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ABSTRACT BOOK

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Keynote Presentations

Molecular Mechanism of Immunological Tolerance by SOCS and NR4a

Akihiko Yoshimura

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Abstract

Immunity is dependent on the balance between positive and negative immune reactions. Immune tolerance is the suppression of specific immune responses to self or harmless substances such as pollen, food and self. When immune tolerance breaks down, autoimmune and allergic diseases will be developed. T cells are the central cells in immune tolerance, and multiple molecular mechanisms of tolerance are known. PD-1 and CTLA4, targets of cancer checkpoint inhibitors, are one of them, and are involved in so called T cell exhaustion or anergy. There are three major signals for immune reactions; TCR signal, co-stimulatory signal by CD28, and cytokine signal. PD-1 inhibits TCR signal, while CTLA-4 inhibits CD28 signal. We discovered the CIS/SOCS family as negative regulators of the cytokine signal pathway. Haploinsufficiency of the SOCS1 gene was found to cause early autoimmune diseases, confirming that SOCS1 also plays an important role in immune tolerance. The transcription factors that control the expression of checkpoint molecules have been unknown. We identified NR4a family nuclear receptors as a strong inducer of regulatory T cells, which are essential cells for immune tolerance. In collaboration with Dr. Anjana Rao, we have shown that Nr4a factors are important for T cell exhaustion. Nr4a binds to the PD-1 enhancer and contributes to stable expression of PD-1. In addition, Nr4a suppresses the expression of effector cytokines. Nr4a-deficiency in $CD8^{+}$T cells resulted in a reduction of exhausted T cells and stronger anti-tumor immunity.

STEMIN and YAP5SA Synthetic Modified mRNA Novel Treatment for Heart Diseases

Robert J. Schwartz

Hugh Roy and Lillian Cranz Cullen Distinguished Professor, Department of Biology & Biochemistry, University of Houston, TX, USA

Abstract

The adult heart lacks the regenerative capacity to self-repair. Serum Response Factor (SRF) is essential for heart sarcomerogenesis and interacts with NKX2-5 and GATA4 for cardiac gene activity. To weaken their SRF interactions, a mutant *SRF153 (A3)* named *STEMIN*, was generated by alanine scanning mutations and synthetic mmRNA transfected into rat cardiac myocytes induced *Nanog*, and *Oct4*, cell cycle factors and blocked sarcomerogenesis. Co-transfected with the *YAP1* mutant, *YAP5S/A*, stem cell factors C-MYC, SOX2 and KLF4 were induced with many of the DNA replisome and cell cycle factors. Analysis by ATAC sequencing revealed YAP5SA remodeled chromatin for

embryonic growth factor genes, *WNT5/11*, *IGF2/BPs*, *Notch, BMP, JAK and STAT* and *FGF* signaling. STEMIN also remodeled the telomerase genes such as TERT. Together *STEMIN* and *YAP5SA* induced Diaphanous 3 in adult myocytes at the anaphase stage, showing cleavage furrows. Synthetic *STEMIN* and *YAP5SA* mmRNAs together with Lipofectamine Messenger MAX were injected into the left ventricular (LV) myocardium 5 minutes after the LAD ligation of the mouse heart in an open-chest surgery on the starting day of the experiments. EDU was injected after 18 hours in the abdomen. After 24 hours, heart sections were DAPI stained and replicating cardiac myocytes cells were identified along needle tracts by co-immunostaining with alpha-Edu click-it kit, anti-TnnT2 and anti-pHH3. Key components of the DNA repliosome were stained with Anti-Claspin, Anti-Orc2 and anti-MCM2, markers of the pre-initiation pathway in early G1 stage. Confocal microscopy and ImageJ software revealed greater than a 15-fold increase in cardiomyocyte nuclei. Infarcted controls showed a significant decline in their ejection fraction ($P<0.05$ for a two-tailed P value in comparison to the combination group of *STEMIN* and *YAP5SA* mmRNA treatment in the second week post-MI. The combination treatment group improved significantly ($P<0.05$) in comparison to the infarcted controls after 4 weeks; thus, cardiac function was significantly improved by 4 weeks post-infarct, and reduced fibrosis and immune cell infiltration. *STEMIN* and *YAP-5SA* reprogramed cardiac myocytes to replicating myocytes and also blocked myocyte apoptosis. *STEMIN* and *YAP-5SA* mmRNA, improved cardiac function and reduced myocardial fibrosis in left ventricles of infarcted adult mice. The combinatorial use of mmRNA encoding *STEMIN* and *YAP-5SA* has the potential to become a powerful clinical strategy to treat human heart disease.

