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ABSTRACT BOOK

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Design and Therapeutic Potential of Purinergic Receptor Ligands

Kenneth A. Jacobson

Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, MD

Abstract:

The purinergic signaling system ("purinome") includes membrane-bound receptors for extracellular purines and pyrimidines, and enzymes/transporters that regulate endogenous agonist concentration. Receptors include: adenosine (A1, A2A, A2B, and A3) and P2Y (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14) receptors (all GPCRs), as well as P2X receptors (ion channels). Receptor activation, especially accompanying physiological stress or damage, creates a temporal sequence of signaling to counteract this stress and either mobilize (P2Rs) or suppress (ARs) immune responses. Experimentally determined structures represent each of the three receptor families. As an A3AR structure is unavailable, we use homology modeling, docking and molecular dynamics simulations to computationally predict the receptor interactions of newly synthesized or planned orthosteric ligands. Recently synthesized adenosine derivatives are highly selective A3AR agonists (>3000-fold vs other ARs) by virtue of a rigid ribose substitution, i.e. a methanocarba (bicyclo[3.1.0]hexane) ring system that maintains an A3AR-preferred conformation and lowers the binding energy barrier. Our two prototypical A3AR agonists are safe and efficacious in clinical trials for psoriasis and liver conditions. A3AR agonists are a potential, nonaddictive treatment for chronic neuropathic pain. Anti-inflammatory P2Y14R antagonists are being designed using homology modeling based on a P2Y12R template, docking and molecular dynamics simulations. Thus, modulation of the purinergic signaling network has broad potential for treating chronic diseases, and structural approaches to ligand design are employed.

Biography:

Dr. Jacobson is Chief of the Molecular Recognition Section in the Laboratory of Bioorganic Chemistry at the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health in Bethesda, Maryland, USA. Dr. Jacobson is a medicinal chemist with interests in the structure and pharmacology of G protein-coupled receptors, in particular receptors for adenosine and for purine and pyrimidine nucleotides. He obtained a Ph.D. in Chemistry from the University of California, San Diego. He has published more than 800 scientific publications and was inducted into the American Chemical Society's Division of Medicinal Chemistry Hall of Fame in 2009.

Reprogramming and Heterogeneity of Tumor Associated Fibroblasts in Colorectal Cancer

Jorge Moscat

Weill Cornell Medicine, Meyer Cancer Center, NY

Abstract:

Cancer-associated fibroblasts (CAFs) are critical components of the tumor microenvironment due to their ability to promote malignancy. A paradigmatic example is a mesenchymal type of colorectal cancer (CRC) characterized by desmoplasia, immunosuppression, and resistance to immune checkpoint blockade (ICB) therapy, termed CMS4. This type of CRC includes more than 30% of the CRC population and has the poorest prognosis. Although it is believed that targeting the stromal fibroblasts in CRC might lead to sensitivity to ICB therapy, the actual role of CAFs in that process has not been clearly established. Also, the precise transcriptional mechanisms whereby fibroblasts acquire the CAF phenotype still need to be resolved. Furthermore, how the tumor reprograms CAF heterogeneity and how different CAF subpopulations impact the tumor epithelium have not been fully elucidated, which is of great relevance to identify new therapeutic

targets in the stroma. Results will be presented demonstrating how a previously unknown SOX2-driven transcriptional program leads to CAF activation and the generation of a new CAF subpopulation that is fundamental for CRC malignancy and immunosuppression. These results open the possibility of generating new therapeutic strategies aimed at specific molecular determinants of the CAF phenotype in mesenchymal CRC and most likely in other desmoplastic malignancies.

Biography:

Dr. Moscat is a Cancer Biologist focused on identifying non-oncogenic vulnerabilities in cancer, with a particular interest in the interface between inflammation and metabolism in liver and colorectal tumors and their response to immunotherapy. Dr. Moscat presently is the Homer T. Hirst III Professor of Oncology and Vice-Chair for Experimental Pathology at the Weill Cornell Medical College. Before joining Cornell, Dr. Moscat served in several leadership positions at the Sanford Burnham Prebys Medical Discovery Institute in La Jolla (California), including Deputy Director of the Cancer Center and the Institute's Director of Metabolism Initiatives.

Mast Cells and IgE Orchestrate Protective Immune Responses to Venoms and *Staphylococcus aureus*. Is this the "Good Side" of Allergy?

Stephen J. Galli

Department of Pathology, Stanford Univ. School of Medicine, Stanford, CA

Abstract:

Venoms toxins provoke pathology. Venoms also can induce allergic sensitization and production of venom-specific IgE antibodies, which can predispose for the development of anaphylaxis upon subsequent exposure to that venom. Surprisingly, we found that certain innate mast cell functions, including degradation of venom toxins by mast-cell-derived proteases, enhanced the survival of naïve mice injected with any of a spectrum of venoms: from the honeybee, two scorpions, four species of poisonous snakes, or the Gila monster (Science. 2006;313:526-30; J Clin Invest. 2011;121:4180-91; Immunity. 2013;39:963-75; J Allergy Clin Immunol. 2016;137:246-57). We also found that mice injected with sub-lethal amounts of honeybee or Russell's viper venom exhibited enhanced survival upon subsequent challenge with potentially lethal amounts of that same venom. This enhancement of venom resistance required IgE antibodies, FcepsilonRI, and mast cells (Immunity. 2013;39:963-75; J Allergy Clin Immunol. 2016;137:246-57; Allergy. 2021 URL: https://onlinelibrary.wiley.com/doi/10.1111/all.14852). Our findings suggest that mast cells, and their IgE-dependent activation, can participate substantially in defense against the morbidity and mortality induced by certain insect and snake venoms. These findings support the theory proposed by Margie Profet, namely that "allergic reactions" evolved as immune defense mechanisms against noxious substances such as toxins and venoms (M. Profet. Q Rev Biol. 1991;66:23-62). Indeed, this ability to reduce the lethality of certain venoms, as well as the ability of mast cells and IgE to reduce the toxicity of infection with a common pathogenic bacterium (Staphylococcus aureus [Immunity. 2020;53:793-804]), may represent ancient, and beneficial, roles of mast cells, and of IgE-dependent mast cell activation.

Biography:

Dr. Galli studies mast cells and basophils, including their roles in allergic disorders, and the beneficial roles of mast cells and IgE in responses to venoms and certain bacteria. Dr. Galli received Scientific Achievement Awards from the International Association of Allergy & Clinical Immunology (1997) and the World Allergy Association (2011), the ASIP Rous-Whipple Award (2014), and the Austrian Society of Allergology and Immunology's Karl Landsteiner Medal (2014). He is a member of the National Academy of Medicine (USA), a Fellow of the American Association for the Advancement of Science, and a foreign member of Rome's Accademia Nazionale dei Lincei.

In Vivo Cell Lineage Tracing Links ERa Loss in HER2-Positive Breast Cancers to the Arising of a Highly Aggressive Breast Cancer Subtype

Jianming Xu

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

Abstract:

HER2-positive (HER2$^+$) breast cancers (BrCs) contain approximately equal numbers of ERa$^+$HER2$^+$ and ERa$^-$HER2$^+$ cases. An enduring obstacle is the unclear cell lineage-related characteristics of these BrCs. Although ERa$^+$HER2$^+$ BrCs could lose ERa to become ERa$^-$HER2$^+$ BrCs, direct evidence is missing. To investigate ERa dependencies and their implications during BrC growth and metastasis, we generated ERa^{Cre}RFP-T mice that produce RFP-marked ERa$^+$ mammary gland epithelial cell (MGEC) lineage. RCAS virus-mediated expression of Erbb2, a rodent Her2 homolog, first produced comparable numbers of ERa$^+$RFP$^+$Erbb2$^+$ and ERa$^-$RFP$^-$Erbb2$^+$ MGECs. Early hyperplasia developed mostly from ERa$^+$RFP$^+$Erbb2$^+$ cells, and ERa$^-$RFP$^-$Erbb2$^+$ cells in these lesions were rare. The subsequently developed ductal carcinomas in situ had 64% slow-proliferating ERa$^+$RFP$^+$Erbb2$^+$ cells, 15% fast-proliferating ERa$^-$RFP$^+$Erbb2$^+$ cells derived from ERa$^+$RFP$^+$Erbb2$^+$ cells, and 20% fast-proliferating ERa$^-$RFP$^-$Erbb2$^+$ cells. The advanced tumors had mostly ERa$^-$RFP$^+$Erbb2$^+$ and ERa$^-$RFP$^-$Erbb2$^+$ cells and only a very small population of ERa$^+$RFP$^+$Erbb2$^+$ cells. In ERa$^-$RFP$^+$Erbb2$^+$ cells, GATA3 and FoxA1 decreased expression and ERa promoter regions became methylated, consisting with the loss of ERa expression. Lung metastases consisted of mostly ERa$^-$RFP$^+$Erbb2$^+$ cells, a few ERa$^-$RFP$^-$Erbb2$^+$ cells, and no ERa$^+$RFP$^+$Erbb2$^+$ cells. The high metastatic capacity of ERa$^-$RFP$^+$Erbb2$^+$ cells was associated with ERK1/2 activation. These results show that the slow-proliferating, non-metastatic ERa$^+$RFP$^+$Erbb2$^+$ cells progressively lose ERa during tumorigenesis to become fast-proliferating, highly metastatic ERa$^-$RFP$^+$Erbb2$^+$ cells. The ERa$^-$Erbb2$^+$ BrCs with an ERa$^+$ origin are more aggressive than those ERa$^-$Erbb2$^+$ BrCs with an ERa$^-$ origin and thus, they should be distinguished and treated differently in the future.

Biography:

Dr. Xu currently holds a Gordon Cain Endowed Professorship in Cell Biology in Baylor College of Medicine. In 1990s, Dr. Xu was the first who defined the physiological functions of the initially identified steroid receptor coactivators (SRCs) and their roles in mammary gland and prostate tumorigenesis by using genetically manipulated mouse models. Dr. Xu's research interests are focused on understanding the roles and mechanisms of transcription factors including nuclear receptors, Twist, TCF4, and NFY and transcriptional coregulators including SRC-1, SRC-3 and NCOA6 in steroid hormone-promoted breast, prostate and endometrial cancers. Dr. Xu has published 195 research articles.

Elucidating the Role of XRN2-Mediated Invasion Programs

Tuyen Dang

Department of Neurosurgery and Stephenson Cancer Center at OU Health Science Center, Oklahoma City, OK

Abstract:

Glioblastoma multiforme (GBM) is a highly aggressive brain cancer. The standard course of treatment is a combination of radiation and chemotherapy. Even with the dual treatment, the 5-year survival rate of patients with GBM is between 4-7%. Therefore, there is an urgent need to develop novel therapies to increase the survivorship. A possible cause of the low survival rate for GBM patients is the presence of neoplastic cells with metastatic capabilities. They often invade out of the scope of surgery or directed therapies such as

radiation and continue to grow unchecked leading to lethal secondary tumor disease. XRN2 is upregulated in GBMs as compared to normal and other brain cancer types. XRN2 is a 5′-3′ exonuclease that resolve DNA:RNA hybrids (R loops) that arise during transcription, especially at the 3′ end of genes. R-loop biology can affect gene expression by modulating the access of genes to transcription factors, miRNA transcription, and methylation status of genes. Our preliminary data have shown that loss of XRN2 reduces GBM motility. Cell motility is the first step towards metastasis. To understand how XRN2 modulates intracranial metastasis, we conducted RNA-Seq analyses of two GBM cell lines with and without XRN2 expression and found that XRN2 can regulate genes involved in cell motility. We have conducted a mini-cherry picked screen of the XRN2 targets and found at least 16 genes to be required for cell motility. In addition, we found that XRN2 is required for tumor establishment and metastasis in orthotopic models of GBM.

Biography:

Dr. Tuyen Dang is a post-doctoral researcher in Dr. Julio Morales's lab at OU Health Science Center in Oklahoma City, OK. Currently she is investigating the role of transcription in DNA repair and invasion. She did her doctoral training with Dr. Gray Pearson at UT Southwestern Medical Center in Dallas, TX. Her doctoral study was elucidating the mechanism of breast cancer cell invasion. Dr. Dang received her bachelor's degree in Biochemistry/Biophysics at Oregon State University in Corvallis, OR.

Exosomes as Biomarkers and Effectors of Tumor Progression and Metastasis: the Activated Stem Cell State in Breast Cancer Progression

Jay Desgrosellier

Department of Pathology, Moore's Cancer Center, University of California, San Diego, CA

Abstract:

My laboratory is focused on discovering more effective ways of preventing breast cancer recurrence and metastasis, leading to a reduction in mortality from this disease. Considering that normal physiological pathways are often hijacked by tumor cells, we have closely studied the developmental signals that control stem cell behavior in the mammary gland with the goal of identifying new strategies for targeting stem-like cancer cells involved in disease progression. This multidisciplinary approach represents a synthesis of the knowledge and skills developed during my training in developmental biology, pharmacology, and integrin signaling in cancer, with the goal of "changing the game" with respect to identifying new treatment strategies for breast cancer. Toward this end, I have developed an independent and multidisciplinary research program in my laboratory focused on deciphering the role of stem-like cancer cells in metastatic disease and identifying vulnerabilities of these cells by investigating the key cell survival and differentiation signaling pathways required for their function.

Biography:

Dr. Desgrosellier received his B.A. in Biology and Chemistry from Whitman College in Walla Walla, WA. He then received his Ph.D. in Pharmacology from Vanderbilt University in Nashville, TN. As a postdoctoral fellow in Dr. David Cheresh's laboratory at UCSD he pursued his interest in integrin signaling in cancer. In studies published in Nature Medicine he discovered a surprising adhesion-independent role for the integrin αvβ3 that promoted tumor cell metastasis. This finding initiated his interest in adult stem cell biology and its relevance to cancer, now a major topic of his independent research program in the UCSD Department of Pathology.

FBXL16 Protects Oncoproteins against Degradation Mediated by SCF-E3 Ligases, thereby Promoting Cancer Cell Growth and Invasiveness

Weiwen Long

Department of Biochemistry and Molecular Biology, Wright State University, Dayton, OH

Abstract:

F-box proteins are the substrate recognition subunits of the SCF (SKP1-Cullin-F-box protein) E3 ubiquitin ligases in that they bind to SKP1 through the F-box motif and bring the substrate to the E3 ligase complex for ubiquitination and subsequent degradation. They fall into three families depending on their substrate recognition domains: FBXLs (Leucine-Rich Repeats or LRR), FBXWs (WD repeats) and FBXOs (Other domains). For example, FBXW7 (often called FBW7), as a tumor suppressor, is known to target many oncoproteins such as c-Myc and steroid receptor coactivator 3 (SRC-3). FBXL16 is a poorly studied F-box protein. As it does not interact with the scaffold protein Cul1, FBXL16 might not form a functional SCF-E3 ligase complex. Here we have revealed a unique role for FBXL16 in stabilizing multiple oncoproteins targeted by SCF-E3 ligase, including c-Myc and SRC-3 (targeted by FBW7), β-catenin (by β-TRCP) and ERα (by FBXO45). Mechanistically, FBXL16 interacts with these F-Box proteins and antagonizes their SCF-E3 ligase activity. Of clinic significance, FBXL16 is highly upregulated in multiple human cancers, in particular lung adenocarcinomas (LUADs) and invasive ER+ breast carcinomas, and high expression level of FBXL16 is associated with tumor aggressiveness and poor patient survival. Knockdown of FBXL16 led to remarkable inhibition of growth and migration of LUAD cell lines and invasive ER+ breast cancer cell lines. Currently, we are investigating the tumor-promoting role of FBXL16 in vivo utilizing the conditional transgenic FBXL16 mouse models.

Biography:

Dr. Weiwen Long obtained his Ph.D. Degree in structural and cellular biology from Tulane University and conducted postdoctoral training on cancer cell signaling and gene regulation in Dr. Bert O'Malley's laboratory at Baylor College of Medicine. He is currently an Associate Professor in the department of Biochemistry and Molecular Biology, Wright State University, Ohio. Dr. Long's research interests and acquired expertise have been directed to protein kinase signaling (with a focus on atypical MAPKs), protein posttranslational modifications (in particular phosphorylation and ubiquitination) and their roles in cancer development and progression.

Unified Workflow for Scalable Isolation of Extracellular Vesicles from Prokaryotes and Eukaryotes

Dionysios C. Watson

Medical Oncology fellow, University Hospitals, Case Western Reserve University, Cleveland, OH

Abstract:

Cells across the kingdoms of life secrete extracellular vesicles (EVs) as a form of intracellular communication, even across species. Moreover, they can be engineered to carry customized therapeutic payloads. Given these properties, EVs are being developed as clinical biomarkers and therapeutics, requiring standardized EV purification compatible with diverse EV sources. There is currently significant variability in EV isolation methods, hampering translational development. We hypothesized that tangential flow filtration (TFF) and size-exclusion chromatography (SEC) would enable reproducible isolation of EVs from a range of sources. EV isolation consisted of: centrifugation and filtration to remove debris; TFF (100 kDa MWCO) to concentrate starting material; and SEC (Izon-qEV 35 nm) to separate EVs from vesicle-free macromolecules. EVs from human cell lines, gram negative/positive aerobic/anaerobic bacteria, plasma, and fecal slurries were isolated with minor variations to account for unique sample requirements. TFF facilitated processing of conditioned cell culture medium volumes of up to 4 L. EV yield measured by microfluidics resistive pulse sensing ranged

from 109-1011 EVs per mL input. We confirmed the ability of SEC to remove non-vesicular macromolecules using engineered cells expressing both vesicle-associated and vesicle-free reporter proteins. Transmission electron microscopy confirmed the enrichment of EVs in early SEC fractions, and the EV-association of reporter proteins by immunogold labelling. In conclusion, combination a workflow consisting of centrifugation/ filtration, TFF, and SEC enable isolation of EVs derived from different kingdoms of life, and from a variety of biological specimens. Unique requirements of individual specimens and downstream applications may require minor modifications of this workflow.

Biography:

Dionysios (Dennis) Watson is a physician-scientist dedicated to developing novel therapeutics for immunotherapy of cancer. His Ph.D. research on the translational development of heterodimeric interleukin-15 for HIV-1 and cancer was conducted at the National Cancer Institute, through a partnership with the University of Patras, Greece. He is currently a Medical Oncology fellow at University Hospitals Cleveland Medical Center (Case Western Reserve University) and Visiting Postdoctoral Researcher at the Cleveland Clinic. Dennis' current research focuses on developing microbial EVs for cancer immunotherapy.

ENT1 Insertion into Ceramide-rich Platforms Functionalizes Gemcitabine Uptake

Aditya Ganju

Laboratory of Signal Transduction, Memorial Sloan Kettering Cancer Center, New York, NY

Abstract:

Endothelial ASMase activation leads to rapid formation of plasma membrane ceramide-rich platforms (CRPs), macrodomains that organize apoptotic signaling programs. We have evidence that the primary transporter for nucleoside drug uptake in mammalian cells, Equilibrative Nucleoside Transport 1 (ENT1), is not constitutively on, as reported, but requires drug (gemcitabine)-induced membrane alteration for ASMase activation, CRP formation and ENT1 insertion into CRPs for functionalization. Here, we show by confocal microscopy that within seconds of gemcitabine treatment of cultured bovine aortic endothelial cells (BAECs), ENT1 enters a CRP in order to functionalize. Studies using the ASMase inhibitor imipramine or an anti-ceramide Ab, which prevent CRP formation, obviate gemcitabine uptake and apoptotic death suggesting ASMase/Ceramide signaling is obligate for ENT1 functionalization of gemcitabine uptake. In contrast to BAECs, MCA/129 fibrosarcoma cells display minimal basal ASMase activity, and gemcitabine fails to induce CRPs, activate ENT1, or take up gemcitabine and induce cell death. However, addition of exogenous SMase with gemcitabine to MCA/129 cells yields formation of CRPs in which ENT1 colocalizes, conferring drug uptake and apoptosis. Critically, conditioned media from gemcitabine-treated BAECs containing large amounts of secreted ASMase substitutes for exogenous SMase, conferring gemcitabine-induced ENT1-mediated gemcitabine uptake, resulting in apoptotic MCA/129 fibrosarcoma cell death. These studies support a synthetic lethal mechanism of tumor cell death in which chemotherapeutic drug injury to neo-angiogenic endothelium facilitates tumor cell death by providing a critical enzyme necessary for drug uptake.

Biography:

Dr. Aditya Ganju is a Research Associate in the lab of Dr. Richard Kolesnick in the department of Molecular Pharmacology at Memorial Sloan Kettering Cancer Center, New York. He joined MSKCC in 2017 as a Research Fellow. He have been involved in elucidating the role of Acid Sphingomyelinase(ASMase)/Ceramide signaling in drug uptake and tumor drug resistance studies at MSKCC. He did his Ph.D. in Pharmaceutical Science at University of Tennessee Health Science Center, Memphis in December 2016. His thesis work focused on discovery of novel regulatory pathway whereby Protein Kinase D1 (PKD1) regulates Metastasis-associated Protein 1 (MTA1) expression in different cancer types.

The Cytoskeletal Protein CAP1 Fulfills Context-Dependent Functions in the Adhesion and Migration of Colon Cancer Cells

Auburn Ramsey

Department of Biological Sciences, Arkansas State University, Jonesboro, AR

Abstract:

Cyclase-Associated Protein (CAP) promotes actin dynamics and cytoskeletal rearrangement in eukaryotes. The ubiquitous mammalian isoform, CAP1, also regulates cell adhesion, migration, and proliferation. However, these functions are cell context-dependent, and differential regulation of FAK (Focal Adhesion Kinase) and cofilin are at least partially responsible. CAP1 is also implicated in multiple cancer types, while the underlying molecular mechanisms remain elusive. In colon cancer patients, the five-year survival rate drops from 91% at the localized stage to 14% in those with distant metastasis, and mechanistic insights into potential involvement of CAP1 may lead to strategies suppressing the invasive cycle of the disease. We silenced CAP1 in SW480 and HCT116 cancer cells through shRNA, and found that depletion of CAP1 led to activated cofilin and adhesion molecules FAK and Rap1, enhanced cell adhesion and migration in HCT116 cells, while it reduced cell adhesion in SW480 cells. CAP1-knockdown SW480 cells also had enhanced stress fibers and increased number of multi-nucleated cells. PKC (Protein Kinase C) induced CAP1 dephosphorylation, while exerting opposite effects on the adhesion of those cell lines. These results suggest important roles for CAP1 in colon cancer that underlie the invasiveness and are dependent on the sub-type of the disease.

Biography:

Auburn Ramsey is an undergraduate Biotechnology student at Arkansas State University, Jonesboro. She has worked in Dr. Guolei Zhou's lab at the Arkansas Biosciences Institute for two and a half years. Her current research involves working with mammalian cancer cells to study CAP1 (Cyclase Associated Protein 1) and its functions in cancer cell adhesion and migration as well as cell signaling involved with CAP1. Her goals are to pursue a career in cancer research and continue schooling until she earns a Ph.D. This fall she plans to enter the M.S. Biology program at Arkansas State University.

Epigenetic Insights of Mesenchymal Stromal Cell Lineage Commitments and Hematopoiesis

Amitava Sengupta

Stem Cell & Leukemia Lab, Cancer Biology Division, CSIR-Indian Institute of Chemical Biology, India

Abstract:

Bone morphogenic protein (BMP)/transforming growth factor β (TGF-β) signaling determines mesenchymal-stromal-cell (MSC) osteolineage commitment and tissue identity. However, molecular integration of developmental signaling with MSC-intrinsic chromatin regulation remains incompletely understood. SWI/SNF-(BAF) is an ATP-dependent chromatin remodeler implicated in multi-cellular development. We demonstrate that BMPs and long-term osteogenic signals in MSCs selectively induce expression of polybromo BAF (PBAF) components Pbrm1, Arid2, and Brd7. Loss of Pbrm1/Arid2/Brd7 profoundly impairs osteolineage gene expression and osteogenesis without compromising adipogenesis. Pbrm1 loss attenuates MSC *in vivo* ossification. Mechanistically, Pbrm1/PBAF deficiency impairs Smad1/5/8 activation through locus-specific epi-genomic remodeling, which involves Pbrm1 bromodomain function, along with transcriptional downregulation of Bmpr/TgfβrII affecting BMP-early-responsive gene expression. Gain of function of BmprIβ, TgfβrII in PBAF-deficient MSCs restores Smad1/5/8 activation and osteogenesis. Pbrm1 loss further affects hematopoietic stem and progenitor activity through non-cell-autonomous regulation of microenvironment and niche-factor expression. Together, these findings reveal a link illustrating epi-genomic feedforward control of BMP/TGF-β signaling to transcriptional and cellular plasticity in the mesenchymal microenvironment and account for stromal-SWI/SNF in hematopoiesis.

