeptidomimetic into proteins *in cellulo* will be discussed.

Systematic Study of Host Network Perturbations by Human Virus Transcriptional Regulators

Xing Liu[1], Zhaorong Li[1], Santoso Clarissa Stephanie[1], Kerstin Spirohn[2,3], Kimberly Tran[1], Cheng-Che Lee[1], Vikram Srinath[1], David Hill[2,3], Marc Vidal[2,3] and Juan Fuxman Bass[1]

[1]*Boston University, Boston, MA, USA*

[2]*Center for Cancer Systems Biology (CCSB), Dana-Farber Cancer Institute, Boston, MA, USA*

[3]*Harvard Medical School, Boston, MA, USA*

Abstract

Viral transcription regulators (vTRs) play key roles in virus-related human diseases as vTRs not only control viral gene expression, but they can also modulate immune responses and promote cell proliferation. Previously we identified 419 vTRs across 20 virus families. However, the DNA binding profiles, protein-protein interactions, and functional roles have not been determined for the vast majority of vTRs. To understand the general and specific mechanism by which vTRs perturb host gene networks, we are performing a comprehensive functional profiling of vTRs from different viral families. By measuring the transcriptional activity of 95 vTRs, we detected both activating and repressing vTRs with activitities that span four orders of magnitude. To uncover the mechanisms of differential transcriptional activities, we performed yeast two-hybrid assays against ~18,000 human ORFs, resulting in 192 protein-protein interactions involving 38 vTRs and 79 human proteins. Over half of the interacting human proteins were human transcription factors or cofactors. To determine which gene networks are perturbed by vTRs we generated 20 stable cells lines each expressing a different vTR from adenovirus, human papillomavirus, ebolavirus, and coronavirus and determined host gene expression by RNA-seq. We found tens to thousands of differentially expressed genes, depending on the vTR. These genes are associated with gene ontology terms such cell adhesion, migration, proliferation and differentiation, innate immunity, and signaling and response to stimulus. Altogether, the resource generated will expand our undertstanding of vTR functions and of general viral evasion mechanisms.

Regulation of Germline Proteostasis by HSF1 and Insulin/IGF-1 Signaling in Gametogenesis and Reproductive Aging

Jian Li, Stacey L. Edwards, Allison C. Morphis.

Aging and Metabolism Research Program, Oklahoma Medical Foundation, Oklahoma City, OK, USA

Abstract

Gametogenesis is sensitive to nutrient availability and environmental perturbation. We recently show that HSF1, the key regulator of cellular heat shock response (HSR), has important roles in germline progenitor cell proliferation and early meiosis in *C. elegans*. Using the auxin-inducible degron system, we identified a compact but important transcriptional program of HSF1 for nascent folding and protein conformation maintenance in germ cells. Out data indicate that this HSF1 activity is distinct from that in the canonical HSR, and is dictated by the nutrient-sensing, insulin/IGF-1 signaling to support proteostasis in rapid germline growth. Proteotoxic stress and maternal aging impair HSF1 activities in germ cells, which destabilizes proteins that are essential for gametogenesis and oocyte quality consequently causing reproductive defects. We found that reduction of insulin/ IGF-1 signaling post development is sufficient to extend reproductive span. This is at least partially through slowing protein synthesis therefore maintaining proteostasis and reproductive health with compromised HSF1 activity during maternal aging. Collectively, we propose that it is essential to couple protein synthesis regulated by insulin/IGF-1 signaling and protein folding capacity controlled by HSF1 to achieve germline proteostasis.

Folding and Misfolding of Highly-Luminescent Protein NanoLuc

Dimitra Apostolidou and Piotr Marszalek

Duke University, Durham, NC, USA

Abstract

Highly-luminescent NanoLuc has been greatly utilized in cell assays and more, yet no information on its mechanical behavior is known. Here, we present our results on the mechanical stability of NanoLuc and its ability to refold in various polyprotein constructs using the Atomic Force Microscopy-based Single Molecule Force Spectroscopy method. Our resultsstrongly suggest that NanoLuc is less likely to successfully refold when linked to itself, an opposite behavior from when it is separated by other proteins. Additionally, we performed Steered Molecular Dynamics Simulations of NanoLuc, which provided great insight into the pathways in which NanoLuc unfolds at the atomistic level. Lastly, we investigated NanoLuc constructs thermal stability and were able to identify it as a robust chaperone substrate, with recovery of NanoLuc' activity up to 70% of its initial bioluminescence prior to thermal denaturation.

EX16 Maintains Peroxisome Abundance via Pexophagy Regulation

Xiaofan Wei*, Yunash Maharjan, Debra Dorotea, Raghbendra Kumar Dutta, Donghyun Kim, Hyunsoo Kim, Yizhu Mu, Channy Park and Raekil Park

Department of Biomedical Science & Engineering, Gwangju Institute of Science and Technology Gwangju 61005, South Korea

Abstract

Peroxisome abundance is regulated by a homeostasis of balance between biogenesis and degradation processes. Peroxin 16 (PEX16) is a one of the peroxisome proteins that involves in trafficking mem-brane proteins for de novo peroxisome biogenesis. Herein, the present study demonstrates that PEX16 modulates peroxisome abundance through pexophagic degradation. PEX16 knockdown in human retinal pigment ephitilial-1 (RPE-1) cells decreased the peroxisome abundance and function, represented by reductions in the expression of peroxi-

some membrane protein ABCD3 and the levels of cholesterol and plasmalogens, respectively. The activation of pexophagy under PEX16 depleted condition was accentuated by: i) abrogated peroxisome loss under PEX16 knockdown in autophagy deficient ATG5-/- cell lines, ii) increased autophagy flux and co-localization of p62, an autophagy adaptor protein, and ABCD3 in the presence of an autophagy inhibitor, chloroquine. However, the levels of cholesterol and plasmalogens were not recovered despite restoring of peroxisome abundance following chloroquine treatment. Thus, PEX16 is in-dispensable for maintaining of peroxisome homeostasis by regulating not only the commonly known biogenesis pathway, but also the autophagic degradation of peroxisome.

The Research and Application Prospects of Sports Injury Therapy Based on Peripheral Blood-derived Mesenchymal Stem Cells

Jia-kuo Yu

Peking University Third Hospital, China

Abstract

Sports injuries are injuries that occur in athletic activities or exercising. They can result from accidents, poor training technique in practice, inadequate equipment, and overuse of a particular body part. Sports injuries is a general joint disease that can lead to an increased social and economic burden in the modern society. The therapeutic use of peripheral blood-derived mesenchymal stem cells (PB-MSCs) has been applied to many different tissue types that are vulnerable to sports injuries. Avenues of treatment include direct injection of PB-MSCs into the defect, however although minimally invasive, research has highlighted flaws which have been improved upon with the use of scaffolds. PB-MSCs have been applied via many different scaffold types, for example PCL, collagen gel and coral each with advantages and disadvantages of which can be improved through further research. Our report summarizes the research and application prospects of PB-MSCs-based therapies for ligamentous or tendinous healing, meniscal volume restoration, or post-traumatic osteoarthritis amelioration/regression.

Potential Survival Responses Triggered by Respiratory Complex I Inhibition in Ovarian Cancer Models

Manuela Sollazzo

Department of Pharmacy and Biotechnology (FABIT), University of Bologna, 40126 Bologna, Italy

Abstract

In the last years the involvement of mitochondrial respiration has been increasingly recognized during tumor progression and resistance to chemotherapy. In particular, targeting respiratory Complex I (CI) has been proposed as a new therapeutic approach to hinder cancer growth. Our recent work displayed that a severe CI impairment promoted a delay of tumor expansion but not its complete eradication. Indeed, over time CI-defective cancer cells survive and undergo growth reactivation. This observation might be relevant in ovarian cancer (OC), where about 85% of patients develop relapses after standard surgical and pharmacological treatments. Here we show that among all the metabolic and molecular consequences of CI dysfunction, OC cells experience a block of mTORC1-mediated protein synthesis which lead to proliferation arrest. This switch off is likely counterbalanced by the activation of different molecular axis, highlighting the involvement of adaptive responses in cell survival and proliferation upon CI targeting. The dissection of such pathways may offer potential molecular players in synthetic lethality with CI inhibition, thus providing new synergistic strategies for OC treatment.

Altered Proteome S-glutathionylation in Cancer Caused by Loss of Glutaredoxin-2 Leads to Mitochondrial Dysfunction and Conversion to More Indolent Oncocytoma

Monica De Luise[1,2], Greta Tedesco[1,2], Lorena Marchio[1,2], Luigi D'Angelo[2,3], Camelia Alexandra Coada[1,2], Gianandrea Pasquinelli[4], Mila Gugnoni[5], Alessia Ciarrocchi[5], David Meierhofer[6], Ivana Kurelac[1,2], Luisa Iommarini[2,3], Anna Maria Porcelli[2,3,7]and Giuseppe Gasparre[1,2]

[1]*Medical Genetics Unit, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy*

[2]*Center for Applied Biomedical Research (CRBA), University of Bologna, Bologna, Italy*

[3]*Department of Pharmacy and Biotechnology (FABIT), University of Bologna, Bologna, Italy*

[4]*Laboratory of Clinical Pathology, Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy*

[5]*Laboratory of Translational Research, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy*

[6]*Max Planck Institute for Molecular Genetics, Berlin, Germany*

[7]*Interdepartmental Center of Industrial Research (CIRI) Life Science and Health Technologies, University of Bologna, Ozzano dell'Emilia, Italy*

Abstract

A family affected by autosomal dominant Hyperparathyroidism-Jaw Tumor syndrome (HPT-JT), due to a large deletion in *CDC73*, showed recurrence of parathyroid tumors (adenomas or carcinomas) in several members, all with oncocytic phenotype, i.e. consisting of cells characterized by mitochondrial hyperplasia that usually carry pathogenic mitochondrial DNA (mtDNA) mutations. Various degrees of mitochondrial derangement in these tumors were caused by different mtDNA mutations with different load, that inversely correlated with malignant features among subjects. The recurrence of oncocytic tumors is a rare event, thus we sought for nuclear genetic determinants in HPT-JT family and ruled out private mutations through exome sequencing analysis of the family members. However, we identified, within the inherited *CDC73* large deletion, the presence of regulatory elements of an upstream gene, glutaredoxin 2 (GLRX2), encoding a mitochondrial isoform that catalyzes reversible protein S-glutathionylation/deglutathionylation, and whose role in cancer has never been depicted. GLRX2 was absent in all family tumors and its expression was halved in lymphocytes and fibroblasts of HPT-JT subjects. To investigate whether the somatic lack of GLRX2 may impair a proper protein deglutathionylation predisposing to oncocytic phenotype, we generated TPC1 and HCT116 cell models knocked out for GLRX2 by CRISPR/Cas9. In agreement with more indolent slow- growing oncocytomas, KO cells showed a lower growth ability and altered proteome S-glutathionylation with a delay in protein deglutathionylation after oxidative stress stimuli. A pilot *in vivo* experiment showed KO cells with a peculiar oncocytic-like phenotype, characterized by an increased number of deranged mitochondria, suggesting that lack of GLRX2 induces a mitochondrial dysfunction. Glutathionylation has been reported to regulate OXPHOS and TCA cycle enzyme activity. Hence, to understand whether such defective phenotype was due to a differential glutathionylation of these protein complexes, native electrophoresis of mitochondrial proteins was performed. Upon oxidative stress stimulus, a significantly higher glutathionylation of the pyruvate dehydrogenase complex (PDH) was observed in GLRX2 KO cells, in association with a decreased ATP synthesis when pyruvate/malate were used as substrates. Considering the importance of oxidative stress in the pathophysiology of cancer we point to a role for GLRX2 in the regulation of oxidative metabolism in stress-exposed cancer cells.

The Extracellular Matrix in Skin Inflammation and Cancer

Karin Pfisterer

Department of Dermatology, Medical University of Vienna, Austria

Abstract

As one of the main physical barriers, the skin has to effectively combat and respond to pathogenic invaders, while actively maintaining tolerance to the host itself and harmless substances from the environment. If this balance is out of equilibrium chronic inflammatory skin diseases are the consequence. The extracellular matrix (ECM) plays a pivotal role in maintaining tissue homeostasis and as such is an essential player in regulating health and disease. The ECM builds a complex meshwork of fibres and associated glycoproteins, which is unique for each tissue in the human body. In human skin, the ECM consists of two distinct parts: the cell surrounding interstitial matrix that fills the intercellular space in the dermis, and a sheet-like matrix called the basement membrane, which separates the dermis from the epidermis. Long considered a passive contributor to mechanical stability, it is now clear that the ECM is critical for many physiological and pathological processes, such as wound healing, ageing, and cancer development. It builds a protective barrier to prevent pathogen entry, it supports immune cell migration by providing a physical scaffold for cell adhesion, and it is involved in sensing and transduction of mechanical forces.