Functional Genomics and Epitranscriptomics of tRNA from Cells to Microbiomes

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Abstract

tRNAs are adapter molecules between the genetic code and the protein sequence. tRNAs are the most abundant cellular RNA family in copy numbers and are highly adapted for translational regulation. A human tRNA contains on average 13 modifications per molecules, collectively the human tRNAome contains more than 40 chemical modification types. Multiple tRNA properties coordinate in translation and non-translational functions including abundance, modification, aminoacylation, and fragmentation. I will discuss our development of tRNA sequencing methods that simultaneously measure abundance, modification, charging, and fragmentation in the same library, and its applications to basic science such as cellular stress response, clinical studies such as infectious disease, and microbiome characterization.

Oral Presentations

Endothelial-to-Osteoblast Conversion in Osteoblastic Metastasis of Prostate Cancer

Paul Corn

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Abstract

Lethal prostate cancer (PCa) is dominated by complications arising from bone metastasis and treatment for bone-metastatic castrate-resistant PCa (bmCRPC) is a major challenge in oncology. bmCRPC is a "microenvironment-dependent" disease in which epithelial-stromal cell interactions create a bone tumor microenvironment (bone-TME) that promotes lethal disease progression and resistance to therapy. As a result, therapies that have historically targeted tumor cells have modestly improved survival but fallen short of cure. While bone lesions in most solid tumors are phenotypically lytic, PCa bone lesions are uniquely bone-forming (termed "osteogenic"). Work from our group and others showed that PCa-induced aberrant bone overgrowth promotes tumor growth. In support of this, targeting tumor-induced bone with Radium-223 (Ra223), prolongs survival in men with bmCRPC. Ra223 is a high energy α-emitting calcium mimetic that preferentially localizes to hydroxyapatite in newly formed matrix within osteogenic metastases and induces double strand DNA breaks in adjacent cells. To improve therapies for bmCRPC, we seek to elucidate mechanisms of PCa-induced bone formation. PCa-induced bone formation was thought to result from the expansion of existing osteoblasts. Surprisingly, we discovered that PCa-induced bone formation occurs through endothelial–to-osteoblast (EC-to-OSB) transition, during which tumor-associated ECs convert to EC-OSB hybrid cells with unique properties that modulate both tumor and stromal components in the bone-TME. This new insight into stromal cell lineage plasticity highlights the bone-TME as a dynamic component that can be reprogrammed by tumor cells to support tumor progression. Targeting this plasticity represents a rational therapy strategy for lethal PCa.

Translesion Synthesis in Cancer Therapy and Viral Infection

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Abstract

Translesion synthesis (TLS), a DNA damage tolerance pathway, plays an important role in cancer cell survival and development of resistance after chemotherapy, suggesting that targeting TLS is an attractive avenue for improving chemotherapeutics. Employing a high-throughput screening campaign, we discovered a small molecule inhibitor, JH-RE-06, that disrupts TLS. Binding of JH-RE-06 induces REV1 dimerization and blocks the REV1-REV7 interaction and POL ζ recruitment. TLS inhibition by JH-RE-06 alone or in combination with existing therapeutics sensitizes drug-resistant

tumors to treatment. Remarkably, TLS inhibition by JH-RE-06 suppresses SARS-CoV-2 proliferation and dramatically represses the SARS-CoV-2-dependent genome instability, implicating a role of TLS in viral infection.