Biography:

Amitava Sengupta completed Ph.D. in 2008 from Jadavpur University, Kolkata, and moved to Cincinnati Children's, USA, and trained with Jose Cancelas on mammalian HSC biology, niche physiology and leukemia genetics. They identified the role of Rho GTPase signaling in CML stem cell activity and function of signaling adaptor proteins in HSC homing and engraftment. Currently his independent group at IICB is investigating 'human elderly AML epigenetics, targeted therapy & immunomodulation', and 'molecular determinants of stromal-niche immunity'. He was a Visiting Scientist in John Dick's group at Princess Margaret Cancer Centre, Toronto, and their labs are collaborating on AML targeted therapy.

Macrophages and Immune Responses in Uterine Fibroids

Alessandro Zannotti

Department of Experimental and Clinical Medicine, Universita Politecnica delle Marche, Italy

Abstract:

Uterine fibroids represent the most common benign tumors of the uterus. They are considered a typical fibrotic disorder. In fact, the extracellular matrix (ECM) proteins—above all, collagen 1A1, fibronectin and versican—are upregulated in this pathology. The uterine fibroids etiology has not yet been clarified, and this represents an important matter about their resolution. A model has been proposed according to which the formation of an altered ECM could be the result of an excessive wound healing, in turn driven by a dysregulated inflammation process. A lot of molecules act in the complex inflammatory response. Macrophages have a great flexibility since they can assume different phenotypes leading to the tissue repair process. The dysregulation of macrophage proliferation, accumulation and infiltration could lead to an uncontrolled tissue repair and to the consequent pathological fibrosis. In addition, molecules such as monocyte chemoattractant protein-1 (MCP-1), granulocyte macrophage-colony-stimulating factor (GM-CSF), transforming growth factor-beta (TGF-β), activin A and tumor necrosis factor-alfa (TNF-α) were demonstrated to play an important role in the macrophage action within the uncontrolled tissue repair that contributes to the pathological fibrosis that represents a typical feature of the uterine fibroids.

Biography:

Dr. Alessandro Zannotti received his Master degree in Molecular and Applied Biology from Polytechnic University of Marche (UNIVPM), Ancona, Italy in 2017. He currently attends the last year of the Ph.D. course in Biomedical Sciences at UNIVPM. Dr. Zannotti spent a period of study at Life and Health Sciences Research Institute (ICVS), at University of Minho, Braga, Portugal within his Ph.D. internship. His Ph.D. research project is focused on the morphological and molecular characterization of benign leiomyoma and malignant leiomyosarcoma with the aim of identifying potential markers to discriminate them. In particular he studies Raf-1 kinase inhibitor protein (RKIP).

Conserved Regulation of Myofibroblast Function by the Protein Kinase N Family in Embryogenesis and Cancer

Angus Cameron

Kinase Biology Laboratory, Barts Cancer Institute, Queen Mary, University of London, John Vane Science Centre, UK

Abstract:

Fibroblasts are critical regulators of tissue structure and morphogenesis. They are the primary source of

the extracellular matrix, which provides the scaffolding for tissue to develop, and they are contractile and motile allowing them to reshape tissues. We identified that the Rho-effector kinase protein kinase N2 (PKN2) plays critical roles in mesenchymal fibroblasts during embryo development. Loss of PKN2 is associated with reduced proliferation and an inability to adopt myofibroblast phenotypes. We have evidence that these functions for the PKN kinases are well conserved in many mesenchymal cell types, including lung fibroblasts, pancreatic stellate cells, cancer-associated fibroblasts and mesenchymal cancer cells. In pancreatic ductal adenocarcinoma (PDAC), differentiation of pancreatic stellate cells (PSCs) into myofibroblast-like cancer-associated fibroblasts (CAFs) promotes fibrotic, therapy-resistant tumours. We have examined the impact of targeting PKN2 in the pancreatic stroma using our conditional knockout model. This has revealed complex tumour promoting and tumour suppressive roles for myofibroblasts in PDAC, with implications for stromal targeting as a strategy to improve therapy response.

Subclonal Mutations in Lymphoma Reveal Mutation Co-Selection

Philip Webster[1], Joanna Dawes[1], Hamlata Dewchand[1], Barbara Iadarola[1], Claudia Garcia-Diaz[3], Katalin Takacs[1], Bruce Bolt[1], Juan Caceres[2], Jakub Kaczor[1], Laurence Game[1], Thomas Adejumo[1], James Elliott[1], Kikkeri Naresh[1], Ge Tan[1], Gopurajah Dharmalingam[1], Simona Parrinello[3], Alberto Paccanaro[2,] Anthony Uren*[1,2*]

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[2]Royal Holloway, University of London, UK
[3]UCL Cancer Institute, University of London, UK

Abstract

Understanding the causal genotype-phenotype relationships of tumors is hampered by datasets with a diversity of tumour phenotypes and varying clonality of driver mutations. Here we present a novel permutation based strategy for dissecting these relationships using murine leukaemia virus (MuLV) driven lymphoma as a model system. MuLV replication in hematopoietic lineages creates proviral integrations that deregulate nearby genes. These integration mutations cause lymphoid malignancies with 100% penetrance. Cloning virus/genome junctions by ligation mediated PCR allows the mutation profile to be determined with minimal coverage with unparalleled sensitivity for subclonal mutations. From a panel of BCL2 transgenic mice and wild type controls we identified nearly 3,000 clonal mutations and an additional 1,800,000 subclonal mutations over a spectrum of B and T lymphoid malignancies. To identify causal phenotype-genotype relationships, mutations are shuffled between tumour samples whilst constraining for both mutation number and clonality in each sample. The output of these permutations are joint distributions of co-mutation and phenotype-genotype interactions that avoid false positives created when analysing frequently and rarely mutated loci together, and is more conservative than traditional contingency table test approaches. Shuffling constraints can also be placed on subtle tumour phenotype differences such that larger cohorts can be analysed in concert without creating false associations due to unrecognised heterogeneity.

Biography:

Anthony Uren completed his Ph.D. at the Walter and Eliza Hall Institute studying apoptosis and chromosome segregation. As a postdoc at Genentech he studied the role of translocations of MALT lymphoma in NF-kappa B signalling. As a postdoc at the Netherlands Cancer Institute he developed the technology of insertional mutagenesis screens in mice. At Imperial College his group used mouse models of lymphoma to study the relationships between early stage clonal mutations and late stage subclonal mutations.

Modulation of Alternative Splicing as a New Therapeutic Avenue in Cancer

Sebastian Oltean

Institute of Biomedical & Clinical Sciences, University of Exeter Medical School, UK

Abstract:

Alternative splicing is known to occur in more than 94% if genes in humans. Cancers express specific splice isoforms, or the normal ratios of isoforms are disrupted. While some of these events may be just a silent by-product of the cancer progression, many of them have functional significance and indeed, manipulation of their expression or splicing ratios is beneficial to the disease phenotype. Abnormal alternative splicing is therefore a new area where therapeutic interventions may be designed. While there are several approaches to manipulate alternative splicing in cancer, the strategy that we use is to screen for small molecules that can switch splicing from detrimental isoforms characteristic to cancer progression to their normal counterparts and therefore rescue phenotype and inhibit tumor growth. We present here examples from two repositioning screens using splicing sensitive-reporters designed to reproduce aberrant splicing of two genes in prostate cancer – VEGF-A, involved in angiogenesis and FGFR2 – involved in epithelial-mesenchymal transitions. We show that the compounds found to correct this aberrant splicing are able to reverse aggressive cancer cell properties in vitro and to slow tumor growth in nude mice xenografts when administered systemically. In conclusion, we show that knowledge on the molecular mechanisms that connect alternative splicing and various cancer properties may be used as a platform for drug development.

Biography:

Sebastian studied medicine at "Iuliu Hatieganu" Medical School, Cluj-Napoca, Romania and trained as a junior doctor in Nephrology and Dialysis before moving to USA where he obtained a Ph.D. from the University of Nebraska-Lincoln in 2004. This was followed by postdoctoral training at Duke University Medical Center (North Carolina, USA) where he became interested in studying the connections between alternative splicing and cancer. In 2008 he moved to the University of Bristol where he continued to study alternative splicing in vivo, with focus towards the importance of several genes splice isoforms (e.g VEGF, FGFR2) in cancer as well as kidney diseases and development of splice-based therapeutics. In 2012 he was appointed Independent Research Fellow and Principal Investigator and developed his own research group in Bristol before moving to University of Exeter Medical School in 2017.

Endothelial Crosstalk of VEGF and BMPs: A New Player in Hippo Signaling

Johanna Laakkonen

University of Eastern Finland, Finland

Abstract:

Emerging knowledge supports the role of bone morphogenetic proteins (BMPs) in vascular homeostasis and angiogenesis. Dysfunctional BMP signaling is involved in various vascular disorders, such as hereditary hemorrhagic telangiectasia, cerebral cavernous malformation, pulmonary arterial hypertension and atherosclerosis. BMP2/4, BMP receptors ALK1, ALK2, ALK3, or BMPR2 mouse knockouts also lead to severe cardiovascular defects and embryonic lethality. Synergistic effect of VEGF and BMPs on vasculature have been previously detected in bone formation but their role in angiogenesis, particularly crosstalk with VEGFR2 signaling has remained elusive. Recently, BMPR pathway was linked to dysfunctional Hippo-signaling, though the exact extracellular ligands, interaction mechanisms and end-responses remained unknown. Hippo pathway has previously been shown to regulate multiple cellular functions such as cell proliferation, survival, differentiation, migration and apoptosis. Dysregulation of the Hippo pathway has also been linked to cancer metastasis, and to epithelioid hemangioendothelioma. Our study defines crosstalk of BMP6 with VEGFR and Hippo signaling pathways. Regulation of BMP signaling might be beneficial in pro-angiogenic and anti-angiogenic therapies.

Biography:

Assoc. Prof., Dr., Academy Research Fellow Johanna Laakkonen, acts as a junior group leader in Univ. of Eastern Finland, A.I. Virtanen Institute of Molecular Sciences, Finland. Her research group focuses in defining molecular mechanisms involved in pathological angiogenesis.

SHIP2 and its New Partners are Involved in Invadopodia Formation

Antoine Mathieu

Institut de Recherche Interdisciplinaire en Biologie Humaine et moléculaire (IRIBHM), Université Libre de Bruxelles, Belgium

Abstract:

A tight control of the machineries regulating membrane bending and actin dynamics is very important for the generation of membrane protrusions, which are crucial for cell migration and invasion. Protein/protein and protein/phosphoinositides complexes assemble and disassemble to coordinate these mechanisms, the scaffold properties of the involved proteins playing a prominent role in this organization. The PI 5-phosphatase SHIP2 is a critical enzyme modulating PI(3,4,5)P3, PI(4,5)P2 and PI(3,4)P2 content in the cell. The scaffold properties of SHIP2 contribute to the specific targeting or retention of the protein in particular subcellular regions. Through immunoprecipitation experiments we identified IRSp53 and CIN85 as new binding interactors of SHIP2 proline-rich domain. We showed that the SH3-binding polyproline motifs recognized by IRSp53 as well as by CIN85, which modulate interaction with SHIP2, are located at distinct sites. The absence of Mena, a common interactor of both IRSp53 and SHIP2, in MDA-MB-231 cells decreased the intracellular content in F-actin and modified the subcellular localization of SHIP2 and IRSp53 by increasing their relative content at the plasma membrane. Together our data suggest that SHIP2, through interaction with the cell protrusion regulators IRSp53 and Mena, participate to the formation of multi-protein complexes. This ensures the appropriate modulations of PIs which are important for regulation of membrane dynamics mostly in generation of invadopodia; responsible for tissue invasion in cancer development.

Cancer Stem Cells Undergo Metabolic Plasticity Toward the Gaining of the Quiescent State

Ilaria Dando

Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy

Abstract:

Pancreatic ductal adenocarcinoma (PDAC) is typically characterized by high chemoresistance and metastatic spread, features mainly attributable to cancer stem cells (CSCs). It is of central interest the characterization of CSCs and, in particular, the study of their metabolic features in order to selectively identify their peculiarities for an efficient therapeutic approach. In this study, CSCs have been obtained by culturing different PDAC cell lines with a specific growth medium. Cells were characterized for the typical stem/mesenchymal properties at short-, medium-, and long-term culture. Metabolomics, proteomics, analysis of oxygen consumption rate in live cells, and the effect of the inhibition of lactate transporter on cell proliferation have been performed to delineate the metabolism of CSCs. We show that gradually de-differentiated pancreatic cancer cells progressively increase the expression of both stem and epithelial-to-mesenchymal transition markers, shift their metabolism from a glycolytic to an oxidative one, and lastly gain a quiescent state. These quiescent stem cells are characterized by high chemo-resistance, clonogenic ability, and metastatic potential. Re-differentiation reverts these features, re-activating their proliferative capacity and glycolytic metabolism, which generally correlates with high aggressiveness. These observations add an important piece of

knowledge to the comprehension of the biology of CSCs, whose metabolic plasticity could be exploited for the generation of promising and selective therapeutic approaches for PDAC patients.

Biography:

Ilaria Dando is an Assistant Professor in Biochemistry at University of Verona, Italy, since 2018. From the beginning of her scientific career, Ilaria Dando's main interest has been the study of the redox and metabolic characteristics of pancreatic ductal adenocarcinoma, particularly focusing on the characterization of cancer stem cells in order to identify new specific markers and to test new potential therapeutic approaches by using in vitro cultured cell lines and animal models. In addition, Ilaria Dando is studying male infertility and fertility preservation, thanks to the collaboration with the Pediatric Surgeon of University of Verona, Italy. The project aims to study the biological features and hormonal regulation of testicular cells obtained from biopsies of pediatric and adult patients.

Targeting Cancer-Associated Fibroblasts by FAP-Selective Ferritin Nanocages Loaded with Navitoclax

Marta Truffi

Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy

Abstract:

Cancer-associated fibroblasts (CAFs) are key components of the tumor microenvironment. They are emerging as interesting stromal target in many solid tumors since they contribute to cancer progression, drug resistance and immune suppression. Our aim was to develop a nanosized delivery agent to specifically target and kill protumorigenic CAFs, reshaping the tumor microenvironment and favoring cancer eradication. Bionanoparticles of human H-ferritin (HFn) were loaded with the pro-apoptotic drug navitoclax. To steer drug delivery to CAFs, HFn were engineered by surface functionalization with antibody fragments specific for the fibroblasts activation protein (FAP), a recognized marker of protumorigenic CAFs. Targeting capability of functionalized versus bare nanoparticles was assessed by flow cytometry on primary murine CAFs and human activated myofibroblasts overexpressing FAP. The pro-apoptotic activity of the nanodrugs was assessed by confocal microscopy and viability assay in cell culture. Functionalized HFn showed enhanced binding to FAP-overexpressing CAFs as compared to non-functionalized HFn, while they only had limited binding to FAP-negative cancer cells. Navitoclax-loaded HFn exerted efficient pro-apoptotic activity in sensitive cells. In vitro treatment of FAP-overexpressing cells with functionalized nanodrugs improved reduction of cell viability as compared to non-functionalized nanodrugs, while no difference was observed in FAP-negative cells equally treated. Accordingly, a significantly higher uptake of navitoclax was observed in FAP-overexpressing cells incubated with functionalized versus bare nanoparticles. Our results showed that FAP-targeted HFn could be a promising strategy to enhance specific drug delivery into CAFs, thus opening new therapeutic possibilities aimed to remodel the tumor microenvironment.

Biography:

Marta Truffi got a M.Sc. in Sciences, Santé et Applications à finalité Recherche at the University Paris Diderot (2009), a second M.Sc. in Industrial Biotechnology at the University of Milano-Bicocca (2010) and a Ph.D. in Biomolecules, Structural Biology, Pathology and Biotherapy at the University Paris Diderot (2013). After the Ph.D., she moved to the University of Milano (Milano, Italy) to focus her research on targeted nanoparticles for diagnostic and therapeutic applications. Since 2019 she has joint the Laboratory of Nanomedicine at ICS Maugeri (Pavia, Italy), where she applies nanotechnology to biomarker investigation in the fields of medical oncology and neurology.

The Effect of Photobiomodulation at 660 nm on the Levels of Cyclooxygenase 2, Interleukin-6 and Tumour Necrosis Factor-A in *In Vitro* Diabetic Wounded Fibroblast Models

Asma Shaikh-Kader

Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa

Abstract:

In diabetic wounds, persistent inflammation leads to impaired tissue repair. Increased levels of interleukin–6 (IL-6) and tumour necrosis factor alpha (TNF-α) contribute to delayed wound healing in diabetes. Additionally, the enhanced production of inflammatory mediators formed due to cyclooxygenase-2 (cox-2) activity contributes to chronic inflammation. Photobiomodulation (PBM) is the use of light to stimulate cellular processes and may affect tissue repair. In this study, the effect of PBM on the levels of IL-6, TNF-α and cox-2 were determined in four fibroblast models (normal; normal wounded; diabetic and diabetic wounded). Cells in the experimental group were irradiated at 660 nm (5 J/cm^2). Models were evaluated at 0, 24 and 48h after irradiation. Cell morphology (light microscopy), cell viability (Trypan Blue exclusion assay) and cox-2, IL-6 and TNF-α levels (ELISA) were determined. Cell migration improved in the diabetic wounded groups over the 48h interval after PBM; viability increased in the diabetic wounded groups at 0 and 24h ($P \leq 0.05$ and $P \leq 0.01$, respectively); levels of cox-2 increased in the diabetic and diabetic wounded groups at 48h ($P \leq 0.05$ and $P \leq 0.01$, respectively), and IL-6 levels decreased in the diabetic and diabetic wounded groups at 24h ($P \leq 0.05$ and $P \leq 0.001$, respectively) and 48h ($P \leq 0.05$) post-irradiation. TNF-α levels decreased at 48h post-irradiation in both diabetic and diabetic wounded groups; but these differences were not statistically significant. PBM at 660 nm may curb inflammation by reducing the levels of IL-6 in diabetic cells, however an increase in the levels of cox-2 at 48h post-irradiation was noted in this study.

Biography:

Asma Shaikh-Kader graduated with a BSc at the University of the Witwatersrand and thereafter joined the School of Physiology in 2008 to complete a BSc with Honours. In 2011, she graduated with a Master of Science in Medicine at Wits University. Asma is currently a full-time lecturer in the department of Human Anatomy and Physiology at the University of Johannesburg, South Africa. She is also a part-time doctoral student in the Laser Research Centre at the University of Johannesburg and her research focuses on the effect of photobiomodulation on wound healing in diabetes mellitus.

Proteomic Profiling of BRAFV600E Mutant Colon Cancer Cells Reveals the Role of Nucleophosmin in Mediating the Resistance to BRAF Inhibition by Vemurafenib

Mirela Sedic

University of Rijeka Department of Biotechnology, Croatia

Abstract:

BRAFV600E mutations occur in approximately 10% of subjects suffering from colorectal cancer (CRC) and are associated with worse overall prognosis, poor treatment response and development of resistance to currently available therapies. The present study aims to identify novel targetable vulnerabilities of BRAFV600E mutant CRC cells, whose pharmacological inhibition might provide new therapeutic opportunities to increase the efficacy of BRAF inhibitor vemurafenib. Towards this aim, we carried out global proteomic profiling of colon cancer cells harboring BRAFV600E mutation in comparison with wild type (WT) BRAF colon cancer cells including both KRAS mutant and double WT cell lines. In this way, we detected 17 and 10 protein spots that were significantly (ANOVA $p<0.05$) up- and down-regulated, respectively, in BRAFV600E mutant in comparison with WT BRAF cell lines. Based on bioinformatics analyses of obtained proteomics data, nucleophosmin emerged as an important up-regulated feature of BRAFV600E mutant colon cancer cells, whose expression was additionally confirmed to be increased at protein and mRNA levels in tumor tissues

from BRAFV600E mutant colon cancer patients in comparison with WT BRAF tumors. Furthermore, the expression of its phosphorylated form (Thr199) was increased upon exposure to vemurafenib in a time- and dose-dependent manner in two vemurafenib-resistant BRAF mutant CRC cell lines. Expectedly, 4-hour pretreatment of resistant cell lines with nucleophosmin inhibitor NSC348884 reversed the resistance to vemurafenib. Collectively, our data suggest that nucleophosmin mediates resistance to vemurafenib in BRAF mutant CRC and posit that targeting nucleophosmin might increase the efficacy of BRAF inhibitors in BRAF mutant CRC.

Biography:

Dr. Mirela Sedic (born Bauman) (ORCID iD https://orcid.org/0000-0003-4679-1541; https://www.researchgate.net/profile/Mirela_Sedic) is Associate Professor at the University of Rijeka Department of Biotechnology in Croatia. She has authored over 50 scientific publications in peer-reviewed journals including BBA Molecular Basis of Disease, Molecular Cancer, The Journal of Pathology, Molecular Cancer Therapeutics, Journal of Medicinal Chemistry and Cancer Treatment Reviews. She is the PI of the project funded by the Croatian Science Foundation "Dissecting the mechanisms of therapy resistance in BRAF-mutant colon cancer using an integrated –omics approach" and the University of Rijeka grant "Molecular features associated with BRAFV600E-mutated versus wild type BRAF colorectal cancer".

Nuclear Translocation of SRPK1 is Associated with 5-FU Sensitivity in Cancer Cells

Ioanna Sigala

Laboratory of Biochemistry, Department of Chemistry, Aristotelian University, Greece

Abstract:

Serine/arginine protein kinases (SRPKs) phosphorylate Arg/Ser dipeptide-containing proteins that play crucial roles in a broad spectrum of basic cellular processes. The existence of a large internal spacer sequence that separates the bipartite kinase catalytic core and anchors the kinases in the cytoplasm is a unique structural feature of SRPKs. Here, we report that exposure of HeLa cells to DNA damage inducers triggers the nuclear translocation of SRPK1. Furthermore, we show that nuclear SRPK1 did not protect from, but on the contrary, mediated the cytotoxic effects of genotoxic agents, such as 5-fluorouracil (5-FU). Confirming previous data showing that the kinase activity is essential for the entry of SRPKs into the nucleus, SRPIN340, a selective SRPK1/2 inhibitor, blocked the nuclear accumulation of the kinase, thus diminishing the cytotoxic effects of the drug. ATR/ATM-dependent phosphorylation of threonine 326 and serine 408 in the spacer domain of SRPK1 was essential for the redistribution of the kinase to the nucleus. Substitution of either of these two residues to alanine or inhibition of ATR/ATM kinase activity abolished nuclear localization of SRPK1 and conferred tolerance to 5-FU treatment. These findings suggest that SRPK1 may play an important role in linking cellular signaling to DNA damage in eukaryotic cells.

Biography:

Dr. Ioanna Sigala hold a Diploma and Doctorate degree in Chemistry in the field of Biochemistry and currently work as a postdoctoral researcher at Aristotle University of Thessaloniki. Her research interests include the study of biological function of protein kinases in cancer cells and organic compounds as potential drugs for cancer therapy.

MicroRNAs, A potential approach in Obstructing Cell Signaling of Gliblastoma

Atieh Moradimotlagh

Department of Microbiology, School of Biology, College of Science, University of Tehran, Iran

Abstract:

Glioblastoma multiforme (GBM) is the most malignant and aggressive type of brain tumor with an average life expectancy of fewer than 15 months. This is mostly due to the highly mutated genome of GBM, which is characterized by the deregulation of many key signaling pathways involving growth, proliferation, survival, and apoptosis. It is critical to explore novel diagnostic and therapeutic strategies that target these pathways to improve the treatment of malignant glioma in the future. MicroRNAs (miRNAs), play a key role as a post-transcriptional regulator of many genes. Different expression of miRNAs is reported in a variety of malignancies compared to corresponding healthy tissue and it noteworthy that some of these miRNAs have been shown to modulate oncogenes or tumor suppressors. These made us take the advantage of the regulatory function of these molecules in the therapeutic approach and there are promising results for the usage of miRNAs in inhibiting GBM cell signaling.