The Sphingosine Kinase 2 Inhibitor ABC294640 Restores the Sensitivity of BRAFV600E Mutant Colon Cancer Cells to Vemurafenib by Reducing AKT-Mediated Expression of Nucleophosmin and Translationally Controlled Tumour Protein

Mirela Sedic

Centre for Applied Bioanthropology, Institute for Anthropological Research, Ljudevita Gaja 32, 10000 Zagreb, Croatia

Abstract

Vemurafenib (PLX4032) has emerged as a potent anti-cancer agent against metastatic BRAFV600E mutated melanoma. However, its clinical efficacy in metastatic BRAFV600E mutated colorectal cancer is limited due to the development of chemoresistance. Sphingolipids have a demonstrated role as mediators of chemoresistance in colon cancer. In the present study, we explored the role of sphingolipid metabolism and signaling in the development of resistance to vemurafenib in BRAFV600E mutant colon cancer cells. We found significantly increased expression levels of activated forms of sphingosine kinases (SphK1 and SphK2) in resistant cells concomitant with increased abundance of sphingosine-1-phosphate (S1P) and its precursor sphingosine, which was accompanied by increased expression levels of the enzymes regulating the ceramide salvage pathway, in particular ceramide synthases 2 and 6 and acid ceramidase. Pharmacological inhibition of SphK1/SphK2 activities or exogenous C6-ceramide enhanced the anti-proliferative effect of PLX4032 in resistant RKO cells in a synergistic manner. Importantly, inhibition of SphK2 by ABC294640 proved effective at restoring sensitivity of resistant cells to vemurafenib at the largest number of combinations of sub-toxic drug concentrations with minimal cytotoxicity. Furthermore, obtained results showed that enhanced anti-proliferative, anti-migratory, anti-clonogenic and pro-apoptotic effects of combination treatment with ABC294640 and PLX4032 relative to either drug alone were accompanied by the inhibition of S1P-regulated AKT activity and concomitant abrogation of AKT-mediated cellular levels of nucleophosmin and translationally-controlled tumour protein. Collectively, our study suggests the possibility of using the combination of ABC294640 and PLX4032 as a novel therapeutic approach to combat vemurafenib resistance in BRAF mutant colon cancer.

Protein Cargo of Salivary Small Extracellular Vesicles as Potential Functional Signature of Oral Squamous Cell Carcinoma

Simona Fontana[1], Rodolfo Mauceri[2,3,4], Maria Eugenia Novara[1], Riccardo Alessandro[1,5] and Giuseppina Campisi[2]

[1]*Department of Biomedicine, Neurosciences and Advanced Diagnostics; University of Palermo, 90133 Palermo, Italy*

[2]*Department of Surgical, Oncological and Oral Sciences (Di.Chir.On.S.), University of Palermo, 90127 Palermo, Italy*

[3]*Department of Biomedical and Dental Sciences, Morphological and Functional Images, University of Messina, 98124 Messina, Italy*

[4]*Department of Dental Surgery, Faculty of Dental Surgery, University of Malta, 2090 Msida, Malta*

[5]*Institute for Biomedical Research and Innovation (IRIB), National Research Council (CNR), 90146 Palermo, Italy*

Abstract

The early diagnosis of oral squamous cell carcinoma (OSCC) is still an investigative challenge. Saliva has been proposed as an ideal diagnostic medium for biomarker detection by mean of liquid biopsy technique. The aim of this pilot study was to apply proteomic and bioinformatic strategies to determine the potential use of saliva small extracellular vesicles (S/SEVs) as a potential tumor biomarker source. Among the twenty-three enrolled patients, 5 were free from diseases (OSCC_FREE), 6 were with OSCC without lymph node metastasis (OSCC_NLNM), and 12 were with OSCC and lymph node metastasis (OSCC_LNM). The S/SEVs from patients of each group were pooled and properly characterized before performing their quantitative proteome comparison based on the SWATH_MS (Sequential Window Acquisition of all Theoretical Mass Spectra) method. The analysis resulted in quantitative information for 365 proteins differentially characterizing the S/SEVs of analyzed clinical conditions. Bioinformatic analysis of the proteomic data highlighted that each S/SEV group was associated with a specific cluster of enriched functional network terms. Our results highlighted that protein cargo of salivary small extracellular vesicles defines a functional signature, thus having potential value as novel predict biomarkers for OSCC.

The Warburg Phenotype of Tumors – A Metabolic Checkpoint

Kathrin Renner, Nathalie Babl and Marina Kreutz

Department of Internal Medicine III, University Hospital Regensburg, Germany

Abstract

Tumor cells frequently show an accelerated glucose metabolism. However, in contrast to non-malignant cells pyruvate is rather converted into lactate ("Warburg phenotype"), which needs to be efficiently secreted to sustain the glycolytic flux. The main lactate transporters identified so far are the monocarboxylate transporter (MCT) 1 and 4, secreting lactate in co-transport with a proton. Thus, the tumor microenvironment is characterized by rather low glucose levels, the accumulation of lactate and a concomitant acidification ("lactic acid"), negatively affecting T and NK cell function. In line, these metabolic conditions limit the efficacy of immunotherapeutic approaches. We dissected the impact of low glucose versus elevated lactic acid concentrations on T and NK cell function and found lactic acid to be dominating. Low glucose levels reduced solely the proliferation of T cells, whereas lactic acid diminished T cell effector functions, proliferation and viability. Accordingly, the application of glycolytic inhibitors especially in conjunction with checkpoint blockade improved the anti-tumor response of T cells *in vitro* and *in vivo*. In summary, the combination of anti-glycolytic drugs and immunotherapeutic approaches could be a promising strategy in tumors displaying the Warburg phenotype.

Inhibition of Mammalian Target of Rapamycin Induces Insulin-like Growth Factor Receptor-mediated Cell Survival Signaling Through Positive Control of Protein Synthesis

Sang-Oh Yoon

University of Illinois at Chicago, IL, USA

Abstract

Mammalian or mechanistic target of rapamycin (mTOR) plays a crucial role in mRNA translation, metabolism, and cell growth. As a component of mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), dysregulation of mTOR signaling contributes to the progression of diseases including cancer. Thus, drugs that target mTOR signaling networks are used in clinics to treat cancer patients. However, most tumors eventually become resistant to mTOR targeting drugs. Therefore, it is of utmost importance to determine feedback signaling mechanisms that govern cell response to mTOR targeting. Using transcriptome- and proteome-wide analysis, we have found that cells induce unique transcriptional and translational signaling according to different mTOR inhibition such as mTORC1 and dual mTORC1/2 inhibition. Although mTOR is a well-known positive regulator of mRNA translation, inhibition of mTOR upregulates proteins involved in insulin-like growth factor receptor (IGFR) signaling, which induces cell survival. Activation of IGFR signaling by mtTOR inhibition is mainly mediated by non-canonical mRNA translation, not by transcription and protein degradation. This study determines cancer's resistance mechanisms toward mTOR targeting, identifying strategies to overcome the resistance.

SIK2 Inhibition Synergistically Enhances PARP Inhibitor Activity in Ovarian and Triple Negative Breast Cancers

Zhen Lu

Associate Professor, The University of Taxes MD Anderson Cancer Center, Houston, TX

Abstract

Genomic instability is a recognized hallmark of cancer. Germline mutations in critical DNA-repair and DNA-damage response genes predispose to cancer development, but also create vulnerabilities that can be exploited for cancer therapy. Poly (ADP-ribose) polymerase (PARP) inhibitors are selectively active in cells with homologous recombination deficiency (HRD) caused by mutations in BRCA1, BRCA2, and other DNA repair genes. PARP inhibitors elicit significant responses in ovarian and breast cancers from BRCA1 or BRCA2 mutation carriers. However, many cancers that initially respond to PARP inhibitors eventually develop drug resistance. Thus, it is important to develop new strategies to enhance PARP inhibitor sensitivity and to increase the duration of response. We have identified Salt Induced Kinase 2 (SIK2) inhibitors (ARN3236 and ARN3261) that induce double strand breaks (DSBs) in DNA of HR-competent cells and produce synthetic lethality with multiple PARP inhibitors. SIK2 is an AMP-activated protein kinase (AMPK)-related protein kinase that is required for ovarian cancer cell proliferation and metastasis. SIK2 is overexpressed and correlates with poor prognosis in patients with high-grade serous ovarian carcinoma and triple negative breast cancer. SIK2 inhibition enhances paclitaxel sensitivity in both cancer types. We have demonstrated that olaparib-induced-growth inhibition was significantly enhanced by concurrent treatment with either ARN3236 or ARN3261 in each of 12 ovarian and breast cancer cell lines tested, but not in 3 non-tumorigenic cell lines. Co-administration of olaparib with SIK2 inhibitors suppressed tumor growth and increased the survival of mice with human ovarian (OC316) and breast (MDA-MB-231) cancer xenografts without affecting animal weight. ARN3261 produced little toxicity in preclinical toxicology studies. SIK2 inhibitors decrease the phosphorylation of class-IIa HDAC4/5/7 and abolish class-IIa HDAC 4/5/7-associated transcriptional activity of Myocyte Enhancer Factor 2D (MEF2D). Genome-wide chromatin immunoprecipitation (CHIP) sequencing revealed that SIK2 inhibitors reduce MEF2D binding to regulatory regions with high-chromatin accessibility in DNA repair genes, including FANCD2, EXO1 and XRCC4, resulting in repression of critical genes in DNA DSB repair pathway and induction of apoptosis. In addition, SIK2 inhibitors significantly decreased olaparib-mediated PARP activity and enhanced olaparib-induced cytotoxicity. Together, our data argue that the combination of a SIK2 inhibitor and a PARP inhibitor has

the potential to increase the magnitude and duration of PARP inhibitor activity with tolerable toxicity. Use of a SIK2 inhibitor in combination with a PARP inhibitor provides a novel therapeutic strategy for ovarian and triple negative breast cancers with or without BRCA gene mutation.

New Therapeutic Strategy Targeting p53 Mutant Cancer

Andrei V. Bakin[1], Justin Zonneville[1], Moyi Wang[1], Mohammed M. Alruwaili[1], Thomas Melendy[2] and Christos Fountzilas[3]

[1]*Department of Cancer Genetics and Genomics, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*

[2]*Department of Microbiology & Immunology and Biochemistry, University at Buffalo, Buffalo, NY, USA*

[3]*Department of Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*

Abstract

The tumor suppressor p53 is commonly inactivated in multiple cancer types, while therapeutic efforts to target mutant p53 were largely unfruitful. Here we report preclinical data supporting a novel combination therapy strategy for treatment of p53-mutant (p53-mut) cancers. Genomic data revealed high expression of Base-Excision Repair (BER) genes in p53-mut cancers. We developed a new methodology for testing BER in live cells. The BER studies revealed a significant dysregulation in BER-mediated repair in p53-mut cells. Taken advantage in this BER defect, we designed a novel treatment strategy that specifically targets p53-mut cancers. We found that treatment with deoxyuridine analogues induced accumulation of DNA damage selectively in p53-mut cells. Further, inhibitors of poly (ADP-ribose) polymerase (PARPi) greatly enhanced this response and increased cell death, although PARPi as a single agent was not effective. In contrast, normal cells responded to PARPi with activation of the p53-p21 axis and cell cycle arrest. Depletion of p53 in p53 wild-type cell lines conferred the p53-mut phenotype. Preclinical cancer studies revealed that the combination of deoxyuridine analogue with PARPi was more effective in inhibition of tumor growth and metastases than either drug alone. This work illustrates a novel combination therapy strategy that may improve survival rates and outcomes for thousands of cancer patients.

USP15 Antagonizes $CRL4^{CRBN}$-mediated Ubiquitylation of Glutamine Synthetase and Neo-substrates

Thang Van Nguyen

Center for Precision Medicine, Department of Medicine, University of Missouri, Columbia, MO, USA

Abstract

Targeted protein degradation by the ubiquitin-proteasome system represents a new strategy to destroy pathogenic proteins in human diseases including cancer and neurodegenerative diseases via molecular glue and proteolysis-targeting chimaera (PROTAC) mechanisms. The immunomodulatory drugs (IMiDs) have revolutionized the treatment of patients with multiple myeloma (MM) and other hematologic malignancies, but almost all patients eventually develop resistance to IMiDs. CRBN, a substrate receptor of CUL4-RBX1-DDB1-CRBN ($CRL4^{CRBN}$) E3 ubiquitin ligase, is a direct target for thalidomide teratogenicity and antitumor activity of IMiDs (now known as molecular glue Cereblon E3 ligase modulators: CELMoDs). Ubiquitylation of target proteins by ubiquitin E3 ligases is required for the action of CELMoD and PROTAC degraders; however, it is not known whether a deubiquitinating enzyme (DUB) might be involved in this process.

The present study revealed that USP15 antagonizes $CRL4^{CRBN}$-mediated ubiquitylation of natural substrate glutamine synthetase and neo-substrates, thereby preventing their degradation. Notably, USP15 is highly expressed in IMiD-resistant MM cell lines. Depletion of USP15 promotes IMiD-induced degradation of IKZF1/3 and

sensitizes IMiD-resistant MM cells to lenalidomide. Indeed, USP15 counteracts $CRL4^{CRBN}$-dependent ubiquitylation of target proteins, thereby preventing their degradation. This accounts for the intrinsic resistance of IMiDs in MM. Inhibition of USP15 represents a valuable therapeutic opportunity to potentiate IMiD/CELMoD and PROTAC therapies for the treatment of many cancers including glioblastoma, MM, Myelodysplastic syndrome with deletion 5q and acute myeloid leukemia.

In conclusion, the findings not only underscore the important contribution of USP15 to the molecular action of CELMoDs/PROTACs but also highlight the potential of USP15 as a key drug target for cancer therapeutics.