Functional Mapping of Microbial Metabolites to Enhance Cancer Immunotherapy Efficacy

Virginia A. Pedicord, Benjamin Beresford-Jones, Puspendu Sardar, Rahul Roychoudhuri, Paula Kuo, Sarah Whiteside, and Satoshi Suyama

Group Leader - Sir Henry Dale Fellow, Department of Medicine and Cambridge Institute of Therapeutic Immunology and Infectious Disease, University of Cambridge, UK

Abstract

The gut microbiota affects numerous aspects of human health, including host metabolism, immunity and brain function. Next gen sequencing methods have revolutionized our understanding of the phylogeny and taxonomic identities of members of the human gut microbiota and their associations with various diseases. Our research team now focuses on the tasks being performed by gut microbes and the products that mediate their beneficial or harmful effects. We previously identified an enzyme and its downstream muropeptide metabolites from commensal *Enterococcus* that improve both local host responses to intestinal infections and systemic immune responses to cancer immunotherapy. However, important barriers have remained in the microbiota field, namely a lack of resources for functional and mechanistic studies and concerns about the application and relevance to human disease of microbiota research performed in animal models. To overcome these barriers, we cultured and whole-genome sequenced bacterial isolates from conventionally housed laboratory mice to build a Mouse gut bacteria Culture Collection (MCC) and used isolate genomes and metagenome-assembled genome (MAG) synthesis to generate a comprehensive Mouse Gastrointestinal Bacteria Catalogue (MGBC), a public database representing 1,094 gut bacteria species from 56 institutes across 18 countries. Our MCC isolate collection, MGBC database and bioinformatic Toolkit now enable us to map and test functional capabilities of both the mouse and human gut microbiotas to establish how microbial species achieve modulatory effects on the host. Using these new biological and bioinformatic resources we are now elaborating the mechanisms by which metabolites from commensal gut microbes modulate responses to tumour immunotherapy.

Targeting Interleukin-6 to Rescue Antitumor T Cells from Becoming Pathogenic

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Abstract

Immune checkpoint blockade (ICB) improves survival outcomes for patients with multiple malignancies including unresectable metastatic melanoma, but ICB treatment induces immune related adverse events requiring intervention with immunosuppressive medications occur in a subset of patients. To identify strategies to mitigate these toxicities without hindering ICB-mediated antitumor immunity, we performed a deep immune analysis of intestinal, colitis, and tumor tissue from ICB -treated patients with parallel studies in preclinical models. Elevated expression of interleukin-6 (IL-6), neutrophil activation and chemoattractant factors and T helper 17 (Th17) cell score were higher in colitis than normal intestine in patients treated with ICB. In murine preclinical hybrid model of melanoma and experimental autoimmune encephalomyelitis (EAE), combination of IL-6 blockade with ICB decreased experimental autoimmune encephalomyelitis (EAE) symptoms and improved tumor control, indicating that the combination could suppress inflammatory response and potentially enhance antitumor immunity. Mechanistically, IL-6 blockade increased polyclonal and monoclonal CD8+ effector T cells (Tc1), reduced the Th17, macrophages and myeloid suppressor cells number in tumor of mice treated with ICB. CD8+T cells produced higher levels of IFN-γ with specificity to melanoma expressed antigen TRP-2. Interestingly, IL-6 blockade increased IL-17 – and IFN-γ-producing hybrid (Tc1/Tc17) cells in tumors, suggesting Tc1/Tc17 hybrid cells could potentially become polarized toward antitumor activity. These findings are consistent with previous findings that IL-6 is a key cytokine required for mouse Th17 and Tc17 cell differentiation. Thus, targeting IL-6 cytokine pathway can have therapeutic benefits involving multiple immune mediated tissue toxicities.