Critical Roles of Socs1 mRNA Methylation in the Control of Ctokine Storm Niche-Selective Inhibition of Pathogenic Th17 Cells by Targeting Metabolic Redundancy

Lin Wu

Dan Littman Lab, Skirball Institute, New York University School of Medicine, New York, NY

Abstract:

Cellular metabolism is foundational to all cellular activities. Targeting metabolic enzymes represents an effective approach to suppress pathogenic cell behavior and recently has received increasing attention in the fields of immunology and cancer. However, many of the metabolic pathways are essentially required by the majority of, if not all, the cells in the body, which greatly limits the clinical translation of metabolic targeting due to its broad toxicity. In this study, we show that CRISPR-mediated targeting of glycolysis in T cells in mice results in global loss of Th17 cells, whereas deficiency of the glycolytic enzyme Gpi1 selectively eliminates inflammatory encephalitogenic and colitogenic Th17 cells, without substantially affecting homeostatic microbiota-specific Th17 cells. Unlike glycolysis genes such as Gapdh, whose deficiency completely blocks glycolytic flux, Gpi1 encodes a functionally redundant enzyme, whose inactivation still supports adequate production of biosynthetic precursors and ATP in the homeostatic Th17 cells through enhanced compensatory activities of the pentose phosphate pathway and mitochondria respiration. In contrast, inflammatory Th17 cells experience a hypoxic microenvironment known to limit mitochondrial respiration, which is incompatible with loss of Gpi1. Our study suggests that inhibiting glycolysis by targeting Gpi1 could be an effective therapeutic strategy with minimum toxicity for Th17-mediated autoimmune diseases. Moreover, it demonstrates the remarkable plasticity of the metabolic network due to the redundant components which can be regulated by environmental factors, and, more importantly, that metabolic redundancies can be exploited for selective targeting of disease processes.

Proteinopathy in the Retinal Pigment Epithelium (RPE): Implications for Sight-loss in Old Age

J. Arjuna Ratnayaka

University of Southampton, UK

Abstract:

Damage to the retinal pigment epithelium (RPE), which maintains overlying photoreceptors in the retina, is linked with irreversible sight-loss including age-related macular degeneration (AMD), the most common cause of blindness in developed societies. RPE cells internalize and degrade photoreceptor outer segments (POS) as part of the daily photoreceptor renewal, which subjects the RPE to the highest proteolytic burden in the body. However, age and onset of retinopathy correlates with partially degraded POS, which accumulate as lipofuscin and related molecules, constituting a clinically well-documented pathway of RPE death in geographic atrophy AMD that has no effective treatment. To elucidate its molecular mechanisms, we exploited an in-vitro RPE model, which structurally and physiologically recapitulates the native RPE monolayer. Disease conditions linked with AMD including oxidative stress and impaired autophagy were recapitulated using 100µm H_2O_2, oxidatively-modified POS (OxPOS) or 10nM bafilomycin A^1, respectively. We also studied effects of the Alzheimer's-related amyloid beta (Aβ) proteins, which accumulate in aged/AMD retinas. Confocal-immunofluorescence studies combined with ultrastructural imaging revealed the fate of trafficked POS in the phagosome and autophagy-lysosomal pathways, which, depending on the insult, became sequestered in early compartments or were trafficked prematurely to lysosomes. A proportion of POS were also prematurely targeted to autophagy bodies. OxPOS accumulated in late compartments generating increased autofluorescence, which recapitulates aged/damaged RPE in patients. Aβ rapidly

accumulated in lysosomes, which, unlike POS cargos, RPE cells were unable to degrade effectively. Our findings revealed contrasting molecular mechanisms underpinning proteinopathy in the RPE that could be manipulated to develop future treatments.

Biography:

Dr. Ratnayaka is a Cell Biologist who studies how tissues in the retina becomes diseased with old age. He also investigates retina-brain links including conditions such as Alzheimer's disease. His group uses in-vitro cell and mouse models as well as donor tissues; employing techniques such as gene editing, lentiviruses and 3D-imaging for their work. Their discoveries have led to collaborative studies with industry. Dr Ratnayaka serves in several scientific and academic advisory panels, and acts as a peer-reviewer for funding organisations. He is also involved in raising awareness of blinding diseases and dementia through public lectures, workshops and outreach activities.

Proteoliposome-like Structure Derived from Simultaneous Evisceration and Enucleation of Cells: A Top-Down Story

Cherng-Wen Darren Tan

Institute for Synthetic Bioarchitectures, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Austria

Abstract:

Biological membranes are powerful barriers to material exchange. Nonetheless, controlled communication between a cell's internal milieu and its environment still occurs, mediated by membrane proteins. This class of protein is what allows a cell to sense changes its surroundings, and to initiate responses to these perturbations. In turn, these diverse and intricate protein functions confer the membranes with complex behaviors. For this reason, the study of biological membranes often includes the study of their attendant membrane proteins. We are interested in how the membrane proteins, CD4, CXCR4, and CCR5 mediate membrane fusion. This is the major process by which HIV particles, as well as HIV-infected cells, invade target cells. Although live target cells would be suitable models for studying this phenomenon, their complexity makes it difficult to decouple the membrane fusion process from other components and events in the cells. Ideally, we would use a cell-derived structure akin to a giant liposome functionalized with the necessary proteins. One way to create such a structure is the controlled removal of most, if not all, of the cytoplasmic contents of an appropriate cell. We present a method for the simultaneous removal of cytoplasmic and nuclear material from A3R5.7 cells, using Colcemid treatment followed by hypotonic cytolysis. Preliminary assays show that the resultant cell membrane remains intact, presents CD4, CXCR4, and CCR5, and is still capable of CD4-mediated membrane fusion with an HIV model. Such systems might eventually be useful for host-pathogen interaction studies, as well as the development of targeted delivery vehicles.

Biography:

Darren Tan has a degree in Cell and Molecular Biology, and a doctorate in Bioengineering from National University of Singapore. His doctoral work explored the culture and behavioral control of pancreatic β-cells in a microfluidic sheer-free gradient generator. He found himself moving to Europe in 2013, much to his surprise. Today, he is a Senior Scientist at University of Natural Resources and Life Sciences in Vienna, where he is also deputy to the Head of Institute for Synthetic Bioarchitectures. His current research interests include modeling biological membranes and exploring synthetic membrane-based HIV/AIDS treatment. Between research and teaching, he paints.

PD1/PDL Pathway Dysregulation in Celiac Disease, and the Role for Diagnostic and as a Therapeutic Target

Torres Lopez MI

Department of Experimental Biology, University of Jaén, Spain

Abstract:

In this study, we focus on the alteration of the programmed cell death 1 (PD-1)/PD-L1 pathway in celiac disease. The PD-1 and PD-L1 levels in the serum and in intestinal biopsies of CD patients may be relevant to the determination of a possible correlation between markers of the autoimmune response, inflammation, and disease activity. Our results provide the first evidence of high PDL1 expression on intestinal epithelial cells and lamina propria cells with different grade of immunoreaction, as demonstrated by immunohistochemistry in celiac patients. Interestingly, our results demonstrated negative expression of PD1 in intestinal samples of active celiac disease. Levels of soluble PD-1 and PD-L1 were considerably higher in the serum of CD patients compared with healthy controls. We have characterized PD1 mRNA variants profile in CD patients and in response to gluten peptides incubation on *in vitro* experiments. PCR amplification of the human PD-1 coding sequence revealed a correlation between the over-expression of the sPD-1 protein and the PD-1Δex3 transcript in celiac disease. Thus, we have found three novel isoforms of alternative spliced, two of them result in a truncated protein and other isoform with loss of 14 aa of exon 2 and complete exon 3 (Δ3) could encodes a new soluble form of PD1 (sPD-1). It is important to consider that the crucial function of the PD-1 pathway is to limit immunopathological responses in host tissues by promoting the resolution of inflammation and restoration of immune homeostasis.

Biography:

Dr. Torres Lopez, Professor of Cell Biology in Jaén University (Spain) has received his Ph.D. with special award. Research in the field of inflammation and tolerance on two areas: (1) In vitro evaluation the immunotoxic ability of the peptidic fragments derived from gluten); and (2) the role of tolerance molecules in inflammatory bowel disease and celiac disease. Have published over 80 scientific papers, invited reviews and book chapters. Currently serves on the Editorial Board of different journals and as a member of the national and international research public evaluate research committees.

PCSK6 Plays an Important Role in Placenta Development

Linglin Xie

Department of Nutrition, Texas A&M University, College Station, TX

Abstract:

Abnormal placenta development has been indicated in preeclampsia and gestational diabetes (GDM), which are both common yet serious complications in approximately 10% of pregnancies. Proprotein convertase subtilisin/kexin-6 (PCSK6) is a protease and its expression in placenta decreases in highfat diet induced hyperinsulinemia. We hypothesized that PCSK6 plays an important role in placenta development. The current study applied a PCSK6 transgenic mouse model consisting of four groups: normoglycemic (NG) WT and PCSK6 knockout (KO) placenta, and HG WT and PCSK6 KO. Histological examination of placenta disclosed that spiral arteries (SpAs) in PCSK6 KO placentas, under NG and HG, had decreased inner to outer diameter ratio and trophoblast giant cell (TGC) association compared to the WT placentas. Consistently, PCSK6 KO placentas overphosphorylate β-catenin, a key protein to regulate trophoblast migration. In the labyrinth, PCSK6 KO affected fetal capillary area (FCA) while HG affected maternal lacunae area (MLA). PCSK6 KO-HG had increased interhaemal membrane (IHM) thickness. From these factors, the calculated diffusion capacity was increased in PCSK6 KO under NG but decreased under HG. In summary, our study demonstrated that PCSK6 is involved in SpA remodeling and glucose dependent angiogenesis. Our study indicated a potential role of PCSK6 in preeclampsia and gestational diabetes related placenta dysfunctions.

Biography:

Dr. Linglin Xie is an associate professor at the Department of Nutrition, Texas A&M University. She has three research interests: 1. how interactions between genes and maternal environment affect placenta development, which leads to severe pregnancy complications. 2. Maternal nutrition and offspring health focusing on offspring obesity and nonalcoholic fatty liver disease. 3. The interactive gene regulatory network involving Tbx5, Gata4, Osr1, and Hh-signaling in heart development.

Yeast, *Saccharomyces cerevisiae*, as a Model for Research of the Molecular Activity of Potential Drugs

Magdalena Cal

Department of Mycology and Genetics, Institute of Genetics and Microbiology, University of Wroclaw, Poland

Abstract:

Yeast cells show numerous similarities to cells of higher eukaryotes, including human cells, in terms of cell processes and protein functions. Therefore, Saccharomyces cerevisiae (baker's yeast) is an extremely useful eukaryotic model organism in molecular biology. Basic research on the molecular activity of potential drugs can be carried out on this model organism. These studies are important to determine the full mechanism of action of compounds with drug potential on a eukaryotic cell. In our research, which was financed by the Polish National Science Centre (project no. UMO-2015/17/D/NZ2/01985), on a yeast model, we showed that the anti-cancer compound, 3-bromopyruvate, induces DNA damage potentially through reactive oxygen species. These results were also confirmed on human cancer cells. Additionally, we have shown that 3-BP affects mitochondrial function. The use of baker's yeast in this aspect gave an extraordinary opportunity to show the activity of an anti-cancer compound on eukaryotic cells.

PD-1 Expressing $CD8^+CXCR5^+$ Follicular T cells Constitute Effector Rather than Exhaustive Phenotype in Patients with Chronic Hepatitis B

Arshi Khanam

Division of Clinical Care and Research, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD

Abstract:

Classical CD8 T cells are implicated for protective and pathogenic roles in chronic hepatitis B (CHB) infection. Recently, a new subset of CD8 T cells expressing CXCR5 and exhibiting features of follicular T cells has been identified during chronic viral infections. However, in CHB, their roles have not yet been well defined. Here, we characterized circulating $CD8^+CXCR5^+$ and $CD8^+CXCR5^-$ T cells and their association with clinical and viral factors in CHB. We found that CHB infection did not influence the overall frequencies of $CD8^+CXCR5^+$ cells but $CD8^+CXCR5^-$ cells were increased. However, among CHB, $CD8^+CXCR5^+$ cells were higher in patients with low HBsAg and HBV DNA level, patients who were HBeAg negative and had high fibrosis scores. Importantly, these cells showed significant association with HBsAg and HBV DNA reduction. Contrarily, $CD8^+CXCR5^-$ T cells were expanded and positively associated with patients having high HBsAg, HBV DNA and ALT levels. $CD8^+CXCR5^+$ T cells constituted higher frequencies of Tc1, Tc2, Tc17 and Tc22 subsets and overexpressed PD-1. Interestingly, $PD\text{-}1^+CD8^+CXCR5^+$ cells exhibited higher CD69 and secreted more IFN-γ, IL-21 and IL-22 than PD-1- population, which illustrate effector phenotype of these cells; whereas, $CD8^+CXCR5^-$ population displayed lower CD69 and secreted less cytokines irrespective of PD-1 expression, suggesting a phenotype of overall exhaustion. In addition, HBcAg-specific cytolytic function measured by CD107a, perforin and granzyme B expression was higher in $CD8^+CXCR5^+$ than $CD8^+CXCR5^-$ cells; however,

HBsAg-specific cytolytic activity was impaired in both cell types. **Conclusion:** $CD8^+CXCR5^+$ cells are enriched in effector phenotypes with HBV-specific cytokine producing abilities and lytic function, despite increased PD-1 and associate with HBsAg and HBV DNA reduction, which may serve them as potential therapeutic target for CHB.

The Role of RhoA in Angiopoietin 2-Induced Lymphangiogenesis

Constantinos Mikelis

Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Jerry H. Hodge School of Pharmacy, Amarillo, TX

Abstract:

Lymphangiogenesis is an important physiological process, but also a determining factor in vascular-related pathological conditions. Angiopoietin-2 (Ang2) plays important role in lymphatic vascular development and function and its presence and upregulation have been reported in several vascular-related diseases, including cancer. Given the established role of the small GTPase RhoA on cytoskeleton-dependent endothelial functions downstream of important angiogenic mediators, we explored whether RhoA participates in Ang2-induced signaling and functions. Ang2-driven human dermal lymphatic endothelial cell (HDLEC) migration depends on RhoA. Knockdown, pharmacological approaches and protein sequencing experiments demonstrated that Ang2-induced migration is independent on the Tie receptors, but dependent on β1 integrin-mediated RhoA activation. Although the key downstream ROCK/pMLC signaling is activated, its blockade does not abrogate Ang2-driven migratory effect. However, formins, an alternative RhoA target, and especially FHOD1, are strong regulators. Lymphatic endothelial RhoA deficiency blocked Ang2-induced lymphangiogenesis in vivo, highlighting RhoA as an important target for anti-lymphangiogenic treatments.

Experimental and Computational Tools for Acquiring and Analyzing Fluidics and Microscopybased Single-cell Data

Gregory Baker

Laboratory of Systems Pharmacology, Department of Systems Biology, Harvard Medical School, Boston, MA

Abstract:

Accurately profiling immune responses to cancer at single-cell resolution is necessary for understanding mechanisms of tumor immune surveillance and therapeutic response. At the Laboratory of Systems Pharmacology, we have developed a suite of technologies for acquiring and analyzing fluidics and microscopy-based single-cell data. Here we demonstrate the potential for these tools to drive discovery in cancer systems immunology through their application to various preclinical and clinical investigations. Combining a platform for multi-organ immune response profiling (SYLARAS) together with an approach to highly-multiplex immunofluorescence (CyCIF), we have identified alterations in the frequency of circulating B220+ CD8T cells in glioma-bearing mice and shown that these cells infiltrate the brain tumor microenvironment. Through further applications of CyCIF, we have highlighted the ability for the method to detect disease-specific spatial patterns of tumor-infiltrating immune cells and generate training data for pixel-level immune cell classification with variational autoencoders. Our most recent efforts have led to the development of pipelines for multiplex image assembly (MCMICRO) and quality control (CyLinter). Using MCMICRO, we have assembled 1.57TB of raw multiplex immunofluorescence data from a cohort of 25 triple-negative breast cancer tissue biopsies then used CyLinter to remove cells affected by optical or image-processing artifacts prior to unsupervised clustering. Our work shows that microscopy aberrations preclude the accurate detection of biologically-relevant cell states comprising tissue ecosystems and underscores the need for

quality control in quantitative multiplex histology. Using these open-access resources we now aspire to build immune-profile atlases across disease states and therapy.

Biography:

Dr. Gregory Baker obtained his Ph.D. from the Department of Molecular and Medical Pharmacology at the University of California, Los Angeles where he developed and investigated preclinical models of glioblastoma in the laboratory of Drs. Pedro Lowenstein and Maria Castro. Before that, he received his Pharm.D. (Doctorate in Pharmacy) from the University of Rhode Island. He is now a Postdoctoral Research Fellow and Trainee of the Ludwig Center in Peter Sorger's lab at Harvard Medical School. His current research centers on the development of experimental and computational tools for single-cell analysis and their application to cancer systems immunology.

Mapping the Regulatory Landscape of Auditory Hair Cells from Single-cell Multi-omics Data

Joerg Waldhaus

Department of Otolaryngology–Head and Neck Surgery, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI

Abstract:

Auditory hair cells transduce sound to the brain and in mammals these cells reside together with supporting cells in the sensory epithelium of the cochlea, called the organ of Corti. To establish the organ's delicate function during development and differentiation, spatiotemporal gene expression is strictly controlled by chromatin accessibility and cell type-specific transcription factors, jointly representing the regulatory landscape. Bulk-sequencing technology and cellular heterogeneity obscured investigations on the interplay between transcription factors and chromatin accessibility in inner ear development. To study the formation of the regulatory landscape in hair cells, we collected single-cell chromatin accessibility profiles accompanied by single-cell RNA data from genetically labeled murine hair cells and supporting cells after birth. Using an integrative approach, we predicted cell type-specific activating and repressing functions of developmental transcription factors. Furthermore, by integrating gene expression and chromatin accessibility datasets, we reconstructed gene regulatory networks. Then, using a comparative approach, 20 hair cell-specific activators and repressors, including putative downstream target genes, were identified. Clustering of target genes resolved groups of related transcription factors and was utilized to infer their developmental functions. Finally, the heterogeneity in the single-cell data allowed us to spatially reconstruct transcriptional as well as chromatin accessibility trajectories, indicating that gradual changes in the chromatin accessibility landscape were lagging behind the transcriptional identity of hair cells along the organ's longitudinal axis. Overall, this study provides a strategy to spatially reconstruct the formation of a lineage specific regulatory landscape using a single-cell multi-omics approach.

Biography:

Dr. Waldhaus received his Ph.D. in Biology from the Eberhard Karls University of Tübingen, Germany in 2010. Between 2011 and 2017, Dr. Waldhaus was working as a Postdoctoral Fellow, Research Associate and Instructor in the Heller Laboratory at Stanford University investigating development and regeneration of cochlear hair cells and supporting cells. In 2018 Dr. Waldhaus joined the Kresge Hearing Research Institute of the University of Michigan as Assistant Professor and he is affiliated with the Center of Computational Medicine & Bioinformatics (CCMB) for the same time.

The Impact of Clay Minerals on Lung Cells– An Analysis at the Single Cell Level

Karin Ardon-Dryer

Department of Geosciences, Atmospheric Science Group, Texas Tech University, Lubbock, TX

Abstract:

Aerosols particles (Natural and anthropogenic) are a key component of our atmosphere, their presence defines air quality levels and they can affect our health. Small particles penetrate into our lungs and this exposure can cause our lung cells to stress and in some cases leads to the death of the cells and to inflammation. During dust storm events there is an increase in particle concentration, many of them are breathable particles that can penetrate deep into our lungs. Exposure to dust particles can lead to respiratory problems, particularly for people with asthma. Therefore, during and after a dust storm event the number of people who are hospitalized with inflammation and respiratory problems increase. However, the exact mechanism that causes these health problems is still unclear. In this project, we investigated the impacts that clay minerals particles which are common in dust storm of different concentrations (doses) have on human lung cells, performing a new and unique analysis at the single cell level. Individual lung cells are continuously tracked after being exposed to dust particles. We monitor the behavior of the cell over time, identify the cells time of death and type of death as a function of particle concertation (doses) and type. These findings will help us to better understand the health-related consequences of exposure to dust storm events and serve as a baseline for when evaluating other aerosol.

Using Neurotechnology and Artificial Intelligence to Treat Disease

Patrick Ganzer

Battelle Memorial Institute, Columbus, OH

Abstract:

Bioelectronic medicine is an emerging type of neurotechnology using peripheral nerve stimulation to treat disease. This presentation will broadly introduce bioelectronic medicine technology, and specifically outline how we are using it to reverse episodes of myocardial ischemia. Myocardial ischemia is a cardiovascular event that is spontaneous, usually asymptomatic, and contributes to fatal cardiovascular consequences. Our bioelectronic medicine technology leverages artificial intelligence (AI), and works as follows: 1) We first train an artificial neural network (ANN) to reliably decode spontaneous events of myocardial ischemia (~94% accuracy; preclinical model). These events are induced by infusions of catecholamines that modulate the prime determinants of myocardial oxygen consumption. 2) Once myocardial ischemia is detected, the ANN responsively triggers closed-loop vagus nerve stimulation (VNS), providing on-demand bioelectronic medicine to restore myocardial oxygen balance. ANN controlled VNS specifically reduced pathophysiological changes in rate pressure product, electrophysiological correlates of ischemic currents, and arrythmia incidence. 3) Interestingly, preprogrammed open-loop VNS cannot react to spontaneous events of myocardial ischemia, and provided almost no benefit. Disruption of efferent vagal fibers also blocked the beneficial effects of ANN controlled VNS. These results show that VNS timing and nerve fibers engaged are both critical for the beneficial effects of ANN controlled bioelectronic medicine. Overall, we anticipate new innovations in the emerging field of artificially intelligent medicines, where AI systems optimize and help deliver therapy for treating disease and dysfunction.

Biography:

Dr. Patrick Ganzer received his B.S. in Neuroscience from King's College in 2008 (Summa Cum Laude), completed his Ph.D. in Biomedical Engineering from Drexel University in 2013, and finished his post-doctoral fellowship at the University of Texas at Dallas in 2017. He is now a Senior Research Scientist at Battelle Memorial Institute, the world's largest non-profit research organization located in Columbus, Ohio. Dr.

Ganzer's research focuses on neurotechnology, applied artificial intelligence, and bioelectronic medicines. His team's work is now being translated to multiple clinical trial applications to treat individuals with disease and disability.

Mechano-niche in Lung Repair/Regeneration Following Injury

Yong Zhou

Department of Medicine, University of Alabama at Birmingham, AL

Abstract:

Normal structure and function of the lung is maintained in homeostasis and repaired/regenerated following diverse injuries by regionally defined stem/progenitor cells. Stem cells reside in unique tissue microenvironments, known as the stem cell "niche", which constitutes stem cell progeny, other niche-support cells including mesenchymal cells (MCs), and the surrounding extracellular matrix (ECM). The stem cell niche provides instructive cues for stem cell self-renewal and differentiation. Fibrotic lungs undergo substantial changes in the tissue biomechanical properties, manifested by stiffening of the ECM. Cells residing in the stem cell niche sense and respond to alterations of the ECM stiffness, highlighting matrix stiffness as an important mechanical component of the stem cell niche. Our preliminary studies suggest that mechano-interactions of lung MCs and the ECM in the stem cell niche regulate the fate of mesenchymal niche cells and impact on lung stem cell properties. Understanding the mechanisms by which lung stem cells interact with their niches in normal vs. pathological repair of the injured lung will provide novel therapeutic approaches to prevent, treat, and potentially reverse pulmonary fibrosis.

Biography:

Dr. Zhou is tenured Associated Professor of Medicine, Ophthalmology, and Biomedical Engineering. Research in his laboratory focuses on the mechanobiology of tissue injury, repair, and regeneration. His clinical interests are human idiopathic pulmonary fibrosis (IPF) and primary open-angle glaucoma. Research findings from his team have been published on highly influential journals, including J Clin Invest, J Exp Med, Nat Commun, and Am J Respir Crit Care Med, in recent years. His research programs have been funded by NHLBI and NEI of NIH, American Heart Association, American Thoracic Society, and the EyeSight Foundation of Alabama.

Retinal Ribbon Synapses and Phototransduction Gene Network: How Ion Channels-Encoding Genes Mutations Could Impair Retinal Biology

Luigi Donato

Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Division of Medical Biotechnologies and Preventive Medicine, University of Messina, Messina, Italy

Abstract:

Today an increasing number of pathophysiological modifications leading to disease onset have been linked to ion transport alterations. One of the most emerging research field is focused on retinal phototransduction and ribbon synaptic physiology. A relevant group of mutations have been reported in ion channel subunit coding genes, as well as in their interacting modulators, improving the knowledge on a particular class of inherited retinal degenerations defined channelopathies. These mutations result in either a loss- or gain-of channel functions affecting their structure, assembly, trafficking, and localization. We present the last obtained results produced from a wide next generation sequencing experiment, consisting of whole exome

sequencing of twenty Italian and Egyptian families, affected by orphan forms of retinal dystrophies. All patients presented a common clinical-documented phenotype characterized by neurotransmission alterations, which differed in each subject for the involved retinal cytotypes. We found 6 mutated genes, never associated to retinal diseases before, whose variants might alter fundamental Ca^{2+} and Mg^{2+} binding sites of important ion channels, differently distributed through all considered patients. Very intriguingly, such genes resulted crucial nodes in a computed network made of already known causative/associated retinal degeneration genes and shared pathways. In order to evaluate the possible effects of found variants on encoded proteins, we applied a mixed approach based on innovative machine learning and molecular dynamics methods, complementary to chemical-biological-based experimental ones. Despite further required experiments, we believe that our study will help scientists and clinicians to provide new personalized diagnosis and innovative treatments for retinal degenerations.