TMEM97 Ablation Aggravates Oxidant-induced Retinal Degeneration

Colleen M. Cebulla

Department of Ophthalmology and Visual Sciences, Havener Eye Institute, The Ohio State University Wexner Medical Center, Columbus, OH, USA

Abstract

The oxidant damage and degeneration of the retinal pigment epithelium (RPE) is central to the pathogenesis of age-related macular degeneration (AMD). This degeneration of the RPE in the macula leads to loss of the overlying photoreceptors and permanent loss of central vision. Recent meta-analyses identified TMEM97 as a new putative AMD risk locus, though functional studies regarding the expression and role of TMEM97 in the RPE and retina had not been done. We investigated TMEM97 function using the sodium iodate model of oxidant-induced retinal degeneration in TMEM97 knockout (KO) mice. Genetic depletion of TMEM97 significantly enhanced oxidative damage in the retina, with increased reactive oxygen species (ROS) and loss of photoreceptors relative to wild type (WT) controls. The damage could be mitigated with *N*-acetyl cysteine antioxidant supplementation. The mechanism of oxidative damage was associated with reduction of levels of master antioxidant transcription factor NRF2 and its target gene product SOD2, the mitochondrial superoxide dismutase, as well as elevated ROS and apoptosis markers, as shown in CRISPR-mediated TMEM97 KO RPE cells compared with controls. Moreover, TMEM97 KO affected proteins key to mitochondrial and lysosomal stability and impeded autophagy flux. These findings suggest that the absence of TMEM97 in RPE cells disturbs redox-balancing systems, thereby heightening oxidative stress. TMEM97 is a druggable target and further translational work should be performed to evaluate candidate therapeutics for TMEM97 in AMD.

Stabilizing Nuclear p27kip1 with Small Molecule Inhibitors of the SCF-Skp2-Cks1 Ubiquitin E3 Ligase Complex as a Cytostatic Therapeutic Approach to Cancer

Leslie I. Gold[1,2], John Dickson[3], Elmar Nurmemmedov[4], Bing Hao[5], Yunfeng Li[5], Nadim Shohdy[1], Xinyan Huang[1], Julien Dubrelle[1], Unnati Pandya[1,6], Bo Rueda[6], Michael Mancini[7] and Fabio Stossi[7]

[1]*New York University School of Medicine—Langone Health, New York, NY, USA*

[2]*Perlmutter Comprehensive Cancer Center, New York, NY, USA*

[3]*Aptachem Consulting LLC, Apex, NC, USA*

[4]*St. John's Cancer Institute, Santa Monica, CA, USA*

[5]*University of Connecticut, Storrs, CT, USA*

[6]*Harvard Medical School, Boston, MA, USA*

[7]*Baylor College of Medicine, Houston, TX, USA*

Abstract

The cyclin-dependent kinase inhibitor, $p27^{kip1}$ (p27), is a substrate for ubiquitylation [and degradation] by the SCF-Skp2-Cks1 E3 ligase causing uncontrolled proliferation in many human cancers. We have previously shown

that estrogen induces MAPK phosphorylation of p27 at Thr187, a prerequisite for SCF-Skp2-Cks1 ubiquitylation of p27 causing degradation of nuclear p27 in primary endometrial cancer (ECA) primary cells and cell lines. This finding parallels the observed absence of nuclear p27 in ECA, largely an estrogen-induced cancer, providing an explanation for a mechanism involved in endometrial carcinogenesis. To block p27 degradation, based on the crystal structure of Skp1-Skp2-Cks1[SSC]-p27, two approaches were used to identify small molecule inhibitors of SCF-Skp2-Cks1 E3 ligase activity (Skp2E3LIs): (i) a structure-based virtual screen targeting the protein interaction surfaces formed by Skp2-Cks1 [the p27 binding pocket] and separately, the Skp1-Skp2 interface, and (ii) ligand-based virtual screen of previously published compounds, which target the p27 pocket within Skp2-Cks1 [blocked E2-induced endometrial hyperplasia in mice with an increase in nuclear p27] or the Skp1-Skp2 interface [blocked tumor growth in mice]. The virtual screening hits (207 compounds) were subjected to time-resolved-FRET and fluorescence polarization (Skp2-Cks1 pocket interface) and ELISA, (Skp1-Skp2 interface) for evaluation of their binding affinities (IC50) to the SSC complex and hillslope values. 35 hits that elicited IC50s ≤ 120 μM in both assays were further evaluated by high content image analysis (HCA) for toxicity, uridine (EdU) uptake, and number of p27 fluorescent nuclei. Three Skp2E3LIs showed IC50s <60 μM (NYU12, NYU27 against the Skp1/Skp2 interface and NYU2 targeting p27 pocket) were non-toxic by trypan blue, were soluble at their potency concentration [10μM] by a kinetic solubility assay, demonstrated EC50s for growth inhibition of ≤15μM, increased nuclear p27, and blocked retinoblastoma protein (pRb) phosphorylation, thereby preventing S phase progression. Furthermore, Skp2E3LIs block both E2-induced degradation of nuclear p27 and proliferation in ECA primary cells and induced an increase in nuclear p27 in 3D spheroids composed of ECA cells and ECA cancer associated fibroblasts (CAFs). Soluble SKP2E3LIs, structurally similar to NYU12 and NYU2 but functionally inactive were selected to compare with active Skp2E3Lis for pharmacological target validation using the Cellular Thermal Shift Assay (CETSA). Following the addition of Increasing concentrations of Active and Inactive Skp2E3LIs to ECA cell lysates followed by heat challenge, a thermal shift in soluble protein and increase in protein stabilization, detected by immunoblotting with anti-Skp2 and Cks1 antibodies, was observed for the active but not inactive analogs. SKp2E3LIs represent a new class of specific E3 ligase inhibitors that prevent degradation of cell cycle proteins involved in growth control. As cytostatic drugs have been shown to increase survival in human cancers, optimization of Skp2E3LIs by SAR, shows promise as a cytostatic therapeutic approach to cancers characterized by high Skp2 levels and loss of nuclear p27.

The Roles and Transcriptional Mechanisms of TWIST1-regulated Genes in Cancer Cells

Xiaobin Yu and **Jianming Xu**

Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Abstract

Because a progressive change of cell plasticity from epithelial-to-mesenchymal transition (EMT) increases the motility, invasiveness, metastasis, stemness, and drug resistance of cancer cells, it is important to understand the molecular mechanisms that regulate EMT in cancer cells. TWIST1 is one of the master transcription factors that induce EMT, and TWIST1 expression in breast cancer is also associated with metastasis, drug resistance and poor prognosis. A previous study has reported that the acetylated form of TWIST1 interacts and recruits BRD4 to chromatin to enhance P-TEFb/Pol II complex-mediated transcriptional elongation, resulting in an increase in its target gene expression. We have also reported that TWIST1 with unknown acetylation status interacts and recruits the NuRD complex to repress its target genes. However, we still do not know what determines TWIST1 to repress or activate its target genes, and how TWIST1-repressed and TWIST1-activated target genes contribute to breast cancer cell growth and metastasis. In this presentation, we will provide results to demonstrate that: 1) unacetylated TWIST1 selectively interacts with the NuRD HDAC (histone deacetylase) complex and recruits this complex to the chromatin for transcriptional repression of epithelial genes; 2) acetylated TWIST1 selectively interacts and recruits the TIP60 histone acetyltransferase (HAT) complex to the chromatin for transcriptional activation of mesenchymal and growth genes; 3) Both TWIST1-repressed and TWIST1-activated target genes are required for the full function of TWIST1 to promote breast cancer cell growth, invasion, and metastasis; and 4) the N-terminus of TWIST1 (TWIST1-N) interacts with both NuRD and TIP60 complexes and does not bind DNA, and overexpressed TWIST1-N is a potent dominant-negative

inhibitor of the full-length TWIST1 for inhibition of TWIST1-mediated cancer cell EMT, migration, invasion, metastasis, and growth.

A Role for Proteasome 26S Subunit, Non-ATPase 3 (PSMD3), in Disease Progression and Drug Resistance of Myeloid Leukemia

Anna M. Eiring[1-3], Joshua J. Lara[2], James E. Young[3], Jose L. Lopez[2], Vanessa V. Velazquez[2], Mayra A. Gonzalez[1], Idaly M. Olivas[1,3] and Alfonso E. Bencomo-Alvarez[1]

[1]*Center of Emphasis in Cancer, Department of Molecular and Translational Medicine, Texas Tech University Health Sciences Center at El Paso, El Paso, TX, USA*

[2]*Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center at El Paso, El Paso, TX, USA*

[3]*Graduate School of Biomedical Sciences, Texas Tech University Health Sciences Center at El Paso, El Paso, TX, USA*

Abstract

Acute myeloid leukemia (AML) patients with mutations in the FMS-like tyrosine kinase 3 (FLT3) gene can be treated with tyrosine kinase inhibitors (TKIs) targeting FLT3. However, many patients develop resistance or experience adverse side effects. The ubiquitin-proteasome system (UPS) plays an important role in regulating protein homeostasis, cell cycle progression, and apoptosis, thereby representing a potential target for combination therapies. However, similar to FLT3 TKIs, proteasome inhibitors are prone to adverse side effects and drug resistance, highlighting the need for alternative therapeutic strategies. We recently reported an oncogenic role for two members of the 19S regulatory complex, 26S proteasome non-ATPase subunits 1 (PSMD1) and 3 (PSMD3), in disease progression and drug resistance of chronic myeloid leukemia (CML) and various solid tumors. We hypothesized that these genes may also play an oncogenic role in FLT3+ AML.

TCGA data revealed that high levels of PSMD3 but not PSMD1 expression correlated with worse overall survival (OS) in FLT3+ AML (p=0.00031). PSMD3 knockdown impaired colony formation of the FLT3-mutant AML cells lines, MOLM-13 and MOLM-14, correlating with increased OS in xenograft models. In contrast with our data in CML, PSMD3 knockdown had little effect on apoptosis or nuclear factor-kappa B transcription. Rather, mass spectrometry-based proteomics analyses revealed a potential role for PSMD3 in regulating energy metabolism. Consistently, PSMD3 knockdown resulted in reduced oxygen consumption rates in MOLM-14 cells. Altogether, PSMD3 may represent a novel molecular biomarker in FLT3+ AML and may be a novel target for combination therapies.

Targeting Lipocalin-2 in Inflammatory Breast Cancer Cells with Small Interference RNA and Small Molecule Inhibitors

Ginette S. Santiago-Sanchez[1], Ricardo Noriega Rivera[2,3], Eliud Hernandez O'farrill[4], Fatma Valiyeva[3], Blanca Quinones Diaz[2,3], Emilly S. Villodre[5], Bisrat Debeb[5], Andrea Rosado Albacarys[6] and Pablo E. Vivas Mejía

[1]*Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*

[2]*Department of Biochemistry, University of Puerto Rico, Medical Sciences Campus, SJ, PR, USA*

[3]*Comprehensive Cancer Center, University of Puerto Rico, Medical Sciences Campus, SJ, PR, USA*

[4]*Pharmaceutical Sciences, University of Puerto Rico, Medical Sciences Campus, SJ, PR, USA*

[5]*Department of Breast Medical Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX, USA*

[6]*Department of General Sciences, University of Puerto Rico, Rio Piedras Campus, SJ, PR, USA*

Abstract

Inflammatory Breast Cancer (IBC) is an aggressive form of invasive breast cancer, highly metastatic with a dismal clinical outcome. The development of novel targeted therapies is needed to improve patient's survival. Here, we proposed Lipocalin-2 (LCN2) (a secreted glycoprotein aberrantly abundant in different cancers) as a plausible target for IBC. We observed higher LCN2 protein levels in IBC compared to non-IBC cells. SiRNA-LCN2 significantly reduced cell proliferation, migration, and invasion in IBC cells. Furthermore, LCN2 silencing promoted apoptosis and arrested the cell cycle progression in G0/G1 to S phase transition. Four potential LCN2 inhibitors, identified throughout in silico analysis among a set of 25,000 ligands, significantly decreased cell proliferation and viability of SUM149 cells. MCF7 cells ectopically expressing LCN2 and treated with two LCN2 inhibitors showed a significant decrease in cell proliferation, suggesting the selectivity of the LCN2 inhibitors in the IBC cells. Since the evidence indicates that Matrix Metalloproteinase 9 (MMP9) interaction with LCN2 is essential for LCN2 activity, we mutated the cysteine critical for the LCN2-MMP9 heterodimer formation to alanine in an LCN2 vector. Wild type (wt) and mutant (mt) vectors were stably transfected in breast cancer cells (MCF7). We observed a decrease in the invasion of the MCF7-mt compared to MCF7-wt clones when exogenous MMP9 was added to the cells. This result suggests that MMP9 modulates the invasive potential of the LCN2-overexpressing IBC cells. Our findings suggest LCN2 as a promising target for IBC treatment using siRNA and small molecule inhibitors.

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The REGγ Inhibitor NIP30 Increases Sensitivity to Chemotherapy in p53-deficient Tumor Cells

Xiaotao Li and Xiao Gao

Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences, School of Life Sciences, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China

Abstract

A major challenge in chemotherapy is chemotherapy resistance in cells lacking p53. Here we demonstrate that NIP30, an inhibitor of the oncogenic REGγ-proteasome, attenuates cancer cell growth and sensitizes p53-compromised cells to chemotherapeutic agents. NIP30 acts by binding to REGγ via an evolutionarily conserved serine-rich domain with 4-serine phosphorylation. We find the cyclin-dependent phosphatase CDC25A is a key regulator for NIP30 phosphorylation and modulation of REGγ activity during the cell cycle or after DNA damage. We validate CDC25A-NIP30-REGγ mediated regulation of the REGγ target protein p21 in vivo using p53−/− and p53/REGγ double-deficient mice. Moreover, Phosphor-NIP30 mimetics significantly increase the growth inhibitory effect of chemotherapeutic agents *in vitro* and *in vivo*. Given that NIP30 is frequently mutated in the TCGA cancer database, our results provide insight into the regulatory pathway controlling the REGγ-proteasome in carcinogenesis and offer a novel approach to drug-resistant cancer therapy.

Sensitization of Pediatric Brain Cancer to Radiotherapy by Inhibition of the DNA Damage Response Pathway

Jessica Buck[1,2], Hetal Dholaria[1,3], Jacqueline Whitehouse[1,2], Meegan Howlett[1,2], Hilary Hii[1], Mani Kuchibhotla[1], Brooke Carline[1], Jacob Byrne[1], Jessica Lawler[2], Patrick Dyer[1], Gilbert Porter[2], Jennifer Stripay[4], Stephanie Smith[4], Martin Ebert[2,5], Martine Roussel[4], Nick Gottardo[1,3] and Raelene Endersby[1,2]

[1]*Telethon Kids Institute, Australia*

[2]*University of Western Australia, Australia*

[3]*Perth Children's Hospital, Australia*

[4]*St Jude Children's Research Hospital, Memphis, TN, USA*

[5]*Sir Charles Gairdner Hospital, Australia*

Abstract

Medulloblastoma is a pediatric brain cancer with poor survival outcomes. Radiation therapy is an important part of clinical treatment, and radiation is a potent inducer of DNA damage in cancer cells. Since CHK1/2, ATR, and ATM kinases are critical regulators of the DNA damage response pathway, we hypothesized that inhibition of these kinases may enhance radiation-induced cytotoxicity in medulloblastoma. *In vitro* treatment of medulloblastoma cell lines (D425, D283, SU-MB002) with either CHK1/2 inhibitor (Prexasertib), ATR inhibitor (AZD6738), or ATM inhibitor (KU-60019) in combination with radiation showed synergistic cell killing. Prexasertib was selected for further testing, and in combination with radiation was shown to reduce proliferative capacity of medulloblastoma cells. Target inhibition of CHK1/2 was confirmed, and prexasertib blocked radiation-induced cell cycle arrest, leading to increased apoptosis both *in vitro* and *in vivo*. In an orthotopic xenograft model of medulloblastoma, 20 mg/kg/day prexasertib in combination with 20 Gy craniospinal radiotherapy significantly extended survival compared to radiotherapy alone. CHK1/2 inhibition therefore shows promise as a strategy for radiosensitization, which could contribute to increased survival rates in children with medulloblastoma. Future studies will test the effect of more highly brain-penetrant CHK1/2 inhibitors, along with inhibitors of the DNA damage response pathway including ATR, ATM and WEE1.

Nlp Promotes Autophagy through Facilitating the Interaction of Rab7 and FYCO1

Danna Yeerken, Wenchang Xiao[1], Weimin Zhang and Qimin Zhan

Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Laboratory of Molecular Oncology, Peking University Cancer Hospital & Institute, Beijing 100142, China

Abstract

Autophagy is the main degradation pathway to eliminate long-lived and aggregated proteins, aged or malfunctioning organelles, which is essential for the intracellular homeostasis and prevention of malignant transformation. Although the processes of autophagosome biogenesis have been well illuminated, the mechanism of autophagosome transport remains largely unclear. In this study, we demonstrated that the ninein-like protein (Nlp), a well-characterized centrosomal associated protein, was able to modulate autophagosome transport and facilitate autophagy. During autophagy, Nlp colocalized with autophagosomes and physically interacted with autophagosome marker LC3, autophagosome sorting protein Rab7 and its downstream effector FYCO1. Interestingly, Nlp enhanced the interaction between Rab7 and FYCO1, thus accelerated autophagic flux and the formation of autophagolysosomes. Furthermore, compared to the wild-type mice, NLP deficient mice treated with chemical agent DMBA were prone to increased incidence of hepatomegaly and liver cancer, which were tight associated with the hepatic autophagic defect.

Taken together, our findings provide a new insight for the first time that the well-known centrosomal protein Nlp is also a new regulator of autophagy, which promotes the interaction of Rab7 and FYCO1 and facilitates the formation of autophagolysosome.

Presumptive Phosphologic Circuits in DNA Repair and Rapid Cellular Data Processing

Philip Penketh

Professor Emeritus, Department of Pharmacology, Yale University, New Haven, CT, USA

Abstract

The possible utilization of biological logic circuit(s) in the integration and regulation of DNA repair is examined. The author believes that this mode of control/regulation likely applies to many other areas of cell biology, such as rapid data processing, data transfer between cells, memory and decision making in single cells. Currently there is significant experimental data to support the involvement of biological logic circuit(s) in the control of DNA repair. Sequential logic processes always require a clock to orchestrate the orderly processing of events. In the proposed hypothesis, the pulses in the expression of p53 serve this function. Given the many advantages of logic type control, one would expect that if this had not arisen directly in the abiotic phase of evolution, it would have arisen in the course of _3 billion years of evolution, where single cell life forms were likely the only forms of life, a biological logic type control system would have evolved to control at least some biological processes. Several other required components such as: a method to temporarily inactivate repair processes when they are not required e.g., the reversible inactivation of the suicide repair protein O^6-methylguanine-DNA methyltransferase (MGMT), by phosphorylation. This prevents complex DNA repair systems with potentially overlapping repair functions from interfering with each other.

Functional Decline, Senescence of Hematopoietic Stem Cell Compartment and Elevated Central Carbon Metabolism

Laura Beiro Poisa, Jonathan Landry and **Anthony D. Ho**

Department of Medicine V, Heidelberg University, Heidelberg, Germany

Molecular Medicine Partnership Unit, EMBL & Heidelberg University, Heidelberg, Germany

Abstract

Aging is associated with functional decline of hematopoiesis with compromised innate immunity and propensity to development of malignancies. Previous comprehensive of human hematopoietic stem cells (HSCs) from different age groups showed that aging HSCs are characterized by elevated glycolysis and accumulation of glycogen, in addition to other characteristics of aging HSCs.

We have demonstrated that the alterations upon aging are caused by a subpopulation of HSCs that has become more glycolytic than others and not on a per cell basis. Using a novel method to separate HSCs, we have isolated human HSCs in three distinct subpopulations with high, intermediate, and low glucose uptake (GU) capacity (GUhigh, GUinter, GUlow (scRNA-seq) studies and revealed that the GUhigh subset showed a significantly higher expression in myeloid development, inflammation and stress response, anti-apoptosis cell cycle checkpoint, histone regulation, elevated -galactosidase, and significantly lower expressions of genes involved in lymphoid development. demonstrated the same pattern of changes in the senescent cells of the HSC compartment as the GUhigh subset derived from elderly human subjects, i.e., significant upregulation of gene-set expressions for (a) inflammatory response (b) G2M checkpoint, (c) MTORC1, and (d) ROS.

Our series of proteome and single cell transcriptome analyses have demonstrated that in human subjects, the GUhigh subpopulation is highly enriched in senescent HSCs. The dependency of senescent HSCs on elevated central carbon metabolism, on anti-apoptosis and on mTORC1 pathways may represent ideal targets for senolytic therapy strategies.

Small Molecule, Novel Benzimidazole Derivatives Closely Mimic the Biochemical and Functional Activity of BMPs

Sheyda Najafi, Leonard Barasa, Sabesan Yoganathan and Jeanette C. Perron

Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Queens, NY, USA

Abstract

Bone Morphogenetic Proteins (BMPs) are diverse factors regulating important cellular processes like proliferation and apoptosis, stem cell differentiation, monocyte chemotaxis, and growth cone guidance. To date, no small molecule, full agonist of BMP signaling has been identified. The few compounds reported to be positive regulators of BMP receptor activity act merely as enhancers or sensitizers. Increasing or restoring BMP signaling has been shown to be therapuetically useful disease conditions such as pulmonary arterial hypertension (PAH) and in bone fracture repair. Unfortunately, the direct use of these proteins as therapeutic agents has encountered significant obstacles.

Here, we introduce the novel indolyl-benzimidazole small molecules (SY-LB-35 and SY-LB-57), which faithfully mimicked BMP agonistic activity. SY-LB-35 and SY-LB-57 strongly stimulated Smad phosphorylation as well as non-Smad targets such as Akt, ERK, JNK, and p38 MAPK in a BMP receptor-dependent manner. Exploring the functional consequences of activating BMP signaling in 3 different cell lines demonstrated that these compounds not only activated BMP receptor-dependent signaling but appeared to functionally mirror BMPs by inducing proliferation, differentiation, migration, and bone formation similar to that induced by BMP2. The functional similarities between BMPs and these novel compounds suggests that SY-LB-35 and SY-LB-57 are acting as the first-identified, full BMP receptor agonists. These indolyl-benzimidazoles, which can be efficiently formulated and delivered to target diseased areas, could potentially replace recombinant BMPs for the treatment of BMP-related pathologies, like PAH, or for directing the differentiation of stem cells in fracture repair or other wound healing processes.

Synergy between the Anthocyanin and tasiRNA Pathways Expose Hidden Features of *Arabidopsis* Carbon Metabolism

Nan Jiang[1], Aimer Gutierrez-Diaz[1], Eric Mukundi[1], Yun Sun Lee[1], Blake C. Meyers[3,4], Marisa S. Otegui[2] and Erich Grotewold[1]

[1]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA*

[2]*Departments of Botany and Genetics, Laboratory of Molecular and Cell Biology, University of Wisconsin-Madison, WI 53706, USA*

[3]*Donald Danforth Plant Science Center, St. Louis, MO 63132, USA*

[4]*Division of Plant Sciences, University of Missouri, Columbia, MO 65201, USA*

Abstract

Anthocyanin pigments furnish a powerful visual output of the stress and metabolic status of *Arabidopsis thaliana* plants. Essential for pigment accumulation is TRANSPARENT TESTA19 (TT19), a glutathione *S*-transferase proposed to bind and stabilize anthocyanins, participating in their vacuolar sequestration, a function conserved across the flowering plants. Here, we report the identification of genetic suppressors that result in anthocyanin accumulation in the absence of TT19. We show that mutations in *RDR6*, *SGS3*, or *DCL4* suppress the anthocyanin defect of *tt19* by pushing carbon towards flavonoid biosynthesis. This effect is not unique to *tt19* and extends to at least one other anthocyanin pathway gene mutant. This synergy between mutations in components of the RDR6-SGS3-DCL4 siRNA system and the flavonoid pathway reveals genetic/epigenetic mechanisms regulating metabolic fluxes.

Mitochondria Engage the Integrated Stress Response to Sustain Prostate Cancer Growth

M. Cecilia Caino

Department of Pharmacology, University of Colorado Anschutz, Aurora, CO, USA

Abstract

There is a continued need to identify novel therapeutic targets to prevent the mortality associated with prostate cancer. In this context, we recently described a novel signaling pathway that connects Mitochondrial Rho GTPase 2 (MIRO2) and the Integrated Stress Response (ISR). *MIRO2* mRNA was upregulated in metastatic prostate cancer compared to localized tumors, and higher *MIRO2* levels were correlated with poor patient survival. Using human cell lines that represent androgen-independent or -sensitive prostate cancer, we showed that MIRO2 depletion impaired cell growth, colony formation and tumor growth in mice. Network analysis of MIRO2's binding partners identified metabolism and cellular responses to extracellular stimuli as top over-represented pathways. The top hit on our screen, General Control Non-derepressible 1 (GCN1), was overexpressed in prostate cancer, and interacted with MIRO2 in prostate cancer cell lines and in primary prostate cancer cells. Importantly, MIRO2 was necessary for efficient ISR signaling, via GCN1-mediated GCN2 kinase, and induction of transcription factor ATF4 levels. Further, MIRO2's effect on regulating prostate cancer cell growth were mainly mediated by ATF4. *In vivo*, levels of activated GCN2 and ATF4 were correlated with MIRO2 expression; and both MIRO2 and activated GCN2 levels were higher in hypoxic areas of prostate cancer xenografts. Overall, we identified MIRO2/GCN1/GCN2 as a novel mitochondrial signaling pathway that controls androgen-independent and androgen-sensitive prostate cancer cell growth via the GCN2-arm of the ISR. We propose that targeting the MIRO2-ISR axis may be a valuable strategy to halt prostate cancer growth.

Epigenetic Regulation of Cell Identity Through HMGN Proteins

Bing He and Michael Bustin

Protein Section, Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

Abstract:

The chromatin epigenetic landscape plays a key role in the establishment and maintenance of cell identity, yet the factors that regulate the dynamics of the epigenome are not fully understood. Here we report that nucleosome binding proteins HMGN1 and HMGN2 preferentially colocalize with epigenetic marks of active chromatin, and with cell-type specific enhancers and super enhancers. Loss of HMGNs enhances the rate of OSKM induced reprogramming of mouse embryonic fibroblasts (MEFs) into induced pluripotent stem cells (iPSCs), transcription factor ASCL1 induced conversion of fibroblast into neurons, and ameloblast differentiation in mice. During OSKM induced reprogramming, loss of HMGNs accelerates the erasure of the MEF-specific epigenetic landscape and the establishment of an iPSCs-specific chromatin landscape, without affecting the pluripotency and the differentiation potential of iPSCs. Thus, HMGN proteins modulate the chromatin epigenetic landscape and stabilizing cell identity.