Biography:

Luigi Donato, Ph.D. in "Applied Biology and Experimental Medicine", frequents the Molecular Genetics Labs of University of Messina, Italy. He is a researcher of the IEMEST institute in Palermo, Italy, too. He published more than 50 papers in reputed journals and participated in more than 30 national and international congresses, also being in the Organizing Committee in several of them. He was a member of ARVO and he is a member of AIBG. He joined the Editorial Board of several journals, such as "Cell Cycle", "BMC Bioinformatics", "Antioxidants" (Guest) and "Frontiers in Genetics" (Guest). His main research fields are retinal dystrophies and omics sciences.

Microfluidic Methods in Study of Cystic Fibrosis Lung Disease

Yuliang Xie

Roy J. Carver Department of Biomedical Engineering, University of Iowa, IA

Abstract:

Cystic fibrosis (CF) is a life-shortening, genetic disease that affects multiple organs. CF lung disease is the major cause of morbidity and mortality in people with CF. Research on a pig model of CF shows that mucus strands emerging from CF submucosal glands fail to detach from the airway surface, disrupt mucociliary transport, and initiate a cascade of mucus accumulation, infection, inflammation and pathology in CF lungs. Merging microfluidic methods with pulmonary physiology sheds light on the mechanism of defective mucus strands detachment in CF. Particularly, a microfluidic mucus biochemical analysis reveals that mucus from CF pig submucosal glands is abnormally acidic. A microfluidic model of submucosal gland demonstrates that acidic pH during mucus strand formation prevents mucus strand stretch and breakage, impairing mucociliary transport. A trachea-on-a-chip study further reveals that changing pH on the apical surface of CF airways does not improve mucus strand clearance. These findings suggest that submucosal glands are key to mucociliary transport, airway host defense, and have important implications for potential treatments of airway diseases.

Biography:

Yuliang Xie, Ph.D., is an assistant professor in Roy J. Carver Department of Biomedical Engineering, University of Iowa. He received his B.S. degree (2007) in Biological Engineering and M.S. degree (2010) in Biochemical Engineering from Zhejiang University, China. He received his Ph.D. degree (2016) in Chemical Engineering from the Pennsylvania State University, USA. Then he worked as a research associate at Howard Hughes Medical Institute and University of Iowa (2016-2020). By merging engineering with medicine, he developed microfluidic methods to study mucus properties and submucosal gland function in pulmonary diseases.

Osr1 Deletion in the Macrophages Promoted Hepatic Inflammation and Nonalcoholic Steatohepatitis (NASH) Progression

Lin Liu

Department of Nutrition, Texas A&M University, College Station, TX

Abstract:

One third of NAFLD progresses to its inflammatory subtype called non-alcoholic steatohepatitis (NASH). Liver macrophage-mediated inflammation contributes to the pathogenesis of NASH; however their associated pathophysiology and the molecular mechanisms remains unclear. Osr1 is a transcription factor, which expresses in the macrophages. Its expression in liver macrophages increased in mice of NASH. We hypothesize that Osr1 regulates macrophage activation and polarization and thus mediates liver inflammation during NASH development. In BMDMs, Osr1 expression was found positively correlated with the M2 marker genes such as PPAR-γ in macrophage phenotype switch (M0/M1/M2). More interestingly, deleting Osr1 shifts the macrophage to a glycolysis like metabolism profile. These results suggest a potential role of Osr1 in macrophage polarization. The physiological role of Osr1 was further investigated in a myeloid-derived macrophage-specific Osr1-knockout mice model (Osr1ΔMφ). Osr1ΔMφ mice displayed worsened NASH phenotypes compared to their littermates. Furthermore, PPAR-γ and c-Myc were identified as the downstream targets of Osr1 by CHIP-qPCR and luciferase reporter assays. In summary, our results demonstrated that macrophage Osr1 plays an important role in hepatic inflammation and NASH progression via mediating macrophage activation.

Biography:

Lin Liu is currently a Ph.D. candidate in Nutrition, Texas A&M University. His research mainly focusses on the genetic regulation of embryonic heart development, molecular nutrition, and the development of non-alcoholic fatty liver disease.

Structural Basis for the Modulation of Human KCNQ4 by Retigabine and Linopirdine

Huaizong Shen

School of Life Sciences, Westlake University, China

Abstract:

Among the five KCNQ channels, also known as the Kv7 voltage-gated potassium channels, KCNQ2-5 control neuronal excitability. Dysfunctions of KCNQ2-5 are associated with neurological disorders such as epilepsy, deafness, and neuropathic pain. Here, we report the cryo-EM structures of human KCNQ4 and its complexes with the opener retigabine or the blocker linopirdine at overall resolutions of 2.5 Å, 3.1 Å, and 3.3 Å, respectively. In all structures, a phosphatidylinositol 4,5-bisphosphate (PIP2) molecule inserts its headgroup into a cavity within each voltage-sensing domain, revealing an unobserved binding mode for PIP2. Retigabine nestles in each fenestration, inducing local shifts. Instead of staying within the central pore, linopirdine resides in a cytosolic cavity underneath the inner gate. Electrophysiological analyses of various mutants corroborated the structural observations. Our studies reveal the molecular basis for the modulatory mechanism of neuronal KCNQ channels and provide a framework for structure-facilitated drug discovery targeting these important channels.

Biography:

Dr. Huaizong Shen completed both his Ph.D. and postdoc training in Tsinghua University during which period he determined the first structure of a eukaryotic voltage-gated sodium channel, NavPaS and several other important Nav channels including human pain channel, Nav1.7. After that, Dr. Shen joined the School of Life Sciences, Westlake University as a principal investigator. Right now, his interest lies in elucidating the working mechanism of KCNQ channels through both structural and electrophysiological methods. His works were published in high-profile journals including Science, Cell and Molecular Cell.

Interneuron Accumulation of Tau Protein Impairs Adult Hippocampal Neurogenesis

Jie Zheng

Department of Pharmacology, Key Laboratory of Basic Pharmacology of Ministry of Education, Zunyi Medical University, China

Abstract:

Phospho-tau accumulation and adult hippocampal neurogenesis (AHN) impairment both contribute importantly to the cognitive decline in Alzheimer's disease (AD), but whether and how tau dysregulates AHN in AD remain poorly understood. Here, we found prominent accumulation of phosphorylated tau in GABAergic interneurons in the dentate gyrus (DG) of AD patients and mice. Specific overexpression of human tau (hTau) in mice DG interneurons induced AHN deficits but increased neural stem cell-derived astrogliosis, associating with a downregulation of GABA and hyperactivation of neighboring excitatory neurons. Chemogenetic inhibition of excitatory neurons or pharmacologically strengthening GABAergic tempos rescued the tau-induced AHN deficits and improved contextual cognition. These findings evidenced that intracellular accumulation of tau in GABAergic interneurons impairs AHN by suppressing GABAergic transmission and disinhibiting neural circuits within the neurogenic niche, suggesting a potential of GABAergic potentiators for pro-neurogenic or cell therapies of AD.

Biography:

Jie Zheng is an associate professor in Zunyi Medical University, China. He received his Ph.D. from Peking University in 2017, and his research focuses on investigating neurobiological mechanisms underlying neurodegeneration and exploring neuro-regenerative therapies.

Multiscale 'Whole-Cell' Models to Study Neural Information Processing – New Insights from Fly Photoreceptor Studies

Zhuoyi Song[1,2*], Yu Zhou[3], Jianfeng Feng[2] and Mikko Juusola[4,5]

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[4]*Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, UK*

[5]*State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing 100875, China*

Abstract

Dynamical "whole-cell" models are computational models that aim to account for the integrated function of numerous genes or molecules to behave like virtual cells in silico. For neural signalling processes, such models should not only implement the molecular mechanisms at the microscopic level but also reproduce the macroscopic signal processing dynamics at the cellular level. However, because of the limited knowledge available for the biochemical signalling pathways inside neurons, few "whole-cell" neuron models exist to date. Here, I will talk about a "whole-cell" model for a fly photoreceptor, the first "whole-cell" model for a neuron. By simulations, the model revealed several completely novel mechanisms underlying neural information processing in fly photoreceptors. Specifically, in this talk, I will briefly introduce several conclusions that are in contrary to traditional beliefs: 1) how signal refractory period and stochasticity are beneficial, rather than detrimental to neural information processing; 2) how photomechanical movements combat motion blur, rather than causing it. These results gained from studying the model was believed to have changed our understanding of phototransduction in insect vision, illustrating the usefulness of using dynamical "whole-cell" models to study neural information processing.

Biography:

Dr. Zhuoyi Song is currently a research fellow (tenure-track) at the Institute of science and technology for brain-inspired intelligence (ISTBI). Dr. Song obtained her Ph.D. in 2011, majored in automatic control at the University of Sheffield, UK. Then, she conducted research at UCL and the University of Sheffield before joining ISTBI in 2019. Dr. Song's research interests reside in multi-scale computational modeling of neural systems, and the study of adaptive neural encoding mechanisms. She published her results in high-impact journals, such as Current Biology, eLife, Journal of Neuroscience, etc.

How Circadian Clocks Work for a Brain Repair System: Demyelination Regulates BMAL1 to Signal Adult Neural Stem Cells to Enhance Remyelination

Jin Young Kim

Department of Biomedical Sciences, City University of Hong Kong, Hong Kong

Abstract:

Circadian clocks, endogenous oscillators generating cell-autonomous rhythms, are intrinsic in most cells, including neural cells. Under physiological conditions, they are synchronized to local macro- and micro-environments by the action of external cues and regulate various cellular processes like metabolism, proliferation, and differentiation. Demyelination is a common form of central nervous system pathology that has variable degrees of recovery. Demyelination changes the surrounding microenvironment via damaged myelin and activation of glial cells. How these microenvironmental changes affect circadian clocks in the lesions, and their consequences are mostly unknown. Here, we show that circadian clocks are altered in the demyelinating lesions. This initiates molecular signals that induce adult neural stem cells (NSCs) in the subventricular zone (SVZ) to produce oligodendrocyte lineage cells (OLCs). Circadian clocks in the demyelinating lesions shortened the period to transcribe more clock target genes, including the Wnt inhibitors SFRP1 and SFRP5. Unexpectedly, SFRP1 and SFRP5 signaled to the SVZ to reduce levels of the circadian transcription factor BMAL1, and this resulted in switching NSCs to OLCs. Local inhibition of Bmal1 expression in the demyelinating lesions prevented the switching of SVZ NSCs into OLCs, thereby reducing the migration of OLCs to the lesions. Thus, our findings show that communications between the demyelinating lesions and the SVZ via local circadian clocks enhance the remyelination process by supplying OLCs from the SVZ to the lesions.

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Inhibiting the DNA Damage Pathway to Promote Recovery from CNS Injury

Zubair Ahmed

Institute of Inflammation and Ageing, University of Birmingham, UK

Abstract:

DNA double strand breaks are the most deleterious types of DNA damage and are a feature of many acute and long-term neurological disorders, including neurodegeneration after trauma and stroke. In mitotically active cells, double strand breaks trigger the DNA damage response to arrest the cell cycle and mount repair via non-homologous end-joining in G1 or G2 phases or homologous recombination in M and S phases. In non-mitotic neurons, persistent activation of the DNA damage response is a trigger for dysregulation of the cell cycle and aberrant re-entry of neurons into G1 leading to neural dysfunction, apoptosis and senescence. We therefore hypothesised that muting this response might be beneficial to neurons after trauma. Here, I will show that attenuating the DNA damage response by targeting several key molecules in the DNA damage pathway, including meiotic recombination 11 (MRE11), Rad50, Nijmegen breakage syndrome 1 (Nbs1) complex and downstream, ataxia telangiectasia mutated (ATM) and checkpoint-2 (Chk2), promotes neuroprotection, axon regeneration and functional recovery in models of central nervous system trauma. We showed selectivity in the pathway since attenuating Chk1 had no effect. Our studies are the first to show that attenuating the DNA damage pathway after acute neurotrauma is beneficial to functional recovery after CNS trauma.

Biography:

Dr. Zubair Ahmed is currently an Associate Professor is Neuroscience. He is currently the Head of Neuroscience and Ophthalmology at the University of Birmingham in the UK and leads a team of scientists dedicated to

understanding why the CNS does not regenerate after injury using models of optic nerve, spinal cord and brain injury. He is a specialist in the molecular pathways that regulate neuroprotection and axon regeneration and has contributed several important observation in the field.

Involvement of Imprinted Genes in Molecular Mechanism Resulting in Pediatric Brain Arteriovenous Malformation

Concetta Scimone

Department of Biomedical and Dental Sciences and of Morphological and Functional Images, University of Messina, Italy

Abstract:

Brain arteriovenous malformations (bAVM, OMIM #108010) are microvascular lesions characterized by the direct shunt from arterioles to venules, missing the normal capillary bed. Affected vessels show impaired expression of vessel differentiation markers resulting in loss of endothelial cells properties and increased vascular permeability. The high pressure of blood perfusing from arteries increases risk of lesion rupture, resulting in intracerebral haemorrhage. In children, bAVM is more prone to rupture, anticipating the age of symptom onset. Pediatric bAVM represents about 3% of all bAVMs and mainly occur sporadically, probably due to defects during embryo vasculogenesis. To date, mutations in genes involved in TGFβRII signalling are considered the main genetic factor linked to bAVM development. To increase knowledge in this field, we performed whole exome sequencing on 4 affected children. Following bioinformatic analysis, germline variants showing minor allele frequency < 0.01 were considered. Functional enrichment of mutated loci clustered them in pathways NOTCH and TGFβR signalling, cytoskeleton and ion homeostasis. However, further investigations revealed a high mutation rate in genes undergo to differential expression due to imprinting mechanism. Effects of these mutations were in-silico predicted. Functional enrichment annotated imprinted mutated loci in pathways related to ion transport and homeostasis as well as in angiogenesis regulation. In order to decipher inheritance pattern of mutated alleles and their consequent expression, we are now proceeding by genotyping the parents of the patients.

Lysosomes Shape Neuronal Ca^{2+} Handling

Nicoletta Plotegher

Department of Biology, University of Padova, Italy

Abstract:

Homozygous mutations of the lysosomal enzyme glucocerebrosidase (GBA1) cause Gaucher disease (GD), a lysosomal storage disorder which may involve severe neurodegeneration, while heterozygous mutations of GBA1 represent the major genetic risk for Parkinson's disease (PD).

In neurons from GBA1 knockout (gba1-/-) mice we previously showed that autophagy is impaired upstream of the lysosomes and mitochondria are dysfunctional. We now show that stimulation with physiological glutamate concentrations is associated to impaired $[Ca^{2+}]c$ responses, reduction of mitochondrial membrane potential and an irreversible fall in the ATP/ADP ratio. Increases in cysotolic Ca^{2+} levels upon stimulation with glutamate were also associated to a reduction in mitochondrial Ca^{2+} uptake was reduced in gba1-/- neurons, as well as in the level of the mitochondrial calcium uniporter (MCU). gba1+/- neurons behavior was similar to gba1-/- in terms of all variables, consistent with a contribution of these mechanisms to the pathogenesis of both neuropathic GD and PD. These data signpost reduced bioenergetic capacity and $[Ca^{2+}]_c$ dysregulation associated to lysosomal dysfunction as mechanisms driving neurodegeneration.

Biography:

Dr. Nicoletta Plotegher is Research Fellow in the Department of Biology at the University of Padova. She started her Ph.D. in Biosciences in 2010 during which she focused on the study of alpha-synuclein aggregation in vitro and in Parkinson's disease cellular models. In 2015, a Marie Curie Individual Fellowship allowed Dr. Plotegher to move to University College London, where she started studying the role of GBA1 in Parkinson's and Gaucher diseases. In 2018 she was awarded a Veronesi Foundation Postdoctoral Fellowship to go back to Padova, where she is further exploring the role of lysosomes in neuronal physiology and pathophysiology.

Safety and Efficacy of First-In-Human Intrathecal Transplantation of Human Astrocytes (Astrorx®) in ALS Patients: Phase I/IIA Clinical Trial Results

Michal Izrael

VP of R&D, Neurodegenerative Diseases Department at Kadimastem Ltd, Israel

Abstract:

Background: AstroRx® is a cell-based therapy, composed of healthy and functional human astrocytes derived from embryonic stem cells. AstroRx® cells can protect neurons by several mechanisms of action that were demonstrated in in-vitro and in vivo preclinical studies. The clinical study hypothesis is that transplantation of astrocytes (AstroRx®) can compensate for the malfunctioning of endogenous astrocytes by restoring hampered physiological capabilities, i.e. reducing toxic compounds (e.g. reuptake of excessive glutamate), reducing oxidative stress, as well as by secreting multiple neurotrophic and neuroprotective factors. **Methods:** We conducted a Phase I/IIa, Open-Label, dose-escalating Clinical Study to Evaluate the Safety, Tolerability and Therapeutic Effects of Transplantation of AstroRx® in Patients with Amyotrophic Lateral Sclerosis (ALS). Enrolled patients were monitored for 3 months of "run-in period" to record their ALS progression. At the end of the run-in period, 5 patients were injected intrathecally with a single dose of 100*106 AstroRx® cells and 5 patients with a single dose of 250*106 cells. After treatment, the patients were monitored for additional 6 months for recording safety data and assessment of disease progression, as compared to the run-in period and for additional 6 months for long range safety. **Results:** AstroRx® treatment was well tolerated in both doses and no treatment-related serious adverse events nor dose-limiting toxicities related to AstroRx® cells were reported. A clinically meaningful decline in disease progression, assessed by the ALS Functional Rating Scale-Revised (ALSFRS-R), was observed during the first 3 months of the 6-month follow-up period in both cohorts. **Conclusions:** Single-dose transplantation of AstroRx® cells is safe and demonstrated a promising efficacy during the first 3 months. These results support a further, randomized-controlled, clinical trial with repeated doses of AstroRx® in patients with ALS, in order to prolong the time span of the clinical effect observed by the single dose.

Mechanisms of Regulation and Diverse Activities of Tau-Tubulin Kinase (TTBK) Isoforms

Channa Bao

Biogen, Cambridge, MA

Abstract:

Tau-tubulin kinase 1 (TTBK1) is a CNS-specific, kinase that has been implicated in the pathological phosphorylation of tau in Alzheimer's Disease (AD) and Frontotemporal Dementia (FTD). TTBK1 is a challenging therapeutic target because it shares a highly conserved catalytic domain with its homolog, TTBK2, a ubiquitously expressed kinase genetically linked to the disease spinocerebellar ataxia type 11. The present study attempts to elucidate the functional distinctions between the TTBK isoforms and increase our

understanding of them as distinct targets for the treatment of neurodegenerative disease. We demonstrate that in cortical neurons, TTBK1, not TTBK2, is the isoform responsible for tau phosphorylation at epitopes enriched in tauopathies such as Serine 422. In addition, although our elucidation of the crystal structure of the TTBK2 kinase domain indicates almost identical structural similarity with TTBK1, biochemical and cellular assays demonstrate that the enzymatic activity of these two proteins is regulated by a combination of unique extra-catalytic sequences and autophosphorylation events. Finally, we have identified an unbiased list of neuronal interactors and phosphorylation substrates for TTBK1 and TTBK2 that highlight the unique cellular pathways and functional networks that each isoform is involved in. This data addresses an important gap in knowledge regarding the implications of targeting TTBK kinases and may prove valuable in the development of potential therapies for disease.

Biography:

Channa Bao currently works in the Genetic and Neurodevelopmental Disorders research unit at Biogen. She has supported programs on Alzheimer's disease, neuropathic pain, and ischemic stroke.

Epigenetic Regulation of CNS Axon Regeneration

Fengquan Zhou

Department of Orthopedic Surgery and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD

Abstract:

Neurons in the mature mammalian central nervous system (CNS) cannot regenerate their axons after various injuries or degenerative diseases. During maturation, CNS neurons gradually lose their intrinsic regenerative ability due to changed chromatin and transcriptomics landscapes. It is well known that epigenetic modifications of histones or DNAs play key roles in regulation of chromatin structure and gene transcription. Here we investigated the roles of EZH2, a histone methyltransferase, in controlling optic nerve regeneration. We found that overexpression of EZH2 in mature retinal ganglion cells (RGCs) significant promoted optic nerve regeneration. By performing RNA-, ATAC-seq and Reduced Representation Bisulfite-Seq of purified RGCs, we not only revealed different chromatin, transcriptomic and epigenetic landscapes regulated by EZH2, but also identified its unique and novel downstream targets. We identified EZH2 as a master negative regulator of many synaptic related ion channels and transporters, all of which function in mature neurons. Thus, EZH2 overexpression drove the mature RGCs back to their younger state with stronger intrinsic axon growth ability. Moreover, EZH2 acted to suppress all 3 classes of extrinsic regeneration inhibitory factors and their receptors self-secreted by RGCs, such as OMgp (myelin-based inhibitor), Tenascin R (CSPG), and Eph6/7/8 (repulsive cues), as well as Lingo (receptor for Nogo, MAG and OMgp). More importantly, overexpression of OMgp and Lingo, together with EZH2, abolished the enhanced optic nerve regeneration, indicating them as functional downstream targets of EZH2. Collectively, the study identified EZH2 as a major epigenetic regulator governing neuronal intrinsic axon regenerative capacity and extrinsic inhibitory cues/receptors.

Biography:

Dr. Fengquan Zhou is a Professor of Orthopedic Surgery and Neuroscience at Johns Hopkins University School of Medicine. His research interests are 1) understanding the molecular and cellular mechanisms by which neural regeneration is regulated, and 2) identifying novel strategies for enhancing neural regeneration and functional recovery after neural injures or neurological diseases.

RNA: DNA Hybrids Mediate DSB Repair Pathway Choice

Julio Morales

Department of Neurosurgery, Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Abstract:

DNA double strand breaks (DSBs) are an extremely dangerous form of DNA damage as one unrepaired break can lead to cell death. Considering how lethal DSBs are to the cell, they must be repaired in a timely and efficient manner. DSBs are typically repaired by one of two major repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). Whether a DSB is repaired through NHEJ or HR is extremely important, due to the potential loss of genetic material when NHEJ is utilized to repair the break. Our understanding of how the choice between NHEJ and HR far from complete. It is known that DNA damage response factors such as 53BP1 and BRCA1, amongst others, contribute to what pathway will repair the DSB, through their recruitment to the DNA lesion. Recently, is has been shown that along with traditional DNA response elements, through mechanisms unknown at this time, RNA polymerase II (RNAPII) is also recruited to DSB sites. Once recruited to the DSB site, RNAPII will initiate transcription that results in the formation of an RNA:DNA hybrid. We have found that the presence of an RNA:DNA hybrid can also contribute to the recruitment of NHEJ or HR factors to a DSB site. This was found by modulating the expression of the 5'-3' exo-ribonuclease XRN2. One of the major functions of XRN2 is to displace the RNAPII transcription machinery from the DNA template at the 3' end of genes through RNA:DNA hybrid resolution. Recently, we found that XRN2 is also involved in DSB repair, as loss of XRN2 leads to increased sensitivity to ionizing radiation and chromosomal aberrations. Loss of XRN2 also lead to a decrease in the cells ability to utilize the NHEJ repair pathway. We have found that loss of XRN2 leads results in an increase in RNA:DNA hybrid formation at the DSB site. In contrast to the increase in RNA:DNA hybrids formed at the DSB site with loss of XRN2, we find a dramatic decrease in the amount of Ku70. Consistent with the decrease in Ku70, we observe an increase in HR repair factors, such as BRCA1, EXO1 and CtIP at the DSB site. Using cells that express RNaseH1, a protein that resolves RNA:DNA hybrids through degradation of the RNA component, we found that resolution of the RNA:DNA hybrid restores Ku70 binding to the DSB site. Expression of RNaseH1 also restores NHEJ repair efficiency. These data demonstrate that the presence of a RNA:DNA hybrid plays a major role in determining whether NHEJ or HR is utilized to repair DSBs.

Modeling Developmental Brain Disorders Using Human Induced Pluripotent Stem Cell-Derived Mini Brains in Culture Dish

Xiaowen Bai

Medical College of Wisconsin, Department of Cell Biology, Neurobiology & Anatomy, Milwaukee, WI

Abstract:

Maternal alcohol exposure during pregnancy can substantially impact the development of the fetus, causing cognitive dysfunction and psychiatric disorders, with the mechanisms largely unknown. Recently developed 3D human cerebral organoids from induced pluripotent stem cells are similar to fetal brains in the aspects of development and structure. These models allow more relevant in vitro systems for studying FASDs than animal models. We found that 50 mM alcohol exposure for 6 hours induced acute apoptosis on organoids. The apoptotic effects of alcohol depended on alcohol concentration and varied between cell types. Specifically, neurons were more vulnerable to alcohol-induced apoptosis than astrocytes. The alcohol-treated organoids also exhibit disrupted mitochondria cristae and decreased intensity of mitochondrial matrix. Additionally, alcohol resulted in metabolic stress in the organoids as evidenced by 1) decreased mitochondrial oxygen consumption rates being linked to basal respiration, ATP production, proton leak, maximal respiration and spare respiratory capacity, and 2) increase of non-mitochondrial respiration in alcohol-treated organoids compared with control groups. Furthermore, we found that alcohol treatment affected the expression of 199

genes out of 17,195 genes analyzed. Bioinformatic analyses showed the association of these dysregulated genes with 37 pathways related to clinically relevant pathologies. Collectively, this human organoid model allows in-depth analyses of alcohol neurotoxicity at cellular, subcellular, bioenergetic metabolism, and molecular levels. Our findings provide novel insights into alcohol-induced pathologic phenotypes in developing human brains and potential neuroprotective strategy by targeting affected mitochondrial metabolisms and molecular networks.

Negative Regulation of TLR4 Receptor Signaling in Mast Cells: Participation of Opioid, Nicotinic and Cannabinoid Receptors

Zyanya Espinosa-Riquer

Center for Research and Advanced Studies, Mexico

Abstract:

Mast cells (MCs) are important elements of the immune system that are specialized on the secretion of inflammatory mediators. Best known for their participation on allergies through the activation of the high-affinity IgE receptor (FcεRI), they are also activated by innate immune stimuli, exerting protective reactions against distinct pathogens in diverse experimental models. The most studied pattern recognition receptor (PRR) in MCs is Toll-like 4 (TLR4) receptor, which is activated by Lipopolysaccharide (LPS/endotoxin). Triggering of TLR4 receptor on MCs turns-on the MyD88-dependent pathway with the consequent activation of NFκB transcription factor and the release of inflammatory cytokines such as TNF-α. Also, it induces the phosphorylation of distinct proteins that regulate not only gene transcription, but also the process of inflammatory mediators' secretion per se. To limit excessive inflammatory responses induced by constant stimulation of TLR4 receptor, we have demonstrated the participation of opioid, nicotinic and cannabinoid receptors. Activation of those molecules prevents LPS-induced TNF-α secretion in vivo and in vitro, by the inhibition of the phosphorylation of important proteins of the TLR4 signaling pathway that regulate transcription and secretion. Remarkably, cannabinoid receptor 2 (CB2) inhibits mast cell-dependent TNF-α release provoked by LPS in vivo and limits TLR4-dependent TNF-α secretion in vitro, lowering protein phosphorylation and inducing the expression of negative regulators known as "tolerance markers". Altogether, our results show that innate immunity responses mediated by mast cells are limited by key regulatory ligands, such as, acetylcholine, opioids and cannabinoids. Supported by Conacyt Project CF 2019-51488.

Biography:

Zyanya P. Espinosa-Riquer obtained a MSc and a Ph.D. in Neuropharmacology and Experimental Therapeutics from Cinvestav, and she is currently a postdoctoral fellow in the same institute. During her Ph.D. studies, she investigated the participation of the endocannabinoid 2-Araquidonoil-glycerol (2-AG) and the cannabinoid type 2 (CB2) receptor on the development of endotoxin tolerance on mast cells. She focused on understanding the molecular mechanisms that control TLR4 signaling on that cellular model. In her current work, she studies the effect of some drugs of abuse and drug adulterants on different aspects of the immune system and rodents´ conduct.

Nuclear Condensates and Gene Expression Regulation - Probing the Connection Using Live-Cell Imaging Approaches

Yaron Shav-Tal

Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, Israel

Abstract:

Nuclear speckles (NS) are membrane-less nuclear bodies that harbor an abundance of splicing factors. The function of these nuclear condensates is unclear. Studies in living cells have proposed a model in which splicing factors can shuttle between NS and transcribing genes, to participate in splicing. To test the influence of NS on transcription and splicing kinetics, we used a detectable, transcriptionally active gene in living cells. We previously found that an mRNA undergoing many splicing events was retained at this gene until the completion of mRNA processing, and this delay in release from the gene was splicing dependent. To determine whether splicing factor availability was the reason for this retention, NS were disassembled and splicing factor dynamics were measured. This disassembly increased the diffusing nucleoplasmic fraction of splicing factors, and reduced their residence times on the active gene. The mRNA that was previously retained on the gene was now rapidly released. In contrast, other perturbations did not affect the dynamics of mRNA release from the gene. Rather, faster release of the mRNA from the gene mediated by increased availability of splicing factors, was dependent on the RS domain of the splicing factors and its phosphorylation state. In conclusion, use of live-cell imaging and quantitative analysis of splicing factor dynamics in the cell nucleus leads us to suggest that nuclear speckles can buffer the availability of splicing factors in the nucleoplasm. As membrane-less structures, nuclear speckles can rapidly respond and regulate the kinetics of mRNA release from the gene after processing.

Biography:

Yaron Shav-Tal received his MSc and Ph.D. degrees from the Weizmann Institute of Science. During his post-doc at Albert Einstein College of Medicine, in the laboratory of Robert Singer, he unraveled the dynamics of mRNA travels in the nucleus. Since 2005 he is at the Faculty of Life Sciences and the Institute of Nanotechnology at Bar-Ilan University, Israel. He is a Full Professor and Vice Dean at the Faculty of Life Sciences. His group is focused on dissecting the kinetics of the gene expression pathway in single living cells using fluorescence live-cell microscopy and tagging of DNA and mRNA molecules.

Activation of a MAPK Hog1 by DNA Damaging Agent and Its Potential Role

Yuqi Wang

Louis University, St. Louis, MO

Abstract:

Hog1 is a mitogen-activated protein kinase in yeast that primarily regulates cellular responses to hyperosmolarity stress. In this study, we have examined the potential involvement of Hog1 in mediating cellular responses to DNA damaging agents. We find that treatment of yeast cells with DNA damaging agent methyl methanesulfonate (MMS) induces a marked and prolonged Hog1 activation. Distinct from stressors such as arsenite that activates Hog1 via inhibiting its phosphatases, activation of Hog1 by MMS is phosphatase-independent. Instead, MMS impairs a critical phosphor-relay process that normally keeps Hog1 in an inactive state. Functionally, MMS-activated Hog1 is not translocated to the nucleus to regulate gene expression but rather stays in the cytoplasm and regulates MMS-induced autophagy and cell adaptation to MMS stress. These findings reveal a new role of Hog1 in regulating MMS-induced cellular stress.

Biography:

Dr. Wang is Professor of Biology at Saint Louis University. His research focuses on mechanisms and regulation of cell signaling.

NLRP3 and AIM2 Inflammasome-Triggered Pathogenic Th17 Immune Response Promotes Severe Immunopathology in Schistosomiasis

Parisa Kalantari

Department of Immunology, Tufts University, Boston, MA

Abstract:

Infection with the parasite Schistosoma mansoni causes morbidity and mortality via a pathogenic host CD4 T cell-mediated immune response directed against parasite egg antigens. The development of a pathogenic Th17 adaptive immune response is associated with immunopathology in schistosomiasis. Although inflammasome's role in exacerbating immunopathology has been reported, which inflammasomes are involved and their impact on Th17 responses are not characterized. Here we demonstrated that schistosome egg-mediated IL-1β secretion and pyroptotic cell death required both NLRP3 and AIM2 inflammasome activation. Schistosome genomic DNA activated AIM2 inflammasome while reactive oxygen species as well as potassium efflux were the major triggers of NLRP3 activation. NLRP3 and AIM2 deficiency led to a significant reduction in pathogenic Th17 responses indicating that these two inflammasomes have a crucial role in triggering pathogenic Th17 differentiation. Additionally, we determined that these two inflammasomes suppress the anti-inflammatory Th2 cytokine IL-4. Our findings establish NLRP3 and AIM2 inflammasomes as central regulators of adaptive immunity that trigger pathogenic Th1 and Th17 responses while suppress Th2 responses, leading to excessive inflammation and severe schistosome immunopathology.

A Genome-wide CRISPR Screen Reveals a Role for the BRD9-containing Non-canonical BAF Complex in Foxp3 Expression and Regulatory T Cell Function

Ye Zheng

Nomis Center for Immunobiology and Microbial Pathogenesis, Salk Institute for Biological Studies, La Jolla, CA

Abstract:

Regulatory T cells (Tregs) play a pivotal role in suppressing auto-reactive T cells and maintaining immune homeostasis. Treg development and function are dependent on the transcription factor Foxp3. Although a large body of work has been devoted to dissecting the molecular mechanisms regulating Foxp3 expression, a systematic, genome-wide approach has not been reported. Here we performed a genome-wide CRISPR/Cas9 knockout screen to identify the regulators of Foxp3 in mouse primary natural Tregs. The screen results not only confirmed a number of known Foxp3 regulators, but also revealed many novel factors that control Foxp3 expression. Gene ontology analysis showed that Foxp3 regulators are highly enriched in genes encoding SWI/SNF and SAGA complex subunits. Among the three SWI/SNF-related complexes, the non-canonical or ncBAF (also called GBAF or BRD9-containing BAF) complex promoted the expression of Foxp3, whereas the PBAF complex repressed its expression. Gene ablation of BRD9 led to reduced Foxp3 expression and compromised Treg function in inflammatory disease and anti-tumor immunity. BRD9 co-localized with Foxp3 in genome-wide binding studies, including at the CNS0 and CNS2 enhancers at the Foxp3 locus. Functional genomics revealed that BRD9 is required for Foxp3 binding and expression of a subset of Foxp3 target genes. Thus, we provide an unbiased analysis of genes and networks regulating Foxp3, and reveal the ncBAF complex as a novel target that could be exploited to manipulate Treg function.

Biography:

The research in Dr. Zheng's lab is focused on regulatory T cells (Tregs), a subset of T lymphocytes that play a pivotal role in maintaining the balance of immune system and preventing autoimmune diseases, such as type-1 diabetes, rheumatoid arthritis, and multiple sclerosis. By studying the molecular pathways that modulate Treg activity, Dr. Zheng's lab is searching for novel therapeutic targets for treatment of autoimmune diseases and other chronic diseases exacerbated by prolonged inflammation.

Shigella flexneri Disruption of Cellular Tension Promotes Intercellular Spread

Brian Russo

Department of Immunology and Microbiology University of Colorado Anschutz Medical Campus, Aurora, CO

Abstract:

During infection, some bacterial pathogens invade into the eukaryotic cytosol and spread between cells of an epithelial monolayer. Intercellular spread occurs when these pathogens push against the plasma membrane, forming protrusions that are engulfed by adjacent cells. Here, we show that IpaC, a *Shigella flexneri* type 3 secretion system protein, binds the host cell-adhesion protein β-catenin and facilitates efficient protrusion formation. *S. flexneri* producing a point mutant of IpaC that cannot interact with β-catenin are defective in protrusion formation and spread. Spread is restored by chemical reduction of intercellular tension or genetic depletion of β-catenin, and the magnitude of the protrusion defect correlates with membrane tension, indicating that IpaC reduces membrane tension, which facilitates protrusion formation. IpaC stabilizes adherens junctions and does not alter β-catenin localization at the membrane. Thus Shigella, like other bacterial pathogens, reduces intercellular tension to efficiently spread between cells.

Biography:

Brian Russo received his Ph.D. from the University of Pittsburgh under direction of Gerard Nau. He did his postdoctoral work with Marcia Goldberg at Massachusetts General Hospital and Harvard Medical School. He is currently an Assistant Professor in the Department of Immunology and Microbiology at the University of Colorado School of Medicine. His research is focused on the mechanisms by which bacterial pathogens interact with their host and cause disease.

Highly Efficient Neuronal Gene Knockout *In Vivo* by CRISPR-Cas9 via Neonatal Intracerebroventricular Injection of AAV in Mice to Expedite Drug Target Validation

Joyce Lo

Biogen, Cambridge, MA

Abstract:

CRISPR-Cas systems have emerged as a powerful tool to generate genetic models for studying normal and diseased central nervous system (CNS). Targeted gene disruption by at specific loci has been demonstrated successfully in non-dividing neurons. Despite its simplicity, high specificity and low cost, the efficiency of CRISPR-mediated knockout in vivo can be substantially impacted by many parameters. Here, we used CRISPR-Cas9 to disrupt the neuronal-specific gene, NeuN, and optimized key parameters to achieve effective gene knockout broadly in the CNS in postnatal mice. Three cell lines and two primary neuron cultures were used to validate the disruption of NeuN by single-guide RNAs (sgRNA) harboring distinct spacers and scaffold sequences. This triage identified an optimal sgRNA design with the highest NeuN disruption in in vitro and

in vivo systems. To enhance CRISPR efficiency, AAV-PHP.B, a vector with superior neuronal transduction, was used to deliver this sgRNA in the Cas9 mice via neonatal intracerebroventricular (ICV) injection. This approach resulted in 99.4% biallelic indels rate in the transduced cells, leading to greater than 70% reduction of total NeuN proteins in the cortex, hippocampus and spinal cord. This work contributes to the optimization of CRISPR-mediated knockout and will be beneficial for fundamental and preclinical research.

Biography:

Joyce Lo, Ph.D. is a research Scientist at Biogen, a biotech company in Cambridge Massachusetts USA specialized in discovery and development of novel therapeutics for the treatment of neurological disorders. Joyce Lo obtained her Ph.D. in University of Missouri-Columbia where she elucidated Nrf2-Keap1 signaling transduction pathway and regulation of E3 ubiquitin ligases. She subsequently pursued her postdoctoral training with Morgan Sheng at Genentech and established a mouse disease model for psychiatric disorders. Joyce Lo has been working to advance gene therapy for the treatment of neuromuscular diseases at Biogen for the past five years. She has also established a CRISPR in vivo platform to facilitate disease target validation for CNS disorders.

GCN5L1 Interacts with WHAMM and KIF5B To Regulate Autolysosome Tubulation

Allen Seylani

National Heart, Lung and Blood Institute, NIH, USA

Abstract:

Lysosome-dependent autophagy, is a nutrient-deprivation induced evolutionarily conserved intracellular recycling program which sequestrates intracellular cargo into autophagosomes (AP), which then fuse with lysosomes to form autolysosomes (ALs) for cargo digestion. To restore free lysosomes, autophagic lysosome reformation (ALR) is initiated by extrusion of tubular structures from autolysosomes at the final stage of autophagy, in a process called lysosomal tubulation (LT). This project aimed to investigate the molecular role of GCN5L1 in LT. GCN5L1 belongs to the BORC multiprotein complexes and is involved in controlling lysosomal trafficking, however, the effect of GCN5L1 on lysosome tubulation remains largely unknown. Genetic ablation of GCN5L1 in the mouse primary hepatocytes showed significantly larger autolysosomes (ALs), decreased lysosome regeneration and absence of lysosomal tubulation. This phenotype suggests the possibility of disruption in lysosome tubulation which results in disturbance of overall lysosome homeostasis. The formation of tubulars from ALs requires kinesin motor protein KIF5B. Immunoprecipitation was employed and confirmed the interaction of GCN5L1 with the ARL8B-KIF5B complex, which recruited KIF5B to ALs. In parallel, GCN5L1 interacted with WHAMM, an actin nucleation promoting factor, which brings actin cytoskeleton to ALs to facilitate LT initiation. Furthermore, impaired LT in GCN5L1 deficient hepatocytes was restored by overexpression of GCN5L1, and this rescue effect was attenuated by knockdown of KIF5B. Additionally, lysosomal mTORC1 activity was upregulated in GCN5L1-/- hepatocytes while inhibition of mTORC1 abrogated the GCN5L1 mediated rescue of LT in knockout hepatocytes. Altogether these findings revealed a novel mechanism of ALR, in which a simultaneous interaction of GCN5L1 with KIF5B and WHAMM is required for LT in parallel with mTORC1 signaling.

Biography:

Allen studied biological sciences with physiology concentration and conducted virology and infectious diseases research for three years at California State University, San Marcos. Later he was awarded a fellowship at the National Institute of Health where he conducts translational research to find solution for rare lysosomal diseases.

Mas-related G Protein-Coupled Receptor-X2 and Adaptor Protein β-arrestin2 Differentially Regulates Mast Cell-Mediated Inflammation and Anaphylaxis

Saptarshi Roy

Department of Basic and Translational Sciences, University of Pennsylvania, School of Dental Medicine, Philadelphia, PA

Abstract:

Alongside high affinity IgE receptor (FcεRI), cutaneous mast cells (MCs) express Mas-related G protein-coupled receptor (GPCR) X2 (MRGPRX2, murine ortholog MrgprB2), which can be activated by a diverse group of cationic ligands including FDA-approved drugs. Activation of MCs via antimicrobial host defense peptide LL-37, has been implicated in the pathogenesis of rosacea but the receptor involved, and the mechanism of its regulation is not well understood. Intradermal injection of LL-37 resulted in the development of rosacea like skin inflammation in wild-type (WT) mice, which was significantly reduced in MrgprB2-/- mice and almost abolished in MC deficient Wsh/Wsh mice. Adapter protein β-arrestin2 (β-arr2) known to participates in GPCR desensitization, however, its role in the regulation of mast cell function is still elusive. We found that LL-37-induced erythema was significantly inhibited in mice with MC-specific deletion of β-arr2 when compared to respective control mice. LL-37-induced enhanced degranulation in peritoneal MCs (PMCs) isolated from β-arr2-/- mice, however, reduced MC chemotaxis, cofilin dephosphorylation and cytokine/chemokine production. Thus β-arr2 act as a positive regulator of MC function. In contrast, in a pseudoallergic model, MCs specific deletion of β-arr2 enhances both mast cell degranulation in response to ciprofloxacin in vitro and pseudoallergic drug reaction in vivo. Moreover, absence of β-arr2 had no effect in antigen/IgE-mediated (FcεRI) Syk phosphorylation and Ca^{2+} mobilization, however, enhanced MCs degranulation in vitro and passive cutaneous anaphylaxis *in vivo*. Thus β-arr2 could act as a negative regulator of MC. In conclusion, MrgprB2 and β-arrestin2 complex could serve as a new target for the development of therapeutics in modulating skin inflammation, pseudoallergy and anaphylaxis.

Biography:

Saptarshi Roy is from India. He completed his master's degree in microbiology and earned his Ph.D. degree (2016) from CSIR-Indian Institute of Chemical Biology, Kolkata, India. His doctoral research was on glycobiological and immunological aspect of host- parasite interaction in Leishmania infection model. In 2017, he joined in Ali laboratory. His major focus is on deciphering the role of adaptor protein β-arrestin2 in regulation of MRGPRX2/MrgprB2 signaling in allergy and asthma. He is also investigating the mechanism of MRGPRX2-mediated mast cell exocytosis.

Maf1 and RNA Polymerase III Transcription Regulates Osteoblast Differentiation and Bone Biology

Ellen Busschers

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

Abstract:

RNA polymerase (pol) III transcribes a variety of non-translated RNAs, including tRNAs. MAF1 is a repressor of RNA pol III-dependent transcription. We have previously shown that MAF1 promotes adipocyte differentiation. We therefore examined whether MAF1 plays a role in the differentiation of other mesenchymal cell types. We find that MAF1 overexpression in mouse long bones results in increased bone volume and it enhances osteoblast differentiation in vitro. Surprisingly, inhibition of RNA pol III-dependent transcription by either chemical inhibition, or by knockdown of the RNA pol III specific transcription factor BRF1, decreases osteoblast differentiation. To determine the basis for these opposing effects, RNA- and tRNA-seq analysis was conducted to determine changes in gene expression prior to and during the differentiation process. These different approaches to manipulate RNA pol III transcription resulted in distinct changes in mRNAs

with limited overlap amongst the different conditions. MAF1 overexpression results in an enrichment of genes involved in osteoblast differentiation and function. The different approaches to alter RNA pol III-dependent transcription also result in distinct changes in tRNA pools. Collectively, these results support a novel role for MAF1 in stimulating osteoblastogenesis in vitro and increasing bone volume in vivo, supporting an important role for RNA pol III-dependent transcription in cell fate determination. Different manners of repressing RNA pol III-dependent transcription result in distinct changes in gene expression profiles and different biological outcomes. We are currently investigating whether the divergent changes in the tRNA pool suggest these opposing biological effects are due to codon biased translation.

Biography:

Ellen Busschers is a Ph.D. candidate in the lab of Dr. Deborah Johnson at Baylor College of Medicine in the Department of Molecular and Cellular Biology.

Lattice Light-Sheet Microscopy Multi-dimensional Analyses (LaMDA) of T-Cell Receptor Dynamics Predict T-Cell Signaling States

Jun Huang

Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL

Abstract:

Lattice light-sheet microscopy provides large amounts of high-dimensional, high-spatiotemporal resolution imaging data of cell surface receptors across the 3D surface of live cells, but user-friendly analysis pipelines are lacking. Here, we introduce lattice light-sheet microscopy multi-dimensional analyses (LaMDA), an end-to-end pipeline comprised of publicly available software packages that combines machine learning, dimensionality reduction, and diffusion maps to analyze surface receptor dynamics and classify cellular signaling states without the need for complex biochemical measurements or other prior information. We use LaMDA to analyze images of T-cell receptor (TCR) microclusters on the surface of live primary T cells under resting and stimulated conditions. We observe global spatial and temporal changes of TCRs across the 3D cell surface, accurately differentiate stimulated cells from unstimulated cells, precisely predict attenuated T-cell signaling after CD4 and CD28 receptor blockades, and reliably discriminate between structurally similar TCR ligands.

Biography:

Jun Huang is an assistant professor of the Pritzker School of Molecular Engineering, Committee on Cancer Biology, Committee on Immunology, and the Graduate Program in Biophysical Sciences of the University of Chicago. His lab performs basic and translational research with the objective of developing effective vaccines and cell immunotherapies for the treatment of cancer, infection, and autoimmunity. He carries out basic immunological research, focusing on molecular mechanisms of T cell recognition and signaling at the single-molecule level. He performs systems immunology, studying the development, differentiation, and metabolism of T cells at the single-cell level. He engineers CAR-T cells, aiming at the treatment of cancer and autoimmunity. He develops new biomaterials, enabling the detection, profiling, and manipulation of T cells and other immune cells for diagnosis and treatment.

Detection of the Small Oligonucleotide Products of Nucleotide Excision Repair in Human Skin

Seon Hee Kim

Department of Bio-Analytical Science, University of Science & Technology, South Korea

Abstract:

UVB radiation results in the formation of potentially mutagenic photoproducts in the DNA of epidermal skin cells. In vitro approaches have demonstrated that the nucleotide excision repair (NER) machinery removes UV photoproducts from DNA in the form of small (~30-nt-long), excised, damage-containing DNA oligonucleotides (sedDNAs). Though this process presumably takes place in human skin exposed to UVB radiation, sedDNAs have not previously been detected in human skin. Using surgically discarded human skin, we have optimized the etection of the sedDNA products of NER from small amounts of human epidermal tissue ex vivo within minutes of UVB exposure and after UVB doses that normally lead to minimal erythema. Moreover, sedDNA generation was inhibited by treatment of skin explants with spironolactone, which depletes the epidermis of the essential NER protein XPB to mimic the skin of xeroderma pigmentosum patients. Time course experiments revealed that a partially degraded from of the sedDNAs could be readily detected even 12 hours following UVB exposure, which indicates that these repair products are relatively stable in human skin epidermis. Together, these data suggest that sedDNA detection may be a useful assay for determining how genetic, environmental, and other factors influence NER activity in human skin epidermis and whether abnormal sedDNA processing contributes to photosensitive skin disorders.

Biography:

Seon Hee Kim is a graduate student in the Department of Bio-Analytical Science at the University of Science &Technology in South Korea. Her research interests are currently focused on the DNA damage response in human cells.