The Aryl Hydrocarbon Receptor (AhR) Regulates Neuroblastoma Differentiation

Kanita A. Chaudhry, Rebecca Wombacher, Anna Bianchi-Smiraglia

Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA

Abstract

Neuroblastoma is a malignancy of the developing sympathetic nervous system and is the most common extracranial solid tumor of childhood. Despite multi-modality therapies, approximately 50% of high-risk patients die

of progressive disease, underscoring the critical need for novel treatment options. Amplification of the *MYCN* oncogene, a master transcriptional driver of neuroblastoma disease progression present in 40-50% of high-risk cases, is associated with unfavorable patient prognosis. However, MycN is a challenging drug target, prompting efforts to indirectly impair its expression or function.

Here, we identify the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, as a novel tumor promoter in neuroblastoma that positively regulates MycN. Our data shows that *MYCN*-amplified neuroblastoma cell lines have higher levels of AhR expression than non-*MYCN*-amplified cells. shRNA-mediated depletion of AhR reduced neuroblastoma cell invasion and clonogenicity, decreased MycN protein expression, and induced differentiation in *MYCN*-amplified cells. AhR depletion diminished neuroblastoma tumor growth in an *in vivo* subcutaneous xenograft murine model. Notably, treatment with clofazimine (CLF), an FDA-approved anti-leprosy antibiotic that we previously identified to be a novel AhR antagonist, showed the same effect. CLF is orally bioavailable, minimally toxic, and cost-effective, representing a potential promising new therapy for neuroblastoma patients. Taken together, our data implicates AhR as a novel therapeutic target in *MYCN*-amplified neuroblastoma. Further study is required to elucidate the molecular mechanisms by which AhR regulates MycN and neuroblastoma differentiation.

GTP Metabolic Enzymes in Control of Cancer Progression

Anna Bianchi-Smiraglia

Department of Cell Stress Biology, Roswell Park Comprehensive Cancer Center, Buffalo, New York, NY, USA

Abstract

GTP is a pleiotropic molecule that plays a key role in many aspects of a cell's life, including as a building block for RNA/DNA synthesis, participating in protein synthesis, and regulating cytoskeleton stability. In particular, we and other have revealed that the levels of GTP and of its metabolic enzymes directly impact the invasive capability of cancer cells through modulation of small GTPases activity. The development of internally ratiometric fluorescent sensors for evaluation of intracellular GTP (GEVALs) has allowed us to assess the dynamic distribution of free intracellular GTP pools during processes such as cancer cells migration and invasion. Our most recent work has revealed that localized GTP production at cell protrusions during invasion is achieved through recruitment of inosine monophosphate dehydrogenase 2 (IMPDH2) and other GTP metabolic enzyme to the cell membrane. This localized GTP production is needed to support GTPase activity and cell invasion and is partly mediated by direct interaction of IMPDH2 with small GTPases. Our findings raise the possibility that selectively disabling IMPDH2's ability to interact with small GTPases and other membrane binding partners may be a novel way to help reducing the invasive capability of tumor cells, minimizing side effects to healthy cells.

In Vitro Injury Models of Human Alveolar Type-2 Organoid Cultures for Drug-Target Validation and Discovery

Anna Engler[1], Magdalena Platzk[1], Carlos Neideck[1], Xin Lin[2], Salil Srivastava[1], Yan Bai[2], Alexis Laux-Biehlmann[1], Markus Koch[3] and Hana Cernecka[3]

[1]*Pulmonary Drug Discovery Lab, Bayer US LLC, Pharmaceuticals, Research & Development, 02115 Boston, MA, USA*

[2]*Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 02115 Boston, MA, USA*

[3]*Bayer AG Pharmaceuticals, Research & Development, 42113 Wuppertal, Germany*

Abstract

Human organoids hold great potential in preclinical and translational research and are a promising resource to address and understand complex diseases processes, discover and validate new therapeutic options and open opportunities for precision medicine. Alveolar Type-2 (AT2) cells are essential for the maintenance of the alveolar epithelium as they have a predominant role in repair after lung injury. Because they are limitation in the

translatability of alveolar biology from classic culturing methods, we opted to implement recently published 3D AT2 cultures (Youk et al 2020, Cell Stem Cell; Katsura et al 2020, Cell Stem Cell). Here, we provide protocols optimized for a higher-throughput utilization of organoid cultures. We compared media high and low in FGF content regarding their colony forming efficiency (CFE) and organoid growth progression. We identified conditions allowing positive and negative assay window observation – showing increased or decreased growth and CFE compared to the experimental control. We compare various starting materials for 3D-cultures, including cell lines, fresh lung isolates and isolates from precision cut lung sections (PCLS). While we found that fresh and frozen lung give high quality cultures, dissociated PCLS were not a suitable source as starting material. Furthermore, we established in vitro injury models by using cigarette smoke extract (CSE)-induced stress stimuli. We compared CSE dose response in short-term (3-days) and long-term (14-days) experiments as well as in comparison with classic 2D, cell line models. We show that organoid cultures are adaptable for medium-throughput use and future optimizations and automations will show whether they can become a high-throughput screening tool for drug discovery.

Improved Methods and Optimized Design for CRISPR Cas9 and Cas12a Homology-Directed Repair

Garrett R. Rettig, Mollie S. Schubert, Bernice Thommandru, Jessica Woodley, Rolf Turk, Gavin Kurgan, Matthew S. McNeill

Integrated DNA Technologies, Coralville, IA, USA

Abstract

CRISPR-Cas proteins – specifically *S.p.* Cas9 and *A.s.* Cas12a – can be used to introduce double-stranded breaks (DSBs) at targeted genomic loci in mammalian cells. Generating edits that mediate specific changes at targeted loci requires homology-directed repair (HDR). To this end a DNA template during repair allows for precise introduction of a desired mutation via the HDR pathway. Here, we describe comprehensive design considerations and optimized methods for highly efficient HDR using single-stranded oligodeoxynucleotide (ssODN) donor templates for several CRISPR-Cas systems including *S.p.* Cas9, *S.p.* Cas9 D10A nickase, and *A.s.* Cas12a delivered as ribonucleoprotein complexes with synthetic guide RNAs. Features relating to guide RNA selection, donor strand preference, and incorporation of blocking mutations in the donor template to prevent re-cleavage were investigated and were implemented in a novel online tool for HDR donor template design. Additionally, we employ chemically modified HDR donor templates in combination with a small molecule to boost HDR efficiency up to 10-fold. These findings allow for highly efficient, precise repair utilizing HDR in multiple mammalian cell lines. Finally, we feature these design rules in a case study to generate multiplex SNP changes via HDR.

Functions of Glutaredoxin Domain-containing Proteins in Hearing and Deafness

Chang Liu, Jinan Li, and **Bo Zhao**

Indiana University School of Medicine, Indianapolis, IN 46202, USA

Abstract

Stereocilia of cochlear hair cells are specialized mechanosensing organelles that convert sound-induced vibration to electrical signals. The base of the stereocilia, which is the site of stereocilia pivoting, is organized by several proteins necessary for human hearing and essential for stereocilia morphogenesis and function. In our studies, we found that glutaredoxin domain-containing cysteine-rich protein 2 (GRXCR2), localized at the base of stereocilia, is critical for the morphogenesis of stereocilia by interacting with taperin, an actin regulatory protein at the base of stereocilia. Interestingly, reducing taperin expression level rescues the morphological defects of stereocilia but only partially restores the hearing of *Grxcr2*-null mouse, suggesting that GRXCR2 interacts with some other proteins required for auditory perception. Recently, we identified a novel interaction between GRXCR2 and CLIC5. Disrupting their interaction *in vivo* has minimal effects on stereocilia morphogenesis but leads to moderate hearing loss at lower frequencies and severe hearing loss at higher frequencies, strongly suggesting

that the interaction between GRXCR2 and CLIC5 is essential for normal hearing. Thus, our study provides a foundation for future investigations of the functions of protein complexes at the basal region of stereocilia critical for auditory perception.

Expression of Presenilins: From the Sea Urchin Embryo to the Mouse Brain and Alzheimer's Disease

Brigitte Ciapa

Paris-Saclay Institute of Neuroscience, CNRS, Université Paris-Sud, Université Paris-Saclay, France

Abstract

Two presenilins, PSEN1 and PSEN2, are expressed in humans where they play a crucial role in Alzheimer's disease (AD). The greatest numbers of mutations carried by FAD (familial forms of AD, 2% of all the AD cases) patients are found in the PSEN1 gene. The remaining AD cases are "sporadic" and linked to aging. Each PSEN is part of the _-secretase complex that has multiple substrates such as Notch or the amyloid precursor protein which gives the amyloid peptides. PSENs also act independently of their γ-secretase activity. In the sea urchin, PSEN is present in unduplicated form and highly similar to that of humans. Its expression must be precisely tuned to control the course of the first mitotic cycles, gastrulation execution and, probably in association with ciliated cells, the setup of tissues during development. By performing in situ hybridization, QPCR and Northern experiments, we found that natural antisense transcription (NAT) corresponding to the PSEN gene can be detected in this model. We then detected the expression of PSEN1 and PSEN2 natural antisense transcripts in mouse brains, a decrease of which may occur during aging. In conclusion, it would be relevant to study the role of PSEN within the gene regulatory networks (GRN) of the sea urchin that control neurogenesis. Studying PSEN1/2 antisense transcription in mouse and human brains could also bring new insights in the AD field, where no therapy has been found so far.

A Weakened Interface in the P182L Variant of HSP27 Leads to Aberrant Binding to Interacting Proteins

Elias Adriaenssens[1], Reid Alderson[2,3], Bob Asselbergh[4], Iva Pritisanac[5], Jonas Van Lent[1], Andrew J. Baldwin[2], Justin LP Benesch[2] and Vincent Timmerman[1]

[1]*Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, Antwerpen, Belgium*

[2]*Chemistry Research Laboratory, University of Oxford, Oxford, UK*

[3]*Laboratory of Chemical Physics, National Institutes of Health, Bethesda, MD, USA*

[4]*Neuromics Support Facility, VIB Center for Molecular Neurology, VIB, Antwerpen, Belgium*

[5]*Molecular Medicine Program, The Hospital for Sick Children, Toronto, ON, Canada*

Abstract

HSP27 is a human molecular chaperone that forms large, dynamic oligomers and functions in many aspects of cellular homeostasis. Mutations in HSP27 cause Charcot-Marie-Tooth (CMT) disease, the most common inherited disorder of the peripheral nervous system. A particularly severe form of CMT disease is triggered by the P182L mutation in the highly conserved IxI/V motif of the disordered C-terminal region, which interacts weakly with the structured core domain of HSP27. Here, we observed that the P182L mutation disrupts the chaperone activity and significantly increases the size of HSP27 oligomers formed in vivo, including in motor neurons differentiated from CMT patient-derived stem cells. Using NMR spectroscopy, we determined that the P182L mutation decreases the affinity of the HSP27 IxI/V motif for its own core domain, leaving this binding site more accessible for other IxI/V-containing proteins. We identified multiple IxI/V-bearing proteins that bind with higher affinity to the P182L variant due to the increased availability of the IxI/V-binding site. Our results provide

a mechanistic basis for the impact of the P182L mutation on HSP27 and suggest that the IxI/V motif plays an important, regulatory role in modulating protein–protein interactions.

Developing the Induced Pluripotent Stem Cell (iPSC) Paradigm as a Personalized Medicine Approach for Late-Onset Alzheimer's Disease

Kai-C. Sonntag[1,2,3], Woo-In Ryu[1,2,3] and Bruce M. Cohen[1,3]

[1]*Department of Psychiatry, Harvard Medical School, Belmont, MA, USA*

[2]*Basic Neuroscience Division, Harvard Medical School, Belmont, MA, USA*

[3]*Program for Neuropsychiatric Research, McLean Hospital, Harvard Medical School, Belmont, MA, USA*

Abstract

The pathology of late-onset Alzheimer's disease (LOAD) is still poorly understood, but it is multifactorial and closely related to changes with age. We developed a cellular platform for LOAD collecting skin fibroblasts or blood cells from LOAD patients and healthy control individuals that are used in the iPSC paradigm to produce brain cells to determine LOAD pathogenic processes in context of age, disease, genetic background, cell development, and cell type. This model has provided evidence for an innate inefficient cellular energy management in LOAD as a potential risk factor for developing neurodegenerative disease with age. One of the findings in LOAD cells was a striking reduction of the redox agent nicotinamide adenine dinucleotide (NAD). Because increase of NAD has been suggested to reduce the risk of aging-associated conditions, we tested nicotinamide riboside (NR), a dietary precursor for NAD *de novo* synthesis, and caffeine, which increases the expression of NMNAT2, an essential enzyme in NAD production. NR and caffeine partially restored NAD levels, however, did not correct the LOAD-associated bioenergetic phenotype. While these data indicate that restoring NAD alone may not be a sufficient control point to address altered energy management in LOAD, they demonstrate the value of the platform in detecting abnormalities and testing interventions. Altogether, this cellular model allows for patient-oriented examination of numerous mechanisms and interactions in LOAD pathogenesis, as a basis for a personalized medicine approach to predict altered aging and risk for development of dementia, and to test or implement (customized) therapeutic or disease-preventive intervention strategies.