Utilizing Improved BRET Approaches to Understand the Complexities Endogenous GPCR Function

Carl White

Harry Perkins Institute of Medical Research and Centre for Medical Research, The University of Western Australia, QEII Medical Centre, Australia

Abstract:

Introduction. Bioluminescence resonance energy transfer (BRET) is widely used to investigate protein or ligand interactions with membrane receptors and/or between cellular proteins. However, a fundamental limitation of current BRET techniques is the requirement for exogenous expression of fusion proteins, which precludes the direct application of this method to study endogenous protein interactions in their native cellular environments. **Aims.** To use NanoBRET based techniques coupled with CRISPR/Cas9-mediated genome engineering to investigate G protein coupled receptor function when expressed under endogenous promotion. **Methods.** CRISPR/Cas9-mediated homology-directed repair was used to insert Nanoluciferase (Nluc) into native genomic loci of HEK293 and/or Hela cells, resulting in Nluc fused to proteins of interest. NanoBRET or Nluc complementation was used to investigate receptor function in population assays using a multilabel plate reader or at the single cell level via bioluminescence imaging **Results.** We demonstrated that receptor ligand binding, receptor conformational changes, receptor internalisation, protein-protein interactions, and receptor trafficking could be monitored in live cells using proteins expressed under endogenous promotion. These approaches do not require over-expression of the proteins of interest, allowing natively expressed proteins to be studied and therefore representing improved models to investigate receptor function. **Discussion.** Using CRISPR/Cas9-mediated genome engineering, NanoBRET can be used to observe various aspects of receptor function using GPCRs found under endogenous promotion. This overcomes a major limitation of existing BRET-based techniques and helps to better understand the influence of cellular context on receptor function.

Biography:

Dr. White is a NHMRC CJ Martin Early Career Research fellow with a research focus on understanding how the cellular context influences G protein-coupled receptor (GPCR) function. He received as Ph.D. in Pharmacology from Monash University (2013) and was awarded the Molly Hollman Medal for best Thesis. He has developed cutting-edge CRISPR/Cas9 genome-editing approaches to, for the first time, allow real-time evaluation of receptor function in native/endogenous cellular contexts using the powerful biophysical technique bioluminescence resonance energy transfer (BRET).

Unconventional Roles of GABARAP-Type Proteins in Surface Protein Trafficking

Jochen Dobner

Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Germany

Abstract:

The y-aminobutyric acid receptor-associated protein (GABARAP) subfamily consisting of GABARAP, GABARAP-like 1 and GABARAP-like 2 is a subfamily of human autophagy-related 8 (Atg8) proteins. Atg8-type proteins are ubiquitin-like modifiers with 14 kDa of size. In contrast to the soluble ubiquitin, however, Atg8s can be conjugated to lipids, enabling them to act as membrane tethers, adaptors as well as fusion-mediating proteins with the latter being intrinsic properties of these proteins themselves. Current research is mainly focused on their roles during the cellular degradation pathway autophagy, but especially GABARAP-type proteins have been originally identified for their involvement during intracellular vesicle-mediated

trafficking processes of cell surface proteins. As cell surface proteins are key elements in intercellular communication and response to extracellular signaling cues, at least another layer is added to the biological functions of these proteins and their involvement in maintaining cell homeostasis. Recently, we and others have described additional autophagy-independent functions of GABARAP-type proteins. By analyzing genome-edited cell lines using various molecular biological, biochemical and live-cell imaging approaches, we demonstrate the role of the whole GABARAP subfamily in maintaining Golgi apparatus morphology and regulation of protein composition at the plasma membrane. Furthermore, we demonstrate that Golgi-dependent vesicular trafficking of fluorescently labelled ceramide is impaired in cells lacking the whole GABARAP subfamily. Overall, our data expands the current knowledge of this intriguing protein family and may open up new avenues for the targeted manipulation of GABARAP-type protein-mediated processes related to health and disease.

Biography:

Born in south-west Germany, Jochen Dobner studied biology (B Sc) and molecular cell and developmental biology (M Sc) at the Leopold-Franzens-University Innsbruck, Austria. After completing the master studies in the field of nutritional biochemistry, he performed his Ph.D. project in the lab of Prof. Dieter Willbold as a stipendiary of the Molecules of Infection (MOI) graduate school. Now, as a member of the Collaborative Research Centre 1208 (CRC1208) at the Heinrich-Heine-University Düsseldorf, Germany, he intensifies his studies on deciphering the roles of the versatile GABARAP subfamily proteins in membrane-related processes beyond autophagy.

Mycobacterial Cell Surface Adhesive Properties at the Nanoscale

Albertus Viljoen

Louvain Institute of Biomolecular Science and Technology, UCLouvain, Belgium

Abstract:

Adhesion to host cells and tissues is an important first step in infection employed by many bacterial pathogens. In mycobacteria, surface hydrophobic properties and specialized adhesins determine the ability of these pathogens to adhere to tissues. We have used state of the art atomic force microscopy (AFM) techniques to unravel the surface adhesive properties of Mycobacterium abscessus, a multidrug-resistant pathogen causing severe lung infections in cystic fibrosis patients. In a first approach, we used fast quantitative imaging (QI) AFM combined with hydrophobic tips to quantitatively map hydrophobic properties on the cell surface at high resolution. We discovered that the transition from a smooth to a rough colony morphology, associated with virulence and caused by the loss of cell envelope associated glycopeptidolipids (GPLs), leads to a dramatic change in surface hydrophobicity; smooth bacteria display unusual nanodomains with varying degrees of hydrophobicity while rough bacteria exhibit uniformly hydrophobic cell surfaces. In a second approach, we have used single-molecule force spectroscopy to study the strength, kinetics and thermodynamics of the interaction between a specialized mycobacterial adhesin, antigen85, and the host extracellular matrix protein, fibronectin. Strong bonds (up to ~500 pN) are observed under high tensile loading, which may favor strong mycobacterial attachment in the lung where cells are exposed to high shear stress or during hematogenous spread leading to a disseminated infection. Together these studies have contributed new insights into how the organization of the mycobacterial outer membrane determines the important cellular function of adhesion.

Biography:

Albertus Viljoen is a postdoctoral fellow at the Catholic University of Louvain in Belgium. He is interested in the biochemistry and biophysics of bacterial cell envelopes and their exposed surfaces. These structures play critical roles in the abilities of bacteria to form biofilms, to resist antibiotics, and to cause infections and are spatially heterogeneous in their chemical composition and biophysical characteristics. Currently, he is contributing to this pioneering field using molecular bacteriology and state-of-the-art atomic force microscopy (AFM) techniques.

Regulation of Histone H1 Subtypes: Lessons Learned from OMICs

Alicia Roque

Biochemistry and Molecular Biology Department, Barcelona Autonomous University, Spain

Abstract:

Histone H1 is involved in the regulation of chromatin higher-order structure and compaction. In humans, there are seven H1 subtypes differentially expressed in somatic cells. However, the regulatory mechanisms that determine the variability of the H1 complement are not fully understood. We have used a new approach based on the integration of OMICs data to address this issue. We have examined the 3D-chromatin structure, the binding of transcription factors (TFs), and the expression of somatic H1 genes in human cell lines using data from public repositories. Analyses of Hi-C, ChIP-seq, and RNA-seq data, have revealed that transcriptional control has a more significant impact on H1 regulation than previously thought. Somatic H1 genes located in topologically associated domains (TADs) show higher expression than in boundary regions. H1 genes are targeted by a variable number of transcription factors, including cell cycle-related TFs, and tissue-specific TFs, suggesting a fine-tuned, subtype-specific transcriptional control. We described that all H1 somatic subtypes are under transcriptional co-regulation, indicating that this phenomenon extends beyond the histone cluster. Transcriptional control and transcriptional co-regulation explain, at least in part, the variability of H1 complement, the fluctuations of H1 subtypes during development, and also the compensatory effects observed, in model systems, after perturbation of one or more H1 subtypes. Analysis of proteomics data suggests that post-transcriptional and translational regulation also contributes to the differential regulation of H1 subtypes.

Biography:

Alicia Roque obtained her Bachelor's Degree in Biochemistry with honors, in 1996, at the Havana University. She began her professional career at the Finlay Institute, developing vaccine candidates for bacterial infections. Afterward, she moved to Spain and obtained her Ph.D. in Biochemistry and Molecular Biology in 2008, which was awarded the Extraordinary Prize. Since 2005, she has combined teaching and research at the Autonomous University of Barcelona. Recently, Alicia has started her research group, interested in the study of the functional specificity and the regulation of H1 variants, as well as their implication in cancer.

CRISPR/Cas9-Based Gene Engineering of Human Natural Killer Cells- Optimization and Approaches for Efficacy Readout

Caroline Leijonhufvud

Center for Hematology and Regenerative Medicine, Department of Medicine, Huddinge, Karolinska Institute, Sweden

Abstract:

Recently cancer immunotherapy has become a fast growing field. The natural killer (NK) cell's ability to naturally detect malignant cells and capacity to perform antibody-dependent cellular cytotoxicity together with low risk of inducing graft-versus-host disease, makes these cells an attractive tool for cancer immunotherapy. NK cells can be used in both allogenic and autologous settings and allows for adpotive infusion using several different preparation protocols. The majority of reports have focused on improving NK cell cytotoxicity by several means including; mismatching KIR and KIR-ligand for induced missing-self, combination with checkpoint blockade and monoclonal tumor targeting antibodies. However, it is equally important to also promote persistance and engrafment or homing to the tumor beering tissue. While genetical modifications of cells have revolutionized T cell-based therapies, NK cells have until now been very difficult to genetically modify due to their defense mechanisms against infections and foreign nucleic acids. We have previously shown that NK cells can be manipulated with transient expression of introduced encoding mRNA electroporation which is less toxic than DNA containing plasmids but unfortunately only yield transient expression. The

recent CRISPR/Cas9 technology for permanent manipulation has great potential for targeted editing to knock-out genes in NK cells. Attractive applications for this are to knock-out check point proteins, homing molecules or other genes of interest for basic biological research. This talk provides insights on how to approach applying the recent CRISPR/Cas9 technology to genetically manipulate NK cells, how to optimize the protocol and how perform reliable readout for addressing knock-out efficacy.

Biography:

Caroline Leijonhufvud gained her M.D. at the Karolinska Institutet (KI) in 2016 where she also began her M.D.-Ph.D.-programme and joined as the first employee when Dr. Mattias Carlsten set up his research group. Her Ph.D. focuses on NK cell targeting specificity and how NK cells can be genetically engineered to further improve their tumor targeting capacity, including antibody-dependent cellular cytotoxicity. Caroline is co-supervised by Dr. Andreas Lundqvist at KI, and Rear Admiral Dr. Richard W. Childs, Assistant Surgeon General of the U.S. and Clinical Director at NHLBI, NIH where she also has spent parts of her Ph.D.

A Ribonuclease Secreted by Leucocytes During Infection Shows a Multifaceted Behaviour, Combining Catalytic and Antimicrobial/Antiviral Activities with Immunomodulation Properties

Ester Boix

Department of Biochemistry and Molecular Biology, Universitat Autonoma de Barcelona, Spain

Abstract:

The human RNase3 is a member of the RNaseA superfamily involved in host immunity. RNase3 is expressed by leukocytes and show broad-spectrum antimicrobial activity. Together with a direct antimicrobial action, RNase3 exhibits immunomodulatory properties. Here, we have analysed the transcriptome of macrophages exposed to the wild-type protein and a catalytic-defective mutant (RNase3-H15A). The analysis of differently expressed genes (DEGs) in treated THP1-derived macrophages highlighted a common pro-inflammatory "core-response" independent of the protein ribonucleolytic activity. Network analysis identified the epidermal growth factor receptor (EGFR) as the main central regulatory protein. Structural analysis suggested that RNase3 can activate the EGFR pathway by direct interaction with the receptor. In addition, we identified a subset of DEGs specifically related to the protein ribonucleolytic activity, characteristic of virus infection response and interferon signalling. Transcriptome analysis revealed an early pro-inflammatory response, not dependant on the protein catalytic activity, followed by a late activation in a ribonucleolytic dependent manner. Next, we demonstrated that overexpression of the macrophage endogenous RNase3 can protect the cells against intracellular infection by Mycobacterium aurum and the human Respiratory Syncytial Virus (RSV). Eventually, comparison of cell infection profiles in the presence of Erlotinib, an EGFR inhibitor, revealed that the receptor activation is required for the antibacterial but not for the antiviral protein action. Moreover, the DEGs related and unrelated to the protein catalytic activity are associated to the immune response to bacterial and viral infection respectively. We conclude that RNase3 modulates the macrophage defence against infection in both catalytic-dependent and independent manners.

Biography:

Ester Boix is leading the "Human host defence RNases" lab at the Universitat Autònoma de Barcelona, Spain. Following 5 years of postdoctoral studies at the National Institutes of Health and the University of Bath, she was awarded a Ramon y Cajal senior researcher contract. She has published more than 80 papers in peer-review journals and has coordinated several monographic issues on the antimicrobial proteins and peptides field. Her research group is pioneer in the identification of RNA recognition sites and antimicrobial properties of host defence RNases. Latest works are focused on the structure-based drug design to target pathogen resistance mechanisms.

Structural Insights into Pseudokinase Domains

Sebastian Mathea

Structural Genomics Consortium, Goethe University, Frankfurt, Germany

Abstract:

Pseudokinases display peculiar signaling mechanisms - and are emerging drug targets. Their features are similar yet different from catalytically active kinases. This becomes particularly evident in the modulated roles of conserved kinase elements, and in the interplay with the co-substrate / co-factor ATP. The focus of the presentation will be on how structural biology can help to enable the pharmacological targeting of pseudokinases. This will be demonstrated by highlighting the pseudokinases ILK, IRAK3, ROR1, RYK and ULK4.

Biography:

Sebastian obtained his Ph.D. for his work on prolyl isomerases at the Max Planck Institute in Halle. As a postdoc, he joined the Structural Genomics Consortium (SGC) in Oxford where he solved several structures of protein kinases. In 2017, he moved to Frankfurt University to work as the pipeline team leader for protein production - structure elucidation - inhibitor screening.

Revisiting PPARγ as a New Friend of GPR120 in the Treatment of Metabolic Disorders: A New Look at an Old Friend

Dayoung Oh

Touchstone Diabetes Center, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX

Abstract:

G Protein-coupled receptor 120 (GPR120; fatty acid receptor 4, FFAR4) and PPARγ agonists both lead to anti-inflammatory and insulin sensitizing effects despite signaling through distinct pathways. We recently reported the overarching idea that these 2 pathways are interactive. Specifically, treatment of obese mice with the PPARγ agonist rosiglitazone (a thiazolidinedione, TZD) in combination with the GPR120 agonist compound A synergistically improves glucose tolerance and insulin sensitivity. We have deconvoluted the mechanisms underlying this feed-forward effect in the study. Taken together, this study shows that low dose TZD administration, in combination with GPR120 agonists, produces additive beneficial effects on glucose tolerance and insulin sensitivity without the undesirable adverse effects of TZD. Our study suggests potential value of combination PPARγ and GPR120 agonists to treat metabolic disease.

Biography:

Dayoung Oh is an Assistant Professor at the UT Southwestern Medical Center, Dallas, TX. Dr. Oh has been working on the diverse signaling pathways of G protein-coupled receptors (GPCRs) and ligand identification for orphan GPCRs using a high throughput system and now she has particular interest in the role of GPCRs in the fields of obesity, inflammation, and Type 2 Diabetes. Her group has had a sharp focus on the role of omega-3 fatty acid receptor, GPR120 in macrophage-mediated chronic inflammation and insulin resistance and has published a number of high impact papers on the subjects. Her group have used various biochemical and physiological approaches including using GPCR knockout animals (global and tissue-specific), molecular biology, nucleic acid/protein biochemistry, and eukaryotic cell-based studies. Her long-term goal is not only to elucidate how GPCRs work in regulating metabolism, but also to identify new avenues for developing therapeutics to treat metabolic syndrome.

Regulation of Nuclear Receptor Function by Astrocyte Elevated Gene-1 (AEG-1)

Devanand Sarkar

Department of Human and Molecular Genetics, Associate Director of Training and Education, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA

Abstract:

Nuclear receptors are ligand-dependent transcription factors. Retinoid X Receptor (RXR) heterodimerizes with many nuclear receptors, regulating functions of vitamins, hormones and lipid metabolites. In the absence of ligand, RXR and its heterodimer partner interact with transcription co-repressors which inhibit transcription. Upon ligand binding, co-activators interact with nuclear receptors initiating transcription. These co-activators harbor a specific 'LXXLL' motif through which they interact with the nuclear receptors. The oncogene Astrocyte elevated gene-1 (AEG-1) is not a co-activator, but it has an 'LXXLL' motif, through which it interacts with RXR and interferes with co-activator recruitment. As such, overexpression of AEG-1 inhibits, while knockout of AEG-1 augments RXR-dependent functions. In vivo, this RXR-inhibitory function of AEG-1 is skewed specifically to PPARα in the liver, so that in a liver-specific AEG-1 transgenic mouse (Alb/AEG-1) PPARα function is inhibited, while in a liver-specific AEG-1 knockout mouse (AEG-1$^{\Delta HEP}$) PPARα function is augmented. PPARα is a master regulator of fatty acid β- oxidation (FAO). In Alb/AEG-1 mouse FAO is inhibited resulting in accumulation of lipids in the liver leading to non-alcoholic steatohepatitis (NASH), while AEG-1$^{\Delta HEP}$ mouse is protected from high fat diet-induced NASH. A hepatocyte targeted nanoparticle delivering AEG-1 siRNA also protects from HFD-induced NASH. NASH is an intermediary step in obesity-induced hepatocellular carcinoma (HCC) and AEG-1 plays a crucial role in both NASH and HCC thereby serving as an important therapeutic target in these disease processes.

Biography:

Devanand Sarkar MBBS, Ph.D. is a Professor of Human and Molecular Genetics and Associate Director for Training and Education at Massey Cancer Center, Virginia Commonwealth University. His research interest is identification of novel genes regulating NASH and HCC, analysis of the function of these genes using complex mouse modeling, and development and evaluation of therapy targeting these genes. Additional key research focus include inflammation, lipid metabolism, RNA binding proteins, regulation of protein translation, next generation sequencing, nanoparticle delivery system and immunotherapy. His research is supported by grants from NCI, NIDDK and DOD.

Frequency and Patterns of Ribonucleotide Incorporation Around Autonomously Replicating Sequences Mark the Division of Labor of Yeast DNA Polymerases

Francesca Storici

School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA

Abstract:

Ribonucleoside monophosphate (rNMP) incorporation in DNA, which occurs across all kingdoms of life, results in DNA structural change and genome instability. Previous studies in yeast showed that the rNMP presence in DNA is strongly induced by misincorporation of replicative DNA polymerases α, δ, and ε, particularly with low-fidelity mutants of these Pols. rNMP presence in DNA is more evident in cells with defects in ribonuclease (RNase) H2, which normally initiates rNMP removal from genomic DNA. Using published rNMP incorporation datasets generated by ribose-seq, emRiboSeq, and RHII-HydEn-seq techniques, we performed a computational analysis of rNMP sites around yeast autonomously replicating sequences (ARSs), where DNA replication starts, and Pols α, δ, and ε synthesize the leading and lagging strands. We analyzed the rNMP incorporation strand biases in both wild-type and RNase H2-mutant libraries. The results show an overall preference of rNMP incorporation on the leading strand in wild-type Pols and Pol ε lowfidelity mutant libraries. Pol α or Pol δ low-fidelity mutant libraries display a preference for rNMP incorporation on the

lagging strand. All the rNMP preferences are reduced around late-firing and low-efficiency ARS's in RNase H2-mutant libraries. Moreover, at the beginning of DNA replication, the leading/lagging-strand ratio of rNMP incorporation increases in wildtype DNA Pols and Pol ε low-fidelity mutant libraries and decreases in Pol δ low-fidelity mutant libraries, which reflects the Pol δ- Pol ε handoff and validates replicative polymerase division of labor in the leading strand synthesis. Moreover, we found the different rNMP incorporation context preferences of different DNA polymerases. Pol δ prefers to incorporate rNMPs after dCMP while Pol ε prefers to incorporate rNMPs after dAMP. Those preferences are strengthened with low-fidelity mutants. Overall, we found the characteristics of rNMP incorporation around ARS's, which are induced by different DNA polymerases, and validate the labor division of DNA polymerases.

This work is supported by NIH, NIEHS R01 ES026243, and Howard Hughes Medical Institute Faculty Scholars Award, HHMI 55108574 to F. Storici.

Insight into Pathological Integrin [α]IIb[β]3 Activation from Safeguarding the Inactive State

Tobias Ulmer

Department of Physiology and Neuroscience, Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA

Abstract:

The inhibition of physiological activation pathways of the platelet adhesion receptor integrin αIIbβ3 may fail to prevent fatal thrombosis, suggesting that the receptor is at risk of activation by yet an unidentified pathway. Here, we report the discovery and characterization of a structural motif that safeguards the receptor by selectively destabilizing its inactive state. At the extracellular membrane border, an overpacked αIIb(W968)-β3(I693) contact prevents αIIb(Gly972) from optimally assembling the αIIbβ3 transmembrane complex, which maintains the inactive state. This destabilization of approximately 1.0 kcal/mol could be mitigated by hydrodynamic forces but not physiological agonists, thereby identifying hydrodynamic forces as pathological activation stimulus. As reproductive life spans are not generally limited by cardiovascular disease, it appears that the evolution of the safeguard was driven by fatal, hydrodynamic force-mediated integrin αIIbβ3 activation in the healthy cardiovascular system. The triggering of the safeguard solely by pathological stimuli achieves an effective increase of the free energy barrier between inactive and active receptor states without incurring an increased risk of bleeding. Thus, integrin αIIbβ3 has evolved an effective way to protect receptor functional states that indicates the availability of a mechanical activation pathway when hydrodynamic forces exceed physiological margins.

IFN γ is Critical for CAR T Cell Mediated Myeloid Activation and Induction of Endogenous Immunity

Darya Alizadeh

Department of Hematology and Hematopoietic Cell Transplantation, City of Hope, Duarte, CA

Abstract:

Chimeric antigen receptor (CAR) T cell therapy has led to encouraging clinical outcomes, especially against hematological malignancies. However, CAR T cell therapy for solid tumors, including brain tumors, faces many challenges, including tumor heterogeneity, a suppressive tumor microenvironment and the lack of T cell persistence. We have developed a syngeneic immunocompetent mouse model, which recapitulates glioma microenvironment (TME) in patients to further comprehend the impact of tumor-promoting immunosuppressive networks on CAR T cell therapies. Analogous to the human IL13Rα2-CAR being evaluated in our clinical trials, murine IL13Rα2-CAR T cells (mIL13Rα2-CAR T) are composed of a mouse IL-13

tumor-targeting domain, murine 4-1BB and CD3ζ signaling domains. The mIL13Rα2-CAR T cells efficiently target IL13Rα2+ murine gliomas in vitro and in vivo. Characterization of the tumor microenvironment post-CAR T therapy reveals activation of endogenous immune cells such as cytotoxic CD8 T and myeloid cells. IFNy production by CAR T cells and IFNy-responsiveness of host immune cells is critical for tumor immune landscape remodeling to promote a more activated and less suppressive tumor microenvironment. The clinical relevance of these observations is supported by studies showing that human IL13Rα2-CAR T cells activate patient-derived endogenous T cells and monocyte/macrophages through IFNy-signaling, as well as induce the generation of tumor-specific T cell responses in a responding patient with GBM. These studies establish that CAR T therapy has the potential to shape the tumor microenvironment, creating a context permissible for eliciting endogenous antitumor immunity.

Biography:

Dr. Darya Alizadeh is an Assistant Research Professor in the Department of Hematology & Hematopoietic Cell Transplantation and a member of the CAR T therapy program at City of Hope. Dr. Alizadeh received her Ph.D. from University of Arizona and completed her postdoctoral studies at City of Hope. Dr. Alizadeh's expertise is in immunotherapy and understanding the immunosuppressive tumor microenvironment in solid tumors. Her research focuses on elucidating the interplay between CAR T cells and endogenous immune cells in brain tumors. Her primary efforts involve developing strategies to enhance CAR T cell function for solid tumors such as glioblastoma.

What microRNAs Could Tell us About the Human X Chromosome

Chiara Siniscalchi

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Italy

Abstract:

MicroRNAs (miRNA) are small non-coding RNAs endowed with great regulatory power, thus playing key roles not only in almost all physiological pathways, but also in the pathogenesis of several diseases. Genomic distribution analysis revealed the highest density of miRNA sequences on the X chromosome; this feature is evolutionary conserved in mammalians and equips females with a larger miRNA machinery than males. However, miRNAs contribution to some X-related conditions, properties or functions is still poorly explored. We analyzed the literature and databases about X-linked miRNAs, trying to understand how miRNAs could contribute to emerging gender-biased functions and pathological mechanisms, such as immunity and cancer. A fine map of miRNA sequences on the X chromosome was reported; in addition, bioinformatics functional analyses of the whole X-linked miRNA targetome (predicted and validated) were performed, relating it to different biological pathways, mainly cancer and cell cycle control but also immunity.

The emerging scenario points to different gaps in the knowledge that should be filled with future experimental investigations, also in terms of possible implications and pathological perspectives for X chromosome aneuploidy syndromes, such as Turner and Klinefelter syndromes.

Biography:

Chiara Siniscalchi is a Ph.D. student in Molecular Life Sciences working in Molecular Biology Laboratory, under the supervision of Professors Nicoletta Potenza and Aniello Russo. During the last two years she has been dedicated to investigating the role of microRNAs and lncRNAs in different pathological pathways, mainly cancer that led the group to publish five papers; however, her main research is focused on microRNAs involved in X-chromosome aneuploidy syndromes.

Molecular Recognition of Sugar Binding in the Melibiose Permease MelB

Lan Guan

Center for Membrane Protein Research, Texas Tech University Health Sciences Center, Lubbock, TX

Abstract:

The melibiose transporter MelB is a well-studied symporter of the major facilitator superfamily (MFS) of transporters, and its mammalian homologues play important roles in the delivery of essential omega-3 fatty acids into the brain and eyes. MelB catalyzes an obligatory cotransport (symport) of galactosides and monovalent cations (H+, Li+, or Na+). Recent studies on thermodynamic ligand binding showed that the symport mechanism is the cooperative binding of sugars and cations. 3-D crystal structure of Salmonella typhimurium MelB (MelBSt) with bound sugar analogs determined from a uniporter mutant carrying a compromised cation site reveals a Na+-free, galactoside-bound binary complex of MelB. Combined with a large body of functional data, it can be concluded that both of the N- and C-terminal 6-helix bundles are likely engaged in forming the sugar- and cation-specificity determinant pockets. The sugar specificity is recognized by a salt-bridge/hydrogen-bonding network and aromatic-stacking interactions, and the polar contacts mainly exist within the galactosyl moiety. An important finding is the close connection between the sugar- and cation-specific binding sites, which lays a solid foundation for our understanding of the cooperative binding mechanism, which is the core in cotransport.

Biography:

Lan Guan, M.D., Ph.D., Professor, Vice Chair, and Incoming Interim Chair of the Department of Cell Physiology and Molecular Biophysics, and Director of the Center for Membrane Protein Research at Texas Tech University Health Sciences Center, School of Medicine, Lubbock, Texas. Her laboratory focuses on cation-coupled nutrient cotransporters--their structures and mechanisms using x-ray crystallography, thermodynamics, and other approaches. Her laboratory determined the first 3-D high-resolution crystal structure of a Na+-coupled major facilitator superfamily transporter. She published more than 88 peer-reviewed research articles, reviews, and book chapters. Her work is currently funded by NIH Grants.

Activation of the Antiviral Factor RNase L Promotes Translation Outside Coding Sequences

Nicholas R. Guydosh[1]*, Agnes Karasik[1,2] and Grant D. Jones[1]

[1]National Institute of Diabetes and Digestive and Kidney Diseases/NIH, Bethesda, MD
[2]National Institute of General Medical Sciences/NIH, Bethesda, MD

Abstract:

Viral infection can trigger the activation of a ribonuclease, RNase L, which plays an important role in the innate immune response. The activated form of RNase L cleaves single stranded regions of viral and host RNAs and this activity is thought to promote clearance of the virus and apoptosis. Widespread cleavage of messenger RNAs (mRNA) leads to broad changes in their abundance but it remains unknown how this reduced pool of mRNAs is translated. Activation of RNase L was previously shown to trigger translation in the 3' untranslated regions (UTRs) of mRNAs in cell lysates. To further investigate this phenomenon in cells, we performed ribosome profiling experiments on RNase L activated cells. We found that RNase L activation leads to substantial accumulation of ribosomes in 3'UTRs and in other non-coding regions, such as 5' UTRs and alternate reading frames within protein coding regions. We also verified that ribosomes were actively translating in non-coding regions of mRNA using in vitro biochemical assays and by computationally dissecting positions of ribosomes. Analysis of published ribosome profiling data on viral infected cells showed that these unconventional translation events also occur during viral infections. Since translation of non-coding regions of mRNAs was dependent on the catalytic activity of RNase L, we favor a model where cleavage of mRNA by RNase L leads to the translation of mRNA fragments. While the function of the synthesized cryptic peptides is unknown, we propose that they may have antiviral roles.

Biography:

As an Investigator at NIDDK/NIH, Dr. Nicholas Guydosh leads a research group focused on understanding how regulation of translation affects gene expression, particularly in response to cellular stress and viral infection. He received his Ph.D. in 2009 from Stanford University and did postdoctoral training at Johns Hopkins University. Recent work from the Guydosh Lab has shown how the cell efficiently recycles ribosomes after termination and detects collisions between ribosomes.

Deamidation Shunts RelA from Mediating Inflammatory Response to Aerobic Glycolysis

Pinghui Feng

Section of Infection and Immunity, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA

Abstract:

Cell proliferation and inflammation are two metabolically demanding biological processes. How these competing processes are selectively executed remains unknown. Here, we report that a trifunctional enzyme containing activity of carbamoyl phosphate synthetase, aspartyl transcarbamoylase and dihydroorotase (CAD), deamidates the RelA subunit to promote cell proliferation via inactivating NF-κ B and activating aerobic glycolysis. CAD catalyzes the first three steps of the de novo pyrimidine synthesis, with the first being rate-limiting. We show that deamidation switches RelA from the expression of NF-κ B-responsive genes to that of glycolytic enzymes, thus shunting inflammatory response to aerobic glycolysis. Enabled by CAD-mediated RelA deamidation, NF-κ B inactivation and aerobic glycolysis are necessary for cell proliferation. Profiling human cancer cells with inhibitors of key glycolytic enzymes substantiates the pivotal role of RelA deamidation, driven by either high CAD expression or natural RelA mutations, in tumorigenesis. This work illuminates a mechanism by which protein deamidation selectively specifies gene expression and consequent biological processes, thus expanding the functional repertoire of protein deamidation and forging molecular link between innate immune response and metabolism.

Angiopoietin-2-induced Lymphatic Endothelial Cell Migration Drives Lymphangiogenesis Via the β1 Integrin-RhoA-Formin Axis

Racheal G. Akwii

Texas Tech University Health Sciences Center, Amarillo, TX

Abstract:

Lymphangiogenesis is an essential physiological process but also a determining factor in vascular-related pathological conditions. Angiopoietin 2 (Ang2) plays an important role in lymphatic vascular development and function and its upregulation has been reported in several vascular-related diseases, including cancer. Given the established role of the small GTPase RhoA on cytoskeleton-dependent endothelial functions, we investigated the relationship between RhoA and Ang2-induced cellular activities. This study shows that Ang2-driven human dermal lymphatic endothelial cell (HDLEC) migration depends on RhoA. We demonstrate that Ang2-induced migration is independent of the Tie receptors, but dependent on β1 integrin-mediated RhoA activation with knockdown, pharmacological approaches, and protein sequencing experiments. Although the key proteins downstream of RhoA, Rho kinase (ROCK) and myosin light chain (MLC), were activated, blockade of ROCK did not abrogate the Ang2-driven migratory effect. However, formins, an alternative target of RhoA, were identified as key players, and especially FHOD1. The Ang2-RhoA relationship was explored in vivo, where lymphatic endothelial RhoA deficiency blocked Ang2-induced lymphangiogenesis, highlighting RhoA as an important target for anti-lymphangiogenic treatments.

Biography:

Ms. Racheal G. Akwii is a Ph.D. candidate in the lab of Dr. Constantinos Mikelis with the Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center. Her research involves vascular biology and cancer research, understanding the cellular and molecular mechanisms regulating the vascular in normal and disease conditions, especially cancer.

Evolving Genetic Code Expansion: Next Generation Technologies for Revealing Molecular O-GlcNAc Modification of Nuclear Pore Complex Accelerates Bidirectional Transport

Tae Yeon Yoo

Department of Systems Biology, Blavatnik Institute, Harvard Medical School, Boston, MA

Abstract:

Macromolecular transport across the nuclear envelope depends on facilitated diffusion through nuclear pore complexes (NPCs). The interior of NPCs contains a permeability barrier made of phenylalanine-glycine (FG) repeat domains that selectively facilitates the permeation of cargoes bound to nuclear transport receptors (NTRs). FG-repeat domains in NPCs are a major site of O-linked N-acetylglucosamine (O-GlcNAc) modification, but the functional role of this modification in nucleocytoplasmic transport is unclear. We developed high-throughput assays based on optogenetic probes to quantify the kinetics of nuclear import and export in living human cells. We found that increasing O-GlcNAc modification of the NPC accelerated NTR-facilitated transport of proteins in both directions, and decreasing modification slowed transport. Superresolution imaging revealed strong enrichment of O-GlcNAc at the FG-repeat barrier. O-GlcNAc modification also accelerated passive permeation of a small, inert protein through NPCs. We conclude that O-GlcNAc modification accelerates nucleocytoplasmic transport by enhancing the nonspecific permeability of the FG-repeat barrier, perhaps by steric inhibition of interactions between FG repeats.

Biography:

Tae Yeon Yoo is a postdoctoral fellow in the laboratory of Timothy Mitchison at Harvard Medical School. He is studying biophysics of nucleocytoplasmic transport using quantitative microscopy assays and molecular probes. Dr. Yoo received his BA in Physics from the University of Chicago and Ph.D. in Applied Physics from Harvard University.

Tuning Insulin Sensitivity by Macrophage-produced Exosomal miRNAs

Wei Ying

Division of Endocrinology & Metabolism, Department of Medicine, University of California, San Diego, CA

Abstract:

Insulin resistance is a major pathophysiologic defect in T2DM/obesity. Weight loss is difficult to maintain and pharmacologic treatments are limited. Anti-inflammatory M2-like macrophages are important in maintaining metabolic homeostasis. Here, we show that M2 polarized bone marrow-derived macrophages (BMDMs) secrete miRNA-containing exosomes (Exos), which improve glucose tolerance and insulin sensitivity when given to obese mice. Depletion of miRNA cargoes blocks the ability of M2 BMDM Exos to enhance insulin sensitivity. We find that miR-690 is highly expressed in M2 macrophage Exos and functions as an insulin sensitizer both in vivo and in vitro. Expressing a miR-690 mimic in miRNA-depleted BMDMs generates Exos

that recapitulate the effects of M2 macrophage Exos on metabolic phenotypes. Nadk is a bona fide target mRNA of miR-690, and Nadk plays a role in modulating macrophage inflammation and insulin signaling. Taken together, these data suggest miR-690 could be a new therapeutic insulin-sensitizing agent for metabolic disease.

Biography:

Dr. Wei Ying received Ph.D. from Texas A&M University and postdoc training with Dr. Jerry Olefsky. Currently he is an Assistant Professor in UCSD. His research mainly focuses on the extracellular RNAmediated inter-organ crosstalk and the cell-specific extracellular RNAs as the biomarkers predicting obesity/Type 2 diabetes-associated metabolic disorders.

GAS5/miR-21 Axis as a Potential Target to Rescue ZCL-082-Induced Autophagy of Female Germline Stem Cells *In Vitro*

Xiaopeng Hu

Shanghai Jiao Tong University, China

Abstract:

The use of small chemical compounds may be a good approach to further investigate the process and mechanism of autophagy in female germline stem cells (FGSCs) development. In this study, we used ZCL-082, a derivative of benzoxaboroles, to treat FGSCs. Using a cell counting kit-8 (CCK8) and 5-ethynyl-2 '-deoxyuri-dine (EdU) assays, we found that ZCL-082 could significantly reduce the viability, proliferation, and number of FGSCs in vitro. Moreover, western blotting revealed that the expression of light chain3 beta2 (LC3B-II) in FGSCs was significantly increased after treatment with ZCL-082 for 3 and 6 h. Meanwhile, the expression of sequestosome-1 (SQSTM1) was significantly decreased. These results suggested that ZCL-082 can induce autophagy of FGSCs in vitro. Regarding the molecular mechanism, ZCL-082 could significantly reduce the expression of growth arrest-specific 5 (GAS5) long non-coding RNA, which could directly bind to microRNA-21a (miR-21a) and negatively regulate each other in FGSCs. Additionally, overexpression of miR-21a significantly enhanced LC3B-II protein expression while significantly reducing the expression of programmed cell death protein 4 (PDCD4) and SQSTM1 protein in FGSCs compared with control cells. The inhibition of miR-21a significantly reduced the basal or ZCL-082-induced upregulated expression of LC3B-II, and it significantly enhanced the expression of PDCD4 while downregulating the basal or ZCL-082-induced expression of SQSTM1 in FGSCs. Taken together, these results suggested that ZCL-082 induced autophagy through GAS5 functioning as a competing endogenous RNA (ceRNA) sponge for miR-21a in FGSCs.

Biography:

Xiaopeng Hu, Assistant researcher of Bio-X Institutes, Shanghai Jiao Tong University, Shanghai 200240, China. Research Interests: The higher structure of Chromatin, histone modification, and RNA modification of differentiation from mammalian pluripotent stem cells or germline stem cells to sperms or oocytes; Exploring the key signals and molecules which regulate spermatogenesis and oogenesis.

Novel Insights into the Regulation of Pancreas Development and Function by the Imprinted *Igf2* Gene

Ionel Sandovici

Metabolic Research Laboratories, MRC Metabolic Diseases Unit, Department of Obstetrics & Gynaecology, University of Cambridge, UK

Abstract:

Imprinted genes, which are expressed in a parent-of-origin-specific manner, are known to have important roles in development, growth and metabolism. However, our knowledge regarding their roles in the control of pancreatic growth and function remains limited. We recently found that many imprinted genes are highly expressed in pancreatic mesenchyme-derived cells and explored the role of the paternally-expressed insulin-like growth factor 2 (Igf2) gene in mesenchymal and epithelial pancreatic lineages using a newly developed conditional Igf2 mouse model and lineage-specific Cre mice. Mesenchyme-specific Igf2 deletion resulted in acinar and β-cell hypoplasia, postnatal whole-body growth restriction and maternal glucose intolerance during pregnancy, suggesting that the mesenchyme is a developmental reservoir of IGF2 used for paracrine signalling. Additionally, primary acinar cells exposed ex-vivo to exogenous IGF2 activated AKT, a key signalling node, and increased their number and amylase production. Igf2 deletions in the developing pancreatic

epithelium or in pancreatic β-cells did not show any discernible growth or functional phenotypes. However, females lacking Igf2 in pancreatic β-cells exhibited failed metabolic adaptations to pregnancy, high-fat diet feeding or congenital leptin deficiency. Based on these findings, we propose that mesenchymal-derived IGF2 is a key developmental regulator of adult pancreas size and function, and that autocrine actions of IGF2 in the β-cell during early development determine their adaptive capacity in adult life.

Biography:

Ionel Sandovici received his MD and Ph.D. degrees from University of Medicine and Pharmacy 'Gr. T. Popa', Iasi, Romania. He was trained in the laboratories of Carmen Sapienza (Temple University, Philadelphia, USA) and Miguel Constância and Wolf Reik (Babraham Institute, Cambridge, UK). He is now a research associate in the laboratory of Miguel Constância at the Institute of Metabolic Science and the Department of Obstetrics and Gynaecology, University of Cambridge, UK, where he studies the role of imprinted genes in development and metabolism, as well as the links between environment and epigenetic regulation of gene activity.

Self-organization and Culture of Mesenchymal Stem Cell Spheroids in Acoustic Levitation

Lousineh Arakelian

Unite de Therapie Cellulaire, Hopital Saint-Louis, Assistance Publique - Hopitaux de Paris; Universite de Paris, Inserm U976 et CIC de Biotherapies CBT501, France

Abstract:

In recent years, 3D cell culture models such as spheroid or organoid technologies have known important developments. These models have shown closer properties to physiological conditions compared to classic 2D cultures on plastic and are therefore considered as important tools for in-vitro, as well as in-vivo applications. However, a reliable use of 3D cell models still requires standardized protocols with well-controlled and reproducible parameters, which are not fully achieved yet. To address this challenge, we hereby propose a robust and scaffold-free approach, based on multi-trap acoustic levitation. We successfully applied this technology to Mesenchymal Stem Cells (MSCs), maintained in acoustic levitation over a 24-h period. During the culture, MSCs spontaneously self-organized from cell sheets to spheroids within about 10 h. Each acoustofluidic chip contained up to 30 spheroids and four chips could be run in parallel, leading to the production of 120 spheroids per experiment. Various biological characterizations showed that the cells within the spheroids were viable, maintained the expression of their cell surface markers and had a higher osteogenic and adipogenic differentiation capacity compared to standard 2D culture conditions. This proof of concept and these encouraging results pave the path to long-time cell culture in acoustic levitation, for obtaining cell sheets, spheroids or organoids of different cell types.

Biography:

Lousineh Arakelian is a biologist who is working at the Saint-Louis Hospital Cell Therapy Unit (AP-HP) and INSERM U976-Stem Cell Biotechnologies team since 2014, under the supervision of Pr. Jérôme Larghero. After completing her Master's degree in cell and molecular biology and cancer research, she moved to her current laboratory where she has been working on several cell therapy and tissue engineering topics, involving different organs such as the heart, esophagus, trachea, liver, as well as new cell culture methods including the acoustic levitation. She is currently completing her Ph.D, at Université de Paris, supervised by Pr. Larghero.

Rabbit Pluripotent Stem Cells: Why and How to Produce Them?

Marielle Afanassieff

Stem cell and Brain Research Institute, University of Lyon, INSERM U1208, France

Abstract:

Pluripotent stem cells (PSCs) possess two main properties: self-renewal and pluripotency. Self-renewal is defined as the ability to proliferate in an undifferentiated state and pluripotency as the capacity to differentiate into cells of the three germ layers: ectoderm, mesoderm, and endoderm. PSCs are derived from early embryos as embryonic stem cells (ESCs) or are produced by reprogramming somatic cells into induced pluripotent stem cells (iPSCs). In mice, PSCs can be stabilized into two states of pluripotency: naive and primed. Naive and primed PSCs notably differ by their ability to colonize a host blastocyst to produce germline competent chimeras; hence, naive PSCs are valuable for transgenesis, whereas primed PSCs are not. Thanks to its physiological and developmental peculiarities similar to those of primates, the rabbit is an interesting animal model for studying human diseases and early embryonic development. Both ESCs and iPSCs have been described in rabbits. They self-renew in the primed state of pluripotency and therefore, cannot be used for transgenesis. The presentation will review the interest of rabbit PSCs, the available data on their pluripotent state and their chimeric ability, the methods developed to improve their capacity to produce germline competent chimeras, and the possible alternatives to exploit them for transgenesis.

Biography:

Marielle Afanassieff has been working on the creation of animal models for human diseases at the French National Institute for Agriculture, Food and Environment since 1992. She is part of a team that studies the molecular and cellular mechanisms responsible for pluripotency of embryonic stem cells in mammals, and has been interested in the rabbit model since 2004. Her work aims to define the signaling pathways, chromatin modifiers and cell cycle regulators that support naïve-state pluripotency in rabbits. She is exploring how these factors can be manipulated to generate embryonic or induced pluripotent stem cells capable of efficiently colonizing host blastocysts.

Unraveling the Origin and Impact of Extrachromosomal Circular DNA on Eukaryotic Genomes

Sam Keating

Section for Ecology and Evolution, Department of Biology, University of Copenhagen, Denmark

Abstract:

Insight into genetic diseases, cancer and evolution relies on our understanding of genetic variation created by mutations. First discovered in childhood tumors, extrachromosomal circular DNA (eccDNA) carrying proto-oncogenes such as MYC and EGFR have since proven to be abundant in cancer. We and others have shown that eccDNA can form from all parts of the genomes of yeast, worms, birds, mice and humans in germline and somatic tissue, ranging in size from a few hundred base pairs to hundreds of thousands of base pairs. Our findings suggest that eccDNA contributes substantially to genetic variation and raises important questions about how eccDNA is formed, maintained and influences the phenotypes of eukaryotic organisms. In human somatic cells, gene-rich chromosomes contribute to more eccDNAs per megabase and the most transcribed protein-coding gene provides the most eccDNAs per gene. While most eccDNAs are unique to the cell they are formed in, some are identified recurrently in several cell lines and individuals, indicating the existence of genomic hotspots for eccDNA formation. Selection for eccDNAs in tumors and yeast can provide transient selective advantages for the cells carrying them and the selective advantages can become permanent when eccDNA reintegrates into chromosomes. We find that eccDNA integrates at a rate that is six fold higher than translocations, suggesting that eccDNA are important intermediates in structural variation such as insertions and gene amplifications. Thus, genomes are rich in chromosome-

derived eccDNAs that might influence phenotypes and genotypes through altered gene copy numbers and transcription of full-length or truncated genes.

Biography:

Sam Keating is a cell biologist and postdoctoral researcher at the University of Copenhagen. Since receiving his Ph.D. from Monash University in 2014, Sam has undertaken postdoctoral training in clinical research at the Folkhälsan Research Center in Helsinki and subsequently at the Department of Internal Medicine at RadboudUMC in the Netherlands. Sam's research has contributed to the understanding of gene regulation in health and disease, identifying novel epigenetic mechanisms involved in cellular memory. He is a coordinator of the Horizon 2020-funded Circular Vision project, which aims to develop new technologies for exploring circular DNA in medicine.

Mesenchymal Stem Cells in Diabetes mellitus Treatment-Several Weapons for One Target

Mohamed Kamal

Pharmacology and Biochemistry Department, Faculty of Pharmacy, The British University in Egypt (BUE), Cairo, Egypt

Abstract:

Diabetes mellitus (DM) is a terribly growing epidemic, currently affecting about 463 million people worldwide, with expected rise to 700 million by the year 2045. Regenerative medicine and stem cell therapy opened new avenues and ignited much hope for patients with DM over the past few years. Several MSCs sources have been proven beneficial in generating insulin producing cells in vitro, treatment of diabetes in vivo and even proven effective and safe in clinical trials. Among these MSCs, Wharton's jelly MSCs are isolated from the umbilical cord tissue. These WJ-MSCs provide several advantages over other MSCs. They have excellent culture properties, they possess immunomodulation properties, and they can generate insulin producing cells. Moreover, they were proven safe and effective in several clinical trials and have shown superior therapeutic efficacy compared to their counterpart; umbilical cord blood MSCs in clinical trials for treatment of diabetes mellitus. Another emerging source is adipose mesenchymal stem cells (Ad-MSCs), which comes with full advantages of MSCs with ease of isolation and suitability for autologous applications. However, some challenges need to be overcome in the clinical practice of MSCs; these include safety issues, generation of cost-effective "clinical grade" cells, as well as route and dosage of administration. Overcoming these challenges will undoubtedly sharpen our weapons in our battle against DM.

Biography:

Dr. Mohamed Kamal, Associate Professor of Biochemistry obtained his Ph.D. in 2011 on glioblastoma cancer stem cells, during which, he joined Majumder's lab in M.D. Anderson Cancer Center, Texas, USA 2008-2010 as Joint Supervision scholarship from Egyptian Government and as a Fulbright postdoctoral fellow 10/2014 – 7/2015. In 2017, he joined the British University in Egypt (BUE) and currently He is director of Center of Drug Research and Development (CDRD) in BUE. In BUE, he managed to initiate line of stem cells and diabetes research. He has authored 21 Scopus indexed publications and peer-reviewer in several journals.

Contrasting Roles of S6K1 and S6K2 in Breast Cancer

Alakananda Basu

Department of Microbiology, Immunology & Genetics, UNT Health Science Center, Fort Worth, TX

Abstract:

Breast cancer is the second leading cause of cancer-related death in women in the United States. The mechanistic target of rapamycin (mTOR), which acts downstream of the phosphatidylinositol-3 kinase (PI3K)/Akt, is frequently deregulated in breast cancer and is an important target for breast cancer therapy. mTOR inhibitors are, however, of limited success, due to feedback activation of survival pathways. The 40S ribosomal protein S6 kinase (S6K) acts downstream of mTOR and exists as two homologs, S6K1 or p70S6K1 and S6K2. Our analysis of The Cancer Genome Atlas database revealed that S6K2 but not S6K1 is overexpressed in breast cancer. This observation was corroborated by immunohistochemistry of human breast tissues. Silencing of S6K1 and S6K2 had opposite effects on Akt phosphorylation and breast cancer cell survival. Inhibition/depletion of S6K2, but not S6K1, caused a substantial increase in the sensitivity of several breast cancer cells to chemotherapeutic agents whereas overexpression of S6K2 protected against cell death. Depletion of S6K2, but not S6K1, decreased the levels of anti-apoptotic Bcl-2 family proteins. S6K2 appears to promote breast cancer cell survival by engaging multiple signaling pathways, including Akt, c-Jun -terminal kinase (JNK) and estrogen receptor-α (ERα). Thus, targeting S6K2 rather than mTOR or S6K1 is expected to be an effective therapeutic strategy for the treatment of breast cancer.