SOX2-mediated 5hmC Dysregulation in GBM Stem Cells

Hernando Lopez-Bertoni[1,2,4], Maria Lugo-Fagundo[1], Sweta Shudir[1], Bachchu Lal[1] and John Laterra[1,2,3,4]

[1]*Hugo W. Moser Research Institute at Kennedy Krieger, 707 N. Broadway, Baltimore, MD, USA*

[2]*Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

[3]*Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

[4]*Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Abstract

Primary brain tumors are among the most devastating forms of cancer and glioblastoma (GBM) represents the most aggressive and lethal form of the disease. We now know that GBM contain small subsets of cells that display tumor-propagating stem-like phenotypes (*i.e.* glioma stem cells or GSCs) that act as critical determinants of GBM resistance to current treatments and tumor recurrence for which there is no proven therapy. Altered patterns of DNA methylation are widely reported in human GBM. Understanding and ultimately targeting the epigenetic mechanisms that induce and maintain these tumor-propagating cell subsets is critical to improving GBM therapy and patient outcomes. DNA methylation is a reversible process and is partially mediated by the ten-eleven translocation (TET) family of enzymes which function as deoxygenases to catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Levels of 5hmC negatively correlates with gli-

oma grade and loss of 5hmC correlates with poor prognosis of GBM patients, strongly suggesting that these enzymes activate tumor suppressing mechanisms. We show that TET2 loss associates with GBM stem cells and correlates with poor survival of GBM patients and identify a SOX2: miR-10b-5p: TET2 axis that represses TET2 expression and induces GBM cell stemness and tumor-propagating potential. *In vivo* delivery of a miR-10b-5p inhibitor normalizes TET2 expression and function to induce regression of orthotopic GSC-derived xenografts and prolongs animal survival. These findings highlight the importance of TET2 and 5hmC loss in Sox2-driven oncogenesis and their potential for therapeutic targeting.

miR-10b

Inhibition of drivers of differentiation and tumor suppressor genes:

- Hyper-methylated promoters of Coding and Non-coding genes
- Inability to recruit Co-factors

Inositol Trisphosphate Receptors (IP3Rs) Regulate Mitophagy and Apoptosis in Pancreatic Beta-Cells

Stanislovas S. Jankauskas[1,2], Jessica Gambardella[1,2], Fahimeh Varzideh[1,2], Urna Kansakar[1,2], Geming Lu[3], Pasquale Mone[1,2], Adolfo Garcia-Ocaña[3], Angela, Lombardi[1] and Gaetano Santulli[1,2]

[1]*Department of Medicine, Einstein–Mount Sinai Diabetes Research Center (ES-DRC), Fleischer Institute for Diabetes and Metabolism (FIDAM), Institute for Aging Research, Albert Einstein College of Medicine, New York, NY, USA*

[2]*Department of Molecular Pharmacology, Division of Cardiology, Wilf Family Cardiovascular Research Institute, Institute for Neuroimmunology and Inflammation (INI), Albert Einstein College of Medicine, New York, NY, USA*

[3]*The Diabetes Obesity Metabolism Institute, The Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Abstract

Insulin resistance is a common feature of type 2 diabetes (T2D). Beta-cell failure in T2D provokes uncontrolled hyperglycemia causing severe complications. Inositol Trisphosphate Receptors (IP3R1, IP3R2, IP3R3) represent a family of Ca^{2+}channels located in the endoplasmic reticulum (ER); their functional role in insulin secretion remains to be determined.

We observed significantly increased expression of IP3Rs in pancreatic islets isolated from T2D patients compared to non-diabetic subjects as well as in two murine models of T2D, namely *db/db* mice and high fat diet (HFD). We tested glucose-stimulated insulin secretion (GSIS) in INS-1 cells subjected to prior incubation with normal or high level of glucose. IP3Rs silencing did not affect GSIS in INS-1 cells on normal glucose, but improved GSIS in hyperglycemia conditions.

We then generated a mouse with a beta-cell specific knockout of IP3R1, IP3R2, IP3R3 ($IP3R^{\beta KO}$). $IP3R^{\beta KO}$ mice were significantly protected from HFD-induced deterioration of glucose tolerance. This effect was associated with increased beta-cell mass in $IP3R^{\beta KO}$ due to a markedly decreased rate of beta-cells apoptosis. Mitophagy flux was higher in $IP3R^{\beta KO}$ on HFD compared to $IP3R^{fl/fl}$ and mitochondrial damage was less severe in $IP3R^{\beta KO}$. We discovered that IP3Rs physically bind Beclin1 and mobilize it to ER. Cytoplasmic levels of Beclin1 were indeed lower in beta-cells from HFD fed $IP3R^{fl/fl}$ mice compared to $IP3R^{fl/fl}$ on normal chow, a phenotype that was rescued by the ablation of IP3Rs.

Taken together, our data indicate for the first time that IP3Rs play a key role in beta cell fitness by regulating mitophagy and apoptosis.

Enhancer LncRNAs Regulate Endothelial Function in Health and Disease

Zhen Bouman Chen, Xiaofang Tang, Yingjun Luo, Kiran Sriram, Naseeb Malhi

Department of Diabetes Complications and Metabolism, City of Hope, Duarte, CA, USA

Abstract

Lining the critical interface between circulating blood and vascular wall, endothelial cells (ECs) play vital functions in health and disease. The optimal gene expression in ECs is essential to maintain endothelial homeostasis, and its dysregulation can lead to EC dysfunction, a common mechanism underlying many metabolic and cardiovascular diseases, e.g. diabetes and diabetes-associated vasculopathy. In our study, we aim to identify the role of long non-coding RNAs (lncRNAs), particularly those derived from enhancers in the functional regulation of ECs. Initially, in ECs subjected to distinct flow patterns promoting or disrupting endothelial homeostasis, we profiled EC transcriptome using RNA-sequencing, in conjunction with chromatin immunoprecipitation (ChIP) and chromatin conformation capture assays (e.g. 4C and Hi-C). We identified an enhancer-associated lncRNA that enhances endothelial nitric oxide synthase (eNOS) expression, aka LEENE. LEENE is co-regulated with eNOS and its enhancer resides in proximity to *eNOS* promoter in ECs. Using various gain- and loss-of-function approach, including CRISPR editing and RNA inference, we demonstrate that LEENE regulates eNOS expression. Mechanistically, LEENE facilitates the recruitment of RNA Pol II to the *eNOS* promoter to enhance eNOS nascent RNA transcription. Knockout of LEENE homologue in mouse resulted in impaired microvascular function, evident in a hindlimb ischemia model. Taken together, our study suggests an emerging important mechanism by which chromatin-associated, enhancer-derived lncRNAs, such as LEENE modulates EC gene expression in physiology and pathological conditions.

Junctophilin Proteins in Cellular Function and Disease Physiology

Stephan E. Lehnart[1,2,3,4,5], Gunnar Weninger[1,2,3], Tatiana Pochechueva[1,2,3], Dana El Chami[1,2,3], Xiaojing Luo[6], Tobias Kohl[1,2,3,4,5], Sören Brandenburg[1,2,3,4,5], Henning Urlaub[7,8], Kaomei Guan[6] and Christof Lenz[7,8]

[1]*Cellular Biophysics and Translational Cardiology Section, Heart Research Center Göttingen, University Medical Center Göttingen, Göttingen, Germany*

[2]*Department of Cardiology & Pneumology, University Medical Center Göttingen, Göttingen, Germany*

[3]*Collaborative Research Center SFB1190 "Compartmental Gates and Contact Sites in Cells", University of Göttingen, Germany*

[4]*Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC2067), University of Göttingen, Germany*

[5]*DZHK (German Centre for Cardiovascular Research), partner site Göttingen, Germany*

[6]*Institute of Pharmacology and Toxicology, Technische Universität Dresden, Dresden, Germany*

[7]*Bioanalytics, Department of Clinical Chemistry, University Medical Center Göttingen, Germany*

[8]*Bioanalytical Mass Spectrometry, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany*

Abstract

Junctophilins (JPHs) are structural proteins connecting the plasma membrane to the endo/sarcoplasmic reticulum (ER/SR). ER/SR tethering to surface membrane structures results in the formation of subcellular junctions with important signaling roles in all excitable cell types. The JPH1-4 isoforms are expressed primarily in

muscle and neuronal cell types. JPH1-4 proteins consist of six membrane-occupation-and-recognition-nexus (MORN) motifs, a joining region connecting a second domain of two MORN motifs, a putative alpha-helical region, a divergent region, and a C-terminal transmembrane tail anchor in the ER/SR membrane. While JPH1-4 play essential roles in developing and maintaining subcellular membrane junctions that control subcellular Ca^{2+} signaling nanodomains, inherited mutations in JPH2 cause hypertrophic or dilated cardiomyopathy, whereas trinucleotide-expansions in the JPH3 gene cause Huntington Disease-Like 2. Decreased JPH1 and JPH2 protein levels can cause skeletal myopathy or heart failure and atrial fibrillation, respectively, and other diseases. JPH1 and JPH2 are Calpain-specific proteolytic targets inside Ca^{2+} release units required for excitation-contraction coupling in skeletal and cardiac myocytes, respectively. Loss of JP2 by Calpain cleavage in heart failure has been reported, however, the precise molecular identity of the Calpain cleavage sites and the (patho-)physiological roles of the proteolytic products remain unclear. We systematically analyzed the JP2 cleavage fragments of Calpain-1/2, revealing that both isoforms preferentially cleave mouse JP2 at R565, but subsequently at three additional secondary Calpain cleavage sites. Moreover, we identified the Calpain-specific primary human JPH2 cleavage products for the first time in iPSC-derived cardiomyocytes. Our data suggest the secondary cleavage event as therapeutic opportunity.

Glucocorticoid-Induced Leucine Zipper and the Failing Heart

Konrad Urbanek

Department of Experimental and Clinical Medicine, University 'Magna Graecia' of Catanzaro, Italy

Abstract

Glucocorticoids (GCs) are essential in regulating functions and homeostasis in numerous biological systems and are among the most powerful drugs for the treatment of autoimmune and inflammatory diseases, but their long-term usage is limited by severe adverse effects. For this reason, to envision new therapies devoid of GC side effects, research has focused on expanding the knowledge of cellular and molecular effects of GCs. GC-induced leucine zipper (GILZ) is a GC-target protein shown to mediate several actions of GCs, including inhibition of the NF-κB and MAPK pathways. GILZ expression is not restricted to immune cells, and it has been shown to play a regulatory role in many organs and tissues, including the cardiovascular system where its contribution to the adaptations of stressed myocardium and the vascular response upon chronic stress is becoming evident. Research on the role of GILZ on endothelial cells has demonstrated its ability to modulate the inflammatory cascade, resulting in a downregulation of cytokines, chemokines, and cellular adhesion molecules. GILZ also has the capacity to protect myocardial cells, as its deletion makes the heart more vulnerable. Moreover, although the available data are limited at present, the role of this GC-target protein in myocardial biology possibly extends beyond its anti-inflammatory/immunosuppressive effects.

The Role of Autophagy in Regulation of Cell Survival/Cell Death in Ovarian Cell Cytotoxicity

Abdel Halim Harrath, Abdulkarem Alrezaki, Lamjed Mansour and Saleh Alwasel

King Saudi University, Department of Zoology, College of Science, Saudi Arabia

Abstract

The exposure to toxicants including petroleum derivatives in our daily life is inevitable due to their extensive use in both industry and at home. Several studies provided evidences for the multiple damage that can display these hydrocarbons on the different tissues and organs, including ovarian cells. Increasing evidence supports the essential role of autophagy in regulating the ovarian toxicity due its key role in carrying normal folliculogenesis as it can favor follicular survival or degeneration. For this purpose, we evaluated autophagic and apoptotic related markers in rat ovarian cells after exposure to different toxicants that are reported to be harmful to the animal and human tissues and cells. We found that the number of growing follicles decreased whereas the number of abnormal follicles increased, leading to faster female reproductive aging. Interestingly, both mechanisms of au-

tophagy and apoptosis have been triggered in ovaries from treated groups compared to control. Indeed, when ovarian cells are subjected to toxicants stress, autophagy is promoted to make a decision on the fate of these cells depending on the magnitude and duration of exposure. Once promoted autophagy can play a protective role and hence contribute to cell adaption and survival. However, when the damage is relatively high, autophagy is promoted to induce cell death by apoptotic pathway, which was associated with oxidative stress-related PI3K/AKT/mTOR signaling pathway.