Biography:

Alakananda Basu, Ph.D. is a professor of Microbiology, Immunology & Genetics at the University of North Texas Health Science Center. She received her Ph.D. in Biochemistry and postdoctoral training in Cancer Biology & Experimental Therapeutics from the University of Pittsburgh School of Medicine. She served as the Program Director of the Cancer Biology program. The primary focus of her research is in signal transduction, especially in the context of cancer therapy. She has been studying how various signaling pathways regulate apoptosis, autophagy and senescence. The ultimate goal of her research is to exploit intracellular signaling systems to benefit cancer therapy.

Characterizing Mechanistic Improvements of Epigenetic PROTAC Degradation Compounds

Sarah D. Mahan*, Kristin M. Riching, James Vasta, Matt Robers, Danette L. Daniels, and Marjeta Urh

Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711

Abstract

Targeted protein degradation is a promising new therapeutic strategy consisting of small molecules, most commonly molecular glues or Proteolysis Targeting Chimeras (PROTACs), which elicit degradation of a target protein. These compounds function to bring together the target protein with an E3 ligase complex component, inducing formation of a ternary complex which serves to ubiquitinate and degrade the target protein via the proteasomal pathway. Significant challenges persist to characterize the cellular mechanism of action and the highly dynamic degradation responses induced by these compounds. Here we demonstrate the use of a live-cell, luminescence-based approach, combined with CRISPR/Cas9 endogenous tagging, to assess key mechanistic differences between epigenetic PROTAC compounds dBET1 and dBET6. Tagging of BET family proteins with the small peptide, HiBiT, which has high affinity for and can complement with the LgBiT protein to produce NanoBiT luminescence, allows for sensitive detection of endogenous protein levels in living cells and can also serve as a BRET energy donor to study protein:protein or protein:small molecule interactions required for successful degradation. We demonstrate the ability to quantitate key degradation parameters for compound triaging and ranking including rate, Dmax, and Dmax50. We further interrogate mechanism by monitoring the kinetics of induced ternary complex formation and target ubiquitination.

Together, these approaches expand the capabilities for understanding the impacts of chemical design on degrader efficacy in live cells.

Biography

Sarah Mahan is a Research Scientist in the Functional Proteomics group at Promega Corporation in Madison, Wisconsin. In her 8 years at Promega, she has spent the last 5 years developing cell-based technologies focused on enabling advancement in the area of targeted protein degradation.

What Triggers the Z-ring Formation in *Escherichia coli*?

Jaan Mannik

Department of Physics and Astronomy, The University of Tennessee, Knoxville, TN

Abstract:

How replication and division processes are coordinated in the cell cycle is a fundamental yet poorly understood question in cell biology. Previous studies in *Escherichia coli* have supported a range of conclusions, from one extreme where these processes are tightly linked to another extreme where these processes are completely independent. Using high-throughput microscopy and cell-cycle modeling, we show that in slowly-growing cells, the replication and division processes are strongly correlated, indicating a significant coupling between them. This coupling weakens when the growth-rate increases. Our data suggest that in slow-growth conditions, the unreplicated chromosome blocks the onset of constriction at mid-cell. We show that the nucleoid-occlusion protein SlmA does not play a role in this process and neither do other known factors involved in positioning bacterial Z-ring relative to the chromosome. Altogether this work reconciles various existing ideas and shows a growth-rate dependence of replication-related control over cell division.

Biography:

Dr. Jaan Männik is an Associate Professor in Biophysics at the University of Tennessee. He received his Ph.D. in Physics from Stony Brook University for work on Josephson junction qubits. He switched to biophysics while doing a postdoc at the Delft University of Technology. His current work focuses on how cell division proteins and DNA self-organize in *E. coli* and how these key macromolecular structures coordinate with each other to implement the cell cycle. This research combines high throughput optical microscopy, microfluidics, molecular biology techniques, and computer modeling.

Delivery of Functional Lysosomal Transport Proteins Via Microvesicles Derived from Baculovirus-Infected Spodoptera Cells to Cultured Fibroblasts and *Ex Vivo* Rabbit Cornea

Jess G. Thoene

Department of Pediatrics, Division of Pediatric Genetics, Metabolism & Genomic Medicine, University of Michigan, Ann Arbor, MI

Abstract:

Transmembrane proteins irreversibly denature when separated from their native lipid membrane. Thoene J, et. al. showed (Mol Genet Metab. 2013 May;109(1):77-85) that the lysosomal transmembrane transporters cystinosin and sialin, defective in cystinosis and ISSD respectively, when expressed via the baculovirus/Spodoptera method, are released during the lytic phase of infection in ~100 nm microvesicles, and deliver functional transporter to lysosomes of cultured human fibroblasts lacking transport activity. Functionality is demonstrated by depletion of stored lysosomal cystine or sialic acid from cystinosin- or sialin-deficient

recipient fibroblasts. LC/MS/MS spectrometry of the vesicles identified insect proteins including moesin, a member of the ERM family which cross-link cytoskeleton and plasma membrane. Human cystinosin was specifically identified in the baculovirus-infected insect microvesicles. Cystinosis is characterized by lysosomal cystine accumulation in all tissues, progressive renal failure, growth failure, and a crystalline cystine keratopathy that causes severe photophobia and foreign body sensation. *Ex vivo* wt NZW rabbit ocular globes incubated at 37 °C in tissue culture medium supplemented with 3 x 1011/ml cystinosinGFP microvesicles display punctate GFP fluorescence consistent with delivery of the cloned protein into the cornea. The GFP fluorescence increases with incubation time, and corneal penetration approaches half the corneal thickness. Use of cystinosin-containing microvesicles as eye drops would represent a significant advance in the treatment of the ocular manifestations of cystinosis, since the drops could be administered as infrequently as once per week. The vesicles may also be effective in delivery of therapeutic proteins to other cell organelles and plasma membrane. US Patent 9,023,798.

Biography:

Dr. Jess Thoene is currently an Active Professor Emeritus at the University of Michigan. From 2000-2006 he was Director of the Hayward Human Genetics Center at Tulane University School of Medicine. He is past Chairman of the Board of Directors of The National Organization for Rare Disorders. He chaired the National Commission on Orphan Diseases, has authored over 100 articles in the peer-reviewed literature on inborn errors of metabolism, published 3 medical reference texts, and holds six U.S. patents. He is certified in Pediatrics and Clinical Biochemical Genetics. He is a member of the American Society of Clinical Investigation.

Zebrafish Cre/lox Conditional Gene Alleles Generated by CRISPR/Cas9 Precision Targeted Integration

Maura McGrail

Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA

Abstract:

The Cre/lox system is widely used for performing cell and tissue-specific conditional analysis of gene function. We have applied our CRISPR/Cas9 directed targeted integration strategy, GeneWeld, to isolate floxed conditional alleles that provide robust gene knockdown and strong loss of function phenotypes. A universal targeting vector, UFlip, containing a floxed mRFP gene trap plus secondary reporter cassette was integrated into an intron in hdac1, rbbp4, and rb1. Active, gene off orientation hdac1-UFlip-Off and rb1-UFlip-Off integration alleles results in >99% reduction of gene expression in homozygotes and recapitulates known indel loss of function phenotypes. Passive, gene on orientation rbbp4-UFlip-On and rb1-UFlip-On integration alleles do not cause phenotypes in trans-heterozygous combination with an indel mutation. Introduction of Cre recombinase by injection leads to recombination at alternating pairs of loxP and lox2272 sites, flipping and locking the cassette into the active, gene off orientation, which again leads to expected mutant phenotypes in combination with a loss of function allele. In combination with our endogenous Cre driver lines expressed in neural progenitor and committed neural cells, we demonstrate rbbp4-UFlip-On and rb1-UFlip-On gene inactivation phenotypes can be restricted to specific neural cell populations. Replacement of the mRFP primary reporter gene trap with a 2A-RFP in rbbp4-UFlip-Off, or 2A-KalTA4 in rb1-UFlip-Off, shows strong expression of RFP in wild type or UAS:RFP injected embryos, respectively. Together these results validate our approach for efficient isolation of Cre/lox responsive conditional gene alleles in order to advance zebrafish Cre recombinase genetics.

Biography:

Dr. McGrail, Associate Professor of Genetics, Development and Cell Biology, is a zebrafish developmental geneticist and genome engineer. Her research includes developing somatic mutagenesis strategies using transposons and genome editing nucleases. Current efforts are focused on CRISPR precision gene editing to advance zebrafish Cre recombinase genetics for conditional gene studies. These tools are being used to build novel genetic models of brain development and neuroinflammation.

Mitochondria-Associated Degradation Pathway (MAD) Function beyond the Outer Membrane

Pin-Chao Liao

Department of Pathology and Cell Biology, Columbia University, New York, NY

Abstract:

The mitochondria-associated degradation pathway (MAD) mediates ubiquitination and degradation of mitochondrial outer membrane (MOM) proteins by the proteasome. We find that the MAD, but not other quality control pathways including macroautophagy, mitophagy, or mitochondrial chaperones and proteases, is critical for yeast cellular fitness under conditions of chronic, low-level oxidative stress in mitochondria produced by treatment with paraquat (PQ). Specifically, inhibition of the MAD increases PQ-induced defects in growth and mitochondrial quality and decreases chronological lifespan. We used mass spectrometry analysis to identify possible MAD substrates as mitochondrial proteins that exhibit increased ubiquitination in response to PQ treatment and inhibition of the MAD. We identified candidate substrates in the mitochondrial matrix and inner membrane and confirmed that two matrix proteins are MAD substrates. These MAD substrates retro-translocate from matrix to cytosol, which is ATP-dependent but membrane potential-independent. Our studies reveal a broader function for the MAD in mitochondrial protein surveillance beyond the MOM and a major role for the MAD in cellular and mitochondrial fitness in a model for aging based on mitochondrial oxidative stress.

Biography:

Dr. Pin-Chao Liao's research interests include mitochondrial quality control in aging and diseases. His Ph.D. training with Dr. Peter Hollenbeck at Purdue University focused on mitochondrial transport and quality control in Drosophila neurons. His postdoctoral training with Dr. Liza Pon at Columbia University focuses on mitochondrial proteostasis in yeast. They identified a novel function for a mitochondrial quality control pathway, Mitochondria Associated Degradation (MAD). He will start his own lab at National Tsing Hua University in Taiwan in 2022 and will continue to study MAD and other mitochondrial quality control pathways in health, disease and aging in yeast and Drosophila.

B Cell Engagement with HIV-1 Founder Virus Envelope Predicts Development of Broadly Neutralizing Antibodies

Samantha Townsley

U.S. Military HIV Research Program, Center of Infectious Disease Research, Walter Reed Army Institute of Research, Silver Spring, MD

Abstract:

Determining which immunological mechanisms contribute to the development of neutralization breadth during HIV-1 infection is a major goal to inform vaccine design. Using samples from a longitudinal HIV-1 acute infection cohort, we identified 16 individuals who were able to neutralize >70% of a multi-subtype panel of 34 viruses (broad neutralizers) and 12 individuals not able to neutralize >35% after 3 years of infection (non-broad neutralizers). B cell populations that recognize HIV-1 founder envelope glycoproteins (founder Env) and total B cell populations were phenotyped at pre-infection, peak viral load (day 11-18), 1 month (day 30-43), and peak breadth time points (day 391-2115). Broad neutralizers maintained reduced peripheral B cell counts throughout infection starting at 1 month ($p=0.02$) compared to non-broad neutralizers. Longitudinally reduced peripheral B cell counts were predictive of the development of neutralization breadth (change per 200 cells/mm^3, $p=0.007$). Infected individuals with <160 B cells/mm^3 1 month after infection were 42 times more likely to become broad neutralizers compared to individuals with B cell counts >160 B cells/mm^3 ($p=0.002$). Increased frequencies of founder Env-reactive B cells within 1 month of initial viremia was associated with the development of neutralization breadth ($p=0.035$), and higher frequencies of founder Env-specific naïve B cells associated with increased activation and differentiation and were predictive of the

development of neutralization breadth (p=0.017). These data demonstrate that initial B cell interactions with founder Env is important for the development of broadly neutralizing antibodies and provide evidence that acute HIV-1 infection events lead to downstream functional outcomes.

Xist Attenuates Acute Inflammatory Response by Female Cells

Seena K. Ajit

Pharmacology & Physiology, Drexel University College of Medicine, Philadelphia, PA

Abstract:

X-inactive specific transcript (XIST) is a long noncoding RNA that randomly inactivates one of the two X-chromosomes in female cells and thus equalizes expression of X-linked genes between females and males. Inflammatory response differs between the sexes and it is well established that incidence of acute inflammation is higher in men but chronic inflammation is elevated in women. Complex regional pain syndrome (CRPS) is a chronic neuropathic condition and the incidence of CRPS is greater in women than in men. We observed significantly higher XIST in blood samples from a subset of female CRPS patients suggesting sex-specific link for XIST to pain and inflammation in CRPS. Our in vitro studies showed that lipopolysaccharide stimulation of J774A.1 female macrophage can upregulate Xist in the cytoplasm where it associates with and delay NF-κB nuclear migration. Expression of 5 kb fragment of the 5' XIST in male cells and adoptive transfer of male splenocytes with Xist reduced acute inflammatory response. These findings suggest that Xist can have a protective anti-inflammatory effect in female cells and could mediate sex-specific differences in acute inflammatory response. Aberrant XIST could contribute to the predominance of pain in women and warrants further investigations into the dual role of XIST under acute and chronic inflammation.

Biography:

Dr. Seena Ajit is currently an Associate Professor in the Department of Pharmacology & Physiology at Drexel University College of Medicine, Philadelphia. Her research focus is on elucidating the molecular mechanisms underlying pain with emphasis on noncoding RNAs and the translation of clinical findings to basic research and vice versa. She is also investigating the role of small extracellular vesicles in intercellular communication, their potential utility as a pain therapeutic and the role of long noncoding RNA XIST in the predominance of chronic pain disorders in women.

Loss of Jedi-1 Impairs Microglial Phagocytosis, Resulting in Reduced Postnatal Neurogenesis in the Subventricular Zone

Vivianne Morrison

Vanderbilt University Department of Biochemistry and Vanderbilt Brain Institute, Nashville, TN

Abstract:

Jedi-1 is an engulfment receptor that mediates phagocytic clearance of apoptotic sensory neurons by satellite glia in the developing murine peripheral nervous system. The clearance of apoptotic debris is also critical for the development and maintenance of the central nervous system (CNS), in particular for postnatal neurogenesis. Neurogenesis relies on the coupling of neural precursor proliferation, newborn neuron apoptosis, and microglial clearance of the apoptotic debris. Using immunofluorescent labeling in brain sections from male and female wildtype (WT) and Jedi-1 knockout mice (JKO) in the first week of postnatal life, we show that Jedi-1 is expressed in WT microglia residing in the postnatal neurogenic niche, the ventricular/subventricular zone (V/SVZ), but absent in the knockout. Therefore, we asked whether

Jedi-1 expression in microglia contributes to this coupling and, thereby regulates neurogenesis. To test whether loss of Jedi-1 hinders microglial phagocytic ability, we employed an in vitro engulfment assay and find that JKO microglia display a significant reduction in engulfment relative to WT microglia. This finding is recapitulated by an accumulation of apoptotic cells in the JKO V/SVZ, as shown by TUNEL assay. To determine whether loss of Jedi-1 and subsequent disruption of microglial phagocytic ability impacts neural precursor proliferation, we performed an EdU pulse at postnatal day 7 in vivo. Our findings demonstrate that JKO mice have fewer proliferating neural progenitors in the SVZ relative to WT mice. Furthermore, JKO mice have reduced numbers of MASH1^{+} newborn neurons when compared to those of WT mice. Together, these data support the hypothesis that postnatal neurogenesis is maintained in part by Jedi-1-dependent microglial phagocytosis of apoptotic newborn neurons.

Lysosomes in Mitosis

Jonathan Stahl-Meyer*, Lya Katrine Kauffeldt Holland, Kenji Maeda and Marja Jäättelä

The Danish Cancer Society Research Center, Cell Death and Metabolism Unit, Denmark

Abstract

Lysosomes are versatile organelles whose integrity is perceived as essential for cell survival due to the degradative potential of their luminal acid hydrolases. Recently, several cytosolic non-lethal roles for lysosomal cathepsins have been identified in inflammation and cell motility during interphase. The function of lysosomes in mitosis are relatively uncharted however, lysosomes remain intact and functional, though no role requiring their degradative capability has been discovered. Here, we show that lysosomes undergo several changes prior to or during mitosis and that lysosomal leakage is involved in chromosome segregation. Lysosomal proteins LAMP1/2, luminal CTSB, and acid sphingomyelinase are reduced in mitotic cells while several lipid classes e.g. several lysoglycerophospholipids and sphingomyelin levels are increased. These changes indicate a decrease in lysosomal membrane stability. Chromatin-proximal lysosomal leakage occurs in the majority of prometaphase cells, leaking CTSB onto the mitotic chromatin where histone H3 is cleaved. Prevention of lysosomal leakage as well as inhibition or depletion of CTSB lead to an increase in telomere-related chromosome segregation defects yielding the post mitotic phenotype of micronuclei formation in cell culture and tissues. Based on these data, we propose a model in which lysosomes are primed for controlled and non-lethal lysosomal leakage contributing to accurate chromosome segregation and maintenance of genomic stability.

Biography:

I got my masters in biochemistry in 2016 from the University of Copenhagen. Following, I rapidly found my way to Marja Jäättelä's group at the Danish Cancer Society Research Center that focuses on lysosomes. Here I worked for a year as a research assistant where I was introduced to a project revolving the intriguing topic of lysosomal leakage in mitosis. I ended up working on this phenomenon as my Ph.D. project. I graduated the 30th of June 2021, and are now working in continuation on the project as a postdoc in the group.

Targeting Mitochondrial Metabolism to Rescue a Drosophila Model of Barth Syndrome

Deena Damschroder

Department of Physiology, Wayne State University, Detroit, MI

Abstract:

Barth syndrome is a rare mitochondrial disease caused by a mutation in the tafazzin gene. Reduced Tafazzin function disrupts the remodeling of cardiolipin and results in impaired mitochondrial function. Mitochondrial

dysfunction in Barth patients often causes severe exercise intolerance that negatively impacts the quality of life. As a result, exercise intolerance is cited as one of the most prominent challenges Barth patients face when trying to live a fulfilling life. This study seeks to identify pharmacological and genetic targets that could increase the exercise tolerance of Barth patients using a Drosophila model of Barth syndrome. We created the first Drosophila endurance training machine, which was used to establish that Drosophila tafazzin (TAZ) mutants have exercise intolerance reminiscent of Barth patients. We report the efficacy of overexpressing a known genetic mimetic of exercise to restore the exercise capacity of TAZ mutants. Additionally, we demonstrate the restorative effects of nicotinamide riboside supplementation and the pathway it works through. Furthermore, we combine these interventions with an exercise regimen to assess the potential for endurance training to synergistically enhance the benefits of these therapies. We found that wild-type TAZ in muscle alone is enough to rescue the exercise capacity of TAZ mutants, indicating that muscle is the key therapeutic target to restore exercise ability. We further show that overexpressing spargel (PGC-1 alpha homolog) specifically in muscle tissue is sufficient to rescue the exercise capacity of TAZ mutants. Finally, we find that the pharmacological agent nicotinamide riboside can restore exercise tolerance in TAZ and that it acts through spargel (PGC-1 alpha homolog) in this context. Together, our results suggests that spargel (PGC-1 alpha homolog) is an important modifier of exercise intolerance in TAZ mutants and should be targeted for future therapies.

Revisiting Platelets and Toll-like Receptors (TLRs): A Spotlight on Platelet-TLRs in Acute Myocardial Infarction

Kathryn Hally

Department of Surgery and Anaesthesia, The University of Otago, New Zealand

Abstract:

Introduction. Platelets are increasingly recognized as important regulators of vascular thrombosis and inflammation via their expression of Toll-like receptors (TLRs). Platelets are also centrally involved in driving pro-inflammatory and pro-thrombotic responses to acute myocardial infarction (AMI). Activation of platelet-TLR pathways during AMI may contribute to these thrombo-inflammatory responses. Here, we examined platelet-TLR expression and TLR-mediated platelet activation in both healthy and AMI subjects, and also investigated how platelets modulate leukocyte responses to TLR stimulation. Methods and Results. We report that increased expression of some platelet-TLRs was an immunological feature of AMI. We also show that, despite treatment with dual anti-platelet therapy (DAPT), platelets from AMI subjects activate in response to TLR2/1 and TLR4 stimulation. Furthermore, the extent of TLR-mediated platelet activation was similar to that seen with non-DAPT-treated platelets. To extend on these results, we conducted a randomized cross-over trial in healthy subjects to determine the extent to which DAPT was protective against TLR-mediated platelet activation. One-week treatment with DAPT did not protect against TLR2/1- or TLR4-mediated platelet activation, indicating the potential for these pathways to activate platelets despite treatment. We also conducted in vitro co-culture experiments and show that platelets can regulate the responses of leukocytes to TLR stimulation. Interestingly, platelets can dampen some leukocyte-mediated responses, challenging the notion that platelets act solely in a pro-inflammatory capacity. Conclusion. Platelet-TLRs can differentially regulate a number of thrombotic and inflammatory responses in healthy and AMI subjects. Importantly, some platelet-TLR pathways remain functional in AMI patients, despite treatment with DAPT.

Biography:

Dr. Kathryn Hally is a Postdoctoral Fellow with The University of Otago, New Zealand. Kathryn completed her Ph.D. at Victoria University of Wellington, also in New Zealand, where she investigated the role of Toll-like receptors in driving thrombotic and immune responses of platelets in the context of cardiovascular disease. Her current research is focused on developing inflammation-based risk scores for predicting optimal myocardial repair following an acute myocardial infarction. More specifically, she utilizes her technical expertise in full-spectrum flow cytometry to interrogate circulating immune cell phenotype for biomarker discovery.

Knockdown of A Disintegrin and Metalloprotease 12 (ADAM12) in 3T3-L1 Cells Reduces Cell Numbers, Delays Differentiation and Increases Lipid Accumulation During Adipogenesis *In Vitro*

Chantal Coles

Murdoch Children's Research Institute, The Royal Children's Hospital, Australia

Abstract:

Matrix metalloproteases are important proteinases active during embryonic development, tissue remodeling and regeneration. ADAM12 (A Disintegrin And Metalloprotease 12) is a proteinase from the matrix metalloprotease family involved in myogenesis and adipogenesis. Expression of ADAM12 is present through all stages of adipogenesis (pre-adipocytes, differentiation and lipid-filling), its expression highest in pre-adipocytes at the onset of terminal differentiation. The molecular pathways implicated in this process are not well understood. We were interested in the role of ADAM12 during adipogenesis. Using an in vitro murine model of adipogenesis, 3T3-L1 cells, we knockdown ADAM12 using small interfering RNA inhibition (siRNAi) in pre-adipocytes, these cells were then differentiated into mature lipid-filled adipocytes and compared to control cells (scrambled transfected). We found knockdown reduced proliferation of pre-adipocytes, delayed differentiation and increase lipid accumulation in mature adipocytes. Using gene expression profiling and metabolic enzymatic markers we have identified pathways impacted by ADAM12 knockdown during proliferation, differentiation and maturation of adipocytes. The pathway most affected by ADAM12 knockdown was regulation of insulin-like growth factor (IGF) by insulin-like growth factor binding proteins (IGFBPs); ADAM12 is known to cleave the IGF binding partners (IGFBP3 and IGFBP5). In addition, the downstream IGF/mTOR pathway was downregulated supporting a role for ADAM12 in the IGFBP/IGF/mTOR axis. Gene expression of the master regulator of adipogeneis, PPARγ, was also downregulated along with the PPARγ signaling pathway. ADAM12 regulates cell proliferation of pre-adipocytes through the IGFBP3/IGF1/mTOR signaling axis and its knockdown delays differentiation/imbalance of lipids in mature adipocytes via reduced PPARγ and adipocytokine signaling pathways.

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