The Strange Cases of Leukocyte Migration and Adhesion: Swimming and Reverse Haptotaxis

Olivier Theodoly[1] and Laurene Aoun[1], Alexander Farutin[2], Nicolas Garcia Seyda[1], Xuan Luo[1], Valentine Seveau de Noray[1], Chaouqi Misbah[2] and Marie-Pierre Valignat[1]

[1]*Laboratory Adhesion & Inflammation (LAI), Aix Marseille University, CNRS, INSERM, Turing Centre for Living Systems, Marseille, France*

[2]*University Grenoble Alpes, CNRS,LIPhy, Grenoble, France*

Abstract

Adhesion plays a crucial role in leukocyte migration and guidance in vivo. Upon recruitment from blood to inflamed zones, leukocytes cross blood vessels endothelium at transmigration portals enriched in integrins ligands and then migrate in tissues with an integrin-dependent manner. Here, we use in vitro approaches to shed further light on the links between adhesion and amoeboid migration. Concerning migration, integrin-mediated adhesion was previously reported to be dispensable, provided that cells were confined within a solid environment. We show here that lymphocytes can also literally "*swim*" in suspension, i.e. that propulsion is possible without any contact to a solid matrix. Propulsion is explained by a mechanism of paddling molecular rather than protrusion scale. Concerning guidance, there exists no direct proof, to our knowledge, of adhesive haptotaxis with leukocytes or more generally with amoeboid cells. We demonstrate here that lymphocytes are sensitive to modulations of adhesion and display adhesive haptotaxis. Moreover, we reveal a unique phenotype of haptotaxis towards lower adhesion zones. This new phenotype of "*reverse haptotaxis*" seems specifically mediated by LFA-1 integrins.

Macrophage Osr1-PPARγ Regulation Plays an Essential Role in Liver Inflammation of NASH Through Regulating Macrophage Polarization and Metabolism

Lin Liu[1], Linghao Hu[2], Brian Kidd[3], Alexandra Walsh[2], Ke Zhang[1,3] and **Linglin Xie**[1]

[1]*Department of Nutrition, Texas A&M University, College Station, TX 77845, USA*

[2]*Department of Biomedical Engineering, Texas A&M University, College Station, TX 77845, USA*

[3]*Texas A&M Institute of Biosciences and Technology, College Station, TX 77845, USA*

Abstract

Liver macrophage-mediated inflammation contributes to the pathogenesis of non-alcoholic steatohepatitis (NASH), which is the most common cause of chronic liver disease in the United States. Odd-skipped related 1 (Osr1), a putative transcription factor, plays important roles in development and tumorigenesis. We previously report more severe NASH phenotype in Osr1 heterozygote mice exposed to high-fat dirt (HFD), however, the underlying mechanisms remain unknown. The current study is focused on the role of Osr1 in the macrophage's polarization and the associated function in the inflammation-induced pathogenesis of NASH. Osr1 is expressed in both hepatocytes and macrophages and exhibited different expression changes in NASH. In HFD-induced nonalcoholic fatty liver disease (NAFLD) and methionine and choline deficient diet (MCD) induced NASH models, deleting Osr1 in the myeloid cells (Osr1ΔMφ), but not the hepatocytes, caused more severe steatohepatitis with exacerbated liver inflammation, highlighting an important role of Osr1 in macrophage mediated liver

inflammation. It is determined that myeloid Osr1 deletion resulted in a polarization switch towards M1 phenotype, associated with energy utilization shift between glycolysis and oxidative phosphorylation (OXPHOS). These inflamed Osr1ΔMφ macrophages further promoted steatosis and inflammation of the hepatocytes via cytokine secretion. We further identified two downstream transcriptional targets of Osr1, c-Myc and PPARγ and established the Osr1-PPARγ regulation in macrophage polarization and liver inflammation in both mouse genetic study and by rosiglitazone treatment in vivo. Consistently and importantly, hepatic overexpression of Osr1 via AAV-delivery significantly repressed the NAFLD/NASH progression in both WT and Osr1ΔMφ mice. Thus, we established that the myeloid Osr1-PPARγ plays a key role in maintaining the balance of liver immune homeostasis and breaking this balance aggravates the progression of NAFLD/NASH.

DNA Repair Gone Bad: Repeat Expansion in the Fragile X-related Disorders

Karen Usdin, Xiaonan Zhao, Inbal Gazy, Carson Miller and Bruce Hayward

National Institute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, MD, USA

Abstract

The Fragile X-related disorders are members of a larger group of genetic disorders known as the Repeat Expansion Diseases, a group of more than 40 different disorders resulting from instability of a disease-specific microsatellite. This microsatellite instability (MSI) is very different from the classic MSI associated with certain types of cancer. Specifically, instability affects a single genetic locus and depends on some of the very same mismatch repair (MMR) factors that protect against cancer-associated MSI. There is growing evidence to support the idea that all of these diseases share a common mutational mechanism. Recent findings from human and mouse models of the Fragile X-related disorders support a model for this MSI in which mis-paired DNA formed during processes like transcription, enters the MMR pathway but is subsequently diverted into a double-strand break repair pathway that produces small, but very frequent expansions as well as larger contractions. This work has identified genetic factors essential for expansion that may be suitable targets for reducing expansion risk.

The Pathogenic Role of PMN Exosomes in Inflammatory Lung Disease

Matthew Madison, Kris Genschmer, Derek Russell, Camilla Margaroli, Liliana Viera, Amit Gaggar, and J. Edwin Blalock

Department of Medicine, Division of Pulmonary, Allergy & Critical Care Medicine, University of Alabama at Birmingham, AL, USA

Abstract

Chronic obstructive pulmonary disease (COPD) is a leading cause of death globally and is associated with high rates of morbidity. A defining clinical feature of COPD is an increased expression of proteolytic enzymes in the airway, which degrade the lung's tissue matrix and progressively destroy its structural integrity. Recent evidence from our laboratory has demonstrated a novel and pathogenic role for exosomes in the initiation and progression of COPD. Exosomes are small extracellular vesicles that participate in various homeostatic functions. In disease states, however, exosomes can be harnessed to promote pathogenesis. Specifically, our laboratory has identified a novel exosome fraction of neutrophilic origin that exists in the airway. In the airways of COPD patients, neutrophil-derived exosomes carry abundant levels of proteases, including neutrophil elastase (NE), on their surface. Exosome-bound NE has an enhanced capacity to bind and break down matrix proteins (e.g., elastin and collagen). Moreover, the orientation of NE on the exosome surface provides the enzyme with resistance against inhibitory factors, like alpha-1 antitrypsin. Our observations in clinical specimens have been further corroborated by animal models of both acute and chronic lung inflammation. Together, these data broaden the understanding of COPD pathogenesis and open opportunities for new therapeutic strategies.

A Single Cell Immune Atlas Reveals the Involvement of Adaptive Immune Cells in Injury Mediated Cyst Progression

Kurt Zimmerman

Assistant Professor, Department of Internal Medicine, Division of Nephrology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Abstract

Inducible disruption of cilia related genes in adult mice results in slowly progressive cystic disease, which can be greatly accelerated by renal injury. To unbiasedly identify modifier cells that may be influencing the differential rate of cyst growth observed in injured versus non-injured cilia mutant kidneys at a time of similar cyst severity, we generated a single cell atlas of cystic kidney disease by sequencing 79,355 cells from control mice and adult induced conditional Ift88 mice (hereafter referred to as cilia mutant mice) that were harvested _7 months post induction or 8 weeks post 30-minute unilateral ischemia reperfusion injury. Analyses of single cell data revealed that adaptive immune cells had more differences in cluster composition, cell proportion, and gene expression than cells of myeloid origin when comparing cystic models to one another and to non-cystic controls. Surprisingly, genetic deletion of adaptive immune cells significantly reduced injury-accelerated cystic disease but had no effect on cyst growth in non-injured cilia mutant mice, independent of the rate of cyst growth or underlying genetic mutation. Using NicheNet, we identify a list of candidate cell types and ligands that were enriched in injured cilia mutant mice compared to aged cilia mutant mice and non-cystic controls that may be responsible for the observed dependence on adaptive immune cells during injury accelerated cystic disease. Collectively, these data highlight the diversity of immune cell involvement in cystic kidney disease.

Mass Cytometry Reveals Distinct Immune Cell Populations Associated with Food Allergy

Xiaoying Zhou[1], Wong Yu[1], Shu-Chen Lyu[1], Claudia Macaubas[2], Bryan Bunning[1], Ziyuan He[1], Elizabeth D. Mellins[2] and Kari C. Nadeau[1]

[1]*Sean N. Parker Center for Allergy & Asthma Research at Stanford University and Division of Pulmonary, Allergy, and Critical Care Medicine, Stanford, CA 94305, USA*

[2]*Department of Pediatrics, Program in Immunology, Stanford University, Stanford, CA 94305, USA*

Abstract

Food allergies are a leading cause of anaphylaxis. It has been understood that IgE mediated food allergies result from a Th2 immune response to protein antigens associated with specific foods. However, little is known about the response of specific antigen presenting cell (APC) subsets to food allergen in the setting of food allergy. To gain insight into the role of APCs in the immune response to food allergen, we compared PBMCs in peanut allergic (PA) and non-allergic (NA) children after stimulation with peanut protein. Using mass cytometry by time of flight (CyTOF), and a Luminex cytokine assay, we observed an increase in monocyte-associated cytokine secretion coupled with a decrease in the frequency of monocytes in PBMCs from PA individuals, but not in NA individuals. Using CFSE labeling, antibody blocking, and T cell depletion, we then demonstrate that in PA individuals, allergen exposure stimulates IL-4/IL-13 secretion by $CD4^+$ T cells, which promote the differentiation of $CD209^+$ monocyte derived dendritic cells (MDDCs) and myeloid DCs expressing CD23. In the case of $CD209^+$ MDDCs, the addition of an anti-CD209 blocking antibody reduced the frequency of peanut activated $CD4^+$ T cells expressing IL-4 and IL-13 from PA subjects. The initiation of oral immunotherapy (OIT) in PA patients is associated with a decrease in $CD209^+$ DCs. These results support a model in which allergen exposure in food allergic individuals results in a positive feedback loop that reinforces the allergic immune response and breaking the cycle of positive feedback is associated with therapeutic effect.

Identification of Lipids Bound to Membrane-associated Cytokinesis Proteins

Andrea Paquola[1,2], Elisabeth Storck[1], Cagakan Özbalci [1,] Stephen J. Terry[1] and Ulrike Eggert[1,2]

[1]*Randall Centre for Cell and Molecular Biophysics, King's College London, New Hunt's House, Guy's Campus, SE1 1UL, UK*

[2]*Department of Chemistry, King's College London, SE1 1UL, UK*

Abstract

As a cell divides, it must rearrange and separate its membrane-bound organelles as well as its plasma membrane to create equivalent daughter cells. Cells actively maintain diverse lipidomes that encompass many thousands of lipids, which reside in these membrane-bound structures. We showed that the lipid composition of dividing cells is altered relative to non-dividing cells. However, the roles of these lipids remain unexplored. It is our hypothesis that specific interactions between lipids and proteins might contribute to their functions during cytokinesis. The first step to test this hypothesis is to determine if specific lipids are bound to cytokinetic proteins. We developed a technique to explore protein-lipid interactions in cytokinesis. This involves detergent free lysis of cells expressing the protein of interest bound to GFP, followed by GFP trap pulldowns and identification of bound lipids by liquid chromatography-mass spectrometry (LC-MS). We validated this approach in HeLa cell lines stably expressing GFP-tagged proteins resident in different organelles or with known lipid-binding domains. All samples resulted in pulldown of expected lipids, validating our experimental setup. Many cytokinetic proteins are associated with the plasma membrane, including RACGAP1 and CHMP4B. We identified which lipids specifically associate with these proteins and started conducting functional studies.

Functional Characterization of Muscle Cells Derived from Healthy and DMD Human Induced Pluripotent Stem Cells

Arnaud Delafenêtre[1], Charles-Albert Chapotte-Baldacci[1], Emmanuelle Massourides[2], Christian Cognard[1], Christian Pinset[2] and Stéphane Sebille[1]

[1]*PRETI laboratory, Université de Poitiers, France*

[2]*INSERM UEVE UMR861, I-STEM AFM, Corbeil-Essonnes, France*

Abstract

Pluripotent stem cells offer great potentials for the development of treatment for rare genetic diseases. Human induced pluripotent stem cells (hiPSCs) produced by genetic reprogramming of healthy donors or patients' cells are widely used to model pathologies. These cellular models are of great interest in the study of Duchenne Muscular Dystrophy (DMD), whose pathophysiology is still poorly understood and for which the actual treatments are not curative. The main objective is to functionally characterize muscle cells derived from hiPSCs generated from DMD patients and healthy individuals. A focus on both myoblast and myotube stages of hiPSCs-derived muscle cells, corresponding to crucial phases of differentiation and maturation of skeletal muscle cells, will be done. A first investigation on the excitability status of cells was initiated through the measurement of membrane potential. Preliminary experiments show that young myotubes exhibit more negative membrane potential as compared to myoblasts. Then, the calcium status of these cells, evaluated by quantitative measurement of calcium levels at rest, with calcium spontaneous activity, or during *in vitro* stimulation has been evaluated. Results show that, at rest, calcium activities were increased in term of calcium release in amplitude for DMD myotubes as compared to healthy cells. When electrically stimulated, the comparison between DMD and healthy cells showed a higher calcium increase in dystrophic cells. All these data suggest that muscle cells derived from hiPSCs display a dysregulation of calcium homeostasis and model some aspects of functional dysregulation in DMD disease.

Fine Tuning of Calcium Constitutive Entry by Optogenetically-Controlled Membrane Polarization: Impact on Cell Migration

Charles-Albert Chapotte-Baldacci[1], Guénaëlle Lizot[1], Cyrielle Jajkiewicz[1], Manuella Lévêque[1,2], Aubin Penna[1], Christophe Magaud[1], Vincent Thoreau[3], Patrick Bois[1], Stéphane Sebille[1] and Aurélien Chatelier[1]

[1]*PRETI laboratory, Université de Poitiers, France*

[2]*Dermatology Department, University Hospital of Poitiers, France*

[3]*NEUVACOD laboratory, Université de Poitiers, France*

Abstract

Anomalies in constitutive calcium entry (CCE) have been commonly attributed to cell dysfunction in pathological conditions such as cancer. Calcium influxes of this type rely on channels, such as transient receptor potential (TRP) channels, to be constitutively opened and strongly depend on membrane potential and a calcium driving force. We developed an optogenetic approach based on the expression of the halorhodopsin chloride pump to study CCE in non-excitable cells. Using C2C12 cells, we found that halorhodopsin can be used to achieve a finely tuned control of membrane polarization. Escalating the membrane polarization by incremental changes in light led to a concomitant increase in CCE through transient receptor potential vanilloid 2 (TRPV2) channels. Moreover, light-induced calcium entry through TRPV2 channels promoted cell migration. Our study shows for the first time that by modulating CCE and related physiological responses, such as cell motility, halorhodopsin serves as a potentially powerful tool that could open new avenues for the study of CCE and associated cellular behaviors.

Developing Patient-specific *CTNNB1* (β-catenin) Mutants for Defining the Role of CD73 in Endometrial Cancer Recurrence

Rebecca M. Hirsch[1,2], Hannah N. Lee[1], Katherine C. Kurnit[3], Pierre D. McCrea[4], Russell R. Broaddus[1,5], and Jessica L. Bowser[1,5]

[1]*Department of Pathology and Laboratory Medicine, The University of North Carolina School of Medicine, Chapel Hill, NC, USA*

[2]*Curriculum in Cell Biology and Physiology, The University of North Carolina School of Medicine, Chapel Hill, NC, USA*

[3]*Department of Obstetrics and Gynecology, Section of Gynecologic Oncology, University of Chicago, Chicago, IL, USA*

[4]*Department of Genetics, University of Texas MD Anderson Cancer Center, TX, USA*

[5]*Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC, USA*

Abstract

Most (80%) endometrial cancers (EC) are diagnosed at an early stage and cured by surgery alone. A gap in knowledge is that 20% of these patients recur, do poorly, and biomarkers to predict recurrence are lacking. While missense mutations in exon 3 of *CTNNB1*, the gene encoding β-catenin, identify patients at higher risk, not all patients recur. We previously reported that ecto-5'nucleotidase (CD73) downregulation in EC with exon 3 mutant β-catenin is a strong predictor of recurrence. Here, using a 4-site (S33A, S37A, T41A, S45A) exon 3 *Xenopus* β-catenin mutant, we show by cellular fractionation and co-IP that CD73 limits nuclear translocation of mutant β-catenin by sequestering it at the membrane with E-cadherin. In EC, single site mutations in exon 3 are common. To interrogate the oncogenic nature of patient-relevant β-catenin mutations in the presence and absence of CD73, we identified common exon 3 *CTNNB1* mutated residues in EC, using the Cancer Genome Atlas

(TCGA), and developed patient-specific β-catenin mutants (D32N, S33F, S33Y, S37C, and S45F) using site-directed mutagenesis. S33F, S33Y, and S37C correspond to phosphorylation sites for glycogen synthase kinase-3β (GSK3β), and S45 is phosphorylated by casein kinase (CK). Characterization of the mutants in EC cancer cells (HEC-1-A) demonstrated decreased or complete absence of phosphorylation at these residues. Further characterization studies with CD73 knockdown have yielded early results demonstrating different oncogenic effects of patient-specific β-catenin mutants. These preliminary data suggest that loss of CD73 is a major oncogenic regulator of β-catenin mutant endometrial tumors.

Comprehensive Mapping of Heterodimer-DNA Binding Using a Novel Yeast-based Method

Anna Berenson, Ryan Lane, Yilin Chen, Mahir Patel, Cosmin Ciausu, Clarissa Santoso and Juan I. Fuxman Bass[1]

Boston University, MA, USA

Abstract

Immune system responses to pathogenic infection are highly regulated at the transcriptional level. Upon infection, transcription factor proteins (TFs) alter the expression of immune response genes by interacting with regulatory DNA regions. Detailed mapping of these interactions is essential for understanding immune responses, as their dysregulation can contribute to immunodeficiency and autoimmunity. Many key TFs involved in immunity, such as NF-κB and AP-1, are known to function as heterodimer complexes, and it is suspected that other TF heterodimers play roles in mediating the transcription of immune response genes. However, a comprehensive mapping of immune gene regulation by TF heterodimers has yet to be achieved. To this end, we have developed the heterodimer yeast one-hybrid (HetY1H) method as a TF-wide approach to detect heterodimer binding to DNA regions of interest. In a pilot screen, we detected 92 heterodimer-DNA interactions between 5 NF-κB and 20 AP-1 heterodimers and 10 promoters of cytokine genes, an important class of immune response genes. Many of these interactions had not been reported in the literature, and we observed varying levels of target specificity between distinct heterodimers. We are currently conducting a comprehensive HetY1H screen of binding between most (~700) known TF heterodimers and 119 cytokine gene promoters. This work will greatly expand our ability to study DNA binding of many TF heterodimers simultaneously and will elucidate how TF heterodimers confer specificity to the process of immune response regulation.

Behavioral Effects of Oxidant and Reduced Chemorepellents on Mutant and Wild-Type *Tetrahymena thermophila*

Ananya Govindarajan

Senior at Pawling High School, New York, NY, USA

Abstract

Tetrahymena thermophila, a single-cell eukaryotic organism, belongs to the Protozoa Kingdom and is used to model sensory input and the effects of environmental conditions such as chemicals and temperature. The G37 gene encoding for a particular receptor in mutant cells showed increased responsiveness to most chemorepellents. Investigating the G37 Tetrahymena gene in various test solutions, including ferric chloride, ferrous sulfate, hydrogen peroxide, tetrazolium blue, potassium chloride, and dithiothreitol were performed to determine the role of oxidants and reducing agents with the mutant and wild-type cells to assess the role of the receptor. The oxidants tested include tetrazolium blue, hydrogen peroxide, and ferric chloride. reducing agents were ferrous sulfate and dithiothreitol. Behavioral assays and recordings processed by ImageJ indicated that ferric chloride, hydrogen peroxide, and tetrazolium blue yielded little to no chemorepellent responses from G37 cells. CU427 cells were over-responsive based on the mean percent of cells (>50% ARs). Reducing agents elicited chemorepellent responses from G37 and CU427, along with potassium chloride. Dithiothreitol yielded unexpected results

as G37 (37.0% ARs) and CU427 (38.1% ARs) had relatively similar responses and were only responsive and not over-responsive to the reducing agent test chemical solution. Ultimately, the G37 receptor is more interactive with molecules that are reducing agents or non-oxidant compounds; G37 is unable to sense and respond to oxidants effectively, further elucidating the pathways of the G37 strain and nature of this receptor. This research can be further applied to neuronal influences and how specific compounds may affect human neurons individually.

Integrated Proteomics and Bioinformatics Analysis Identifies Overexpression of Ezrin and Aberrant Regulation of Actin Cytoskeleton in Vemurafenib-resistant BRAFV600E Mutated Colon Cancer Cells

Iris Car[1], Antje Dittmann[2], Olga Vasieva[3], Luka Bockor[1], Petra Grbcic[4], Marko Klobucar[4], Sandra Kraljevic Pavelic[5], Miran Coklo[1] and Mirela Sedic[1]

[1]*Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia*

[2]*ETH Zurich / University of Zurich, Functional Genomics Center Zurich, Zurich, Switzerland*

[3]*INGENET Ltd, UK*

[4]*University of Rijeka, Department of Biotechnology, Rijeka, Croatia*

[5]*University of Rijeka, Faculty of Health Studies, Rijeka, Croatia*

Abstract

Approximately 12% of patients with metastatic colorectal cancer (CRC) have BRAFV600E mutation that confers poor prognosis and non-responsiveness to standard therapies. Treatment with a single-agent vemurafenib, selective BRAFV600E kinase inhibitor, did not show a meaningful clinical activity in patients with BRAFV600E mutant CRC. In order to improve outcomes in CRC patients with BRAFV600E mutation, there is a critical need to better understand the mechanisms driving the development of resistance to vemurafenib in CRC. The aim of the present study was to identify novel molecular features and druggable targets of vemurafenib-resistant colon cancer cells using integrated proteomics and bioinformatics approach. Bioinformatics analysis of significantly upregulated proteins in resistant RKO colon cancer cells obtained by mass spectrometry-based proteomics suggested CAV1 (Caveolin-1) and EZR (Ezrin) as the first order targets. KEGG pathway analysis revealed that the upregulated proteins were significantly associated with the regulation of actin cytoskeleton (EZR) and focal adhesion (CAV1). Increased baseline levels of EZR and CAV1 in resistant cells were additionally confirmed by western blot and qRT-PCR. Importantly, treatment with vemurafenib significantly upregulated EZR expression at both, protein, and gene level in resistant *versus* sensitive cells after 72 hours. Finally, mRNA expression analysis of colorectal adenocarcinoma in the TCGA dataset using cBioPortal revealed higher expression of EZR in BRAFV600E mutated CRC in comparison with unaltered group. Altogether, we suggest that ezrin-regulated actin cytoskeleton organization could play an important role in the development of resistance to vemurafenib in BRAF-mutant colon cancer cells.

Characterizing How DHPs Inhibit Transforming Growth Factor Beta Signalling

Stephanie Wojtowicz[1], Dennis Schade[2] and John Di Guglielmo[1]

[1]*Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada*

[2]*Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Christian-Albrechts-University of Kiel, Kiel, Germany*

Abstract

Transforming growth factor beta (TGFβ) is a multifunctional cytokine involved in various cellular processes. However, aberrant TGFβ signalling fuels epithelial-to-mesenchymal transition and metastasis and is a distinctive

trait of many epithelial-derived tumors, including lung carcinomas. Although a novel class of 1,4-dihydropyridines (DHPs) has been shown to specifically inhibit TGFβ receptor type II (TβRII) during cardiomyogenesis, the effect of DHPs on non-small cell lung tumorigenesis has not been investigated. This led us to our overall goal to characterize how DHPs affect TGFβ-related functional outcomes in non-small cell lung cancer (NSCLC) cells. Using Western blotting and immunofluorescence microscopy, we observed that DHPs reduced TGFβ-dependent signaling in NSCLC cell lines as well as TGFβ-dependent events such as E-cadherin to N-cadherin shift and decreased the formation of TGFβ-induced actin stress fibers. Interestingly, these outcomes correlated with a significant decrease in TβRII protein levels, although preliminary data suggests that TβRII endocytosis may not be affected by DHPs. Further characterization of the mechanism that DHPs utilize to target TGFβ receptors will lay a foundation for the use of these small molecules as an effective anti-TGFβ lung carcinoma treatment strategy.

The Relationship Between TGFβ and Autophagy in Non-small Cell Lung Cancer

Charles B. Trelford and John Di Guglielmo

Schulich School of Medicine and Dentistry, Department of Physiology and Pharmacology, Western University, Canada

Abstract

Non-small cell lung cancer (NSCLC) is the leading cause of cancer mortality worldwide. Its progression has been linked to abnormalities in transforming growth factor beta (TGFβ) signalling, which promotes epithelial-mesenchymal transition (EMT) and a catabolic process known as autophagy. EMT primes epithelial cancer cells for metastasis whereas autophagy eliminates damaged or non-essential cellular components to produce energy for cancer cell survival. Although TGFβ induces autophagy, the mechanism remained unknown. Therefore, we silenced components of canonical and non-canonical TGFβ signalling and found that Smad4 of the canonical pathway and the non-canonical TGFβ-activated kinase 1-tumour necrosis factor receptor-associated factor 6-p38 mitogen activated protein kinase pathway upregulate autophagy by decreasing mechanistic target of rapamycin activity and increasing uncoordinated 51-like autophagy activating kinase 1 activity. Furthermore, we found that autophagy regulated TGFβ signalling and EMT. Indeed, autophagy inhibition delayed TGFβ receptor internalization and altered receptor trafficking to the early and late endosomes, as well as lysosomes. As such, receptor Smad phosphorylation was dampened, which decreased nuclear Smad activity. Since receptor Smads function as transcription factors, fewer Smad proteins within the nucleus downregulated TGFβ-dependent outcomes such as EMT. In conclusion, we characterized the relationship between TGFβ and autophagy which function in tandem to create a feedforward loop to augment tumourigenesis. The significance of this study is that by characterizing TGFβ induced autophagy we are now closer to understanding how tumours augmented by TGFβ can selectively evade chemotherapeutic toxicities and metastasize.

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United Scientific Group (A non-profit organization)
8105, Rasor Blvd - Suite #112 PLANO, TX 75024, USA
Ph: +1-469-854-2280/81; **Toll Free:** +1-844-395-4102; **Fax:** +1-469-854-2278
Email: organizer@cebconference.com
Web: https://cellexpbiol.unitedscientificgroup.org/