Inhibiting Lung Adenocarcinoma Growth by Inducing Apoptosis Independent of Bcl-2 Proteins

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Abstract

Dysregulation of Bcl-2 protein signaling is frequently implicated in pulmonary tumorigenesis: lung adenocarcinoma cells show an increased ratio of anti-apoptotic to pro-apoptotic Bcl-2 proteins, rendering them resistant to conventional anti-cancer therapies that depend on Bcl-2 proteins. Therefore, an important task in lung cancer research is to develop new treatments that overcome the resistance to cell death displayed by tumor cells due to impaired apoptotic pathways. The opportunistic bacterium, *Pseudomonas aeruginosa*, produces the quorum sensing molecule N-(3-oxododecanoyl)-homoserine lactone (C12). We have found that C12 preferentially induces lung

adenocarcinoma cell apoptosis *in vitro* and inhibits transplanted lung adenocarcinoma growth *in vivo* independent of Bcl-2 proteins, probably through its direct damage to the mitochondrial outer membrane. Importantly, C12 cytotoxicity in lung adenocarcinoma cells is mediated through the lactonase activity of paraoxonase 2 (PON2). PON2 is upregulated in human lung adenocarcinoma, enabling lung adenocarcinoma cells to resist conventional therapeutic drugs. Decreasing PON2 expression in human lung adenocarcinoma cells reduces cell proliferation, lowering the likelihood of drug-induced resistance. Therefore, PON2/C12 interaction represents a novel therapeutic target for discovering drugs that induce apoptosis in PON2-overexpressing lung adenocarcinoma in a fashion independent of the Bcl-2 protein profile.

Role of T Follicular Helper Cells in Anti-tumor Immunity

Roza Nurieva

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Abstract

Clinical relapse and metastases are the major causes of death in melanoma. Currently, adoptive T cell therapy using tumor-infiltrating lymphocytes rich in cytotoxic $CD8^+$ T cells (CTLs) is one of the promising approaches that helps overcome this major challenge in melanoma; however, CTL cell transfer alone does not improve clinical response, suggesting a supportive role of helper $CD4^+$ T cells. There is increasing evidence on the potential contribution of tumor infiltrated $CD4^+$ T follicular helper (Tfh) cells and intratumoral lymphoid structure formation in tumor eradication; however, to date, the role of Tfh cells in anti-tumor immunity has not been clearly elucidated. Remarkably, using a murine melanoma model, we found that the percentage of intratumoral Tfh cells negatively correlates with melanoma growth. In addition, we observed enhanced tumor growth in Bcl6 T-cell conditional knockout mice, which lack Tfh cells as compared to wild-type mice, suggesting that Tfh cells might play a protective role in tumor growth and could serve as a marker for tumor regression. Importantly, in support of mouse studies, in melanoma patients the number of Tfh cells correlates with the rate of patient survival. Importantly, adoptive transfer of antigen specific Tfh cells into tumor bearing mice results in increased number of intratumoral CTLs, as well as in an efficient tumor eradication, indicating the essential role of Tfh cells in promoting CTL expansion and anti-tumor reactivity. Our results thus indicate the therapeutic potential of Tfh cells in promoting CTL-mediated anti-tumor immunity against melanoma and provide the basis for potential usage of these cells to improve current immunotherapy approaches.

Therapeutic Targets in Preclinical Urological Models

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Abstract

Penile Squamous cell carcinoma (PSCC) is a rare aggressive cancer. The cure rate is high if diagnosed early. However, for patients with metastatic disease, the median survival is less than 12 months and less than 6 months after the disease becomes chemorefractory. Human papillomavirus (HPV) infection occurs in about 50% of PSCC cases. High risk HPV genotypes are involved in the carcinogenesis of PSCC through the activity of viral E6 and E7 oncoproteins that are capable of binding to and inactivating p53 and the retinoblastoma-1 tumor suppressor proteins (Rb) respectively. E7's inhibition of the Rb pathway leads to increased expression of the $p16^{INK4a}$ (CDKN2A) protein, (also known as p16). Overexpression of p16 can be detected by immunohistochemical (IHC) staining and has been found to be a reliable marker for high-risk HPV infection in oropharyngeal SCC and PSCC. In addition, PSCC patients with high p16 expression have been found to have a better prognosis than PSCC patients with low expression. Therefore, there is a lot of evidence that targeted therapy possibly based on HPV+ infection could have therapeutic response. We have generated several patient-derived tumor xenograft animal models of both HPV-positive and HPV-negative PSCC. These models resemble the orginial patient tumor tissue and we have found significant differences in expression of cell cycle proteins in our HPV-negative models versus our HPV-positive models.

SEQing New Functions for AhR in MYCN-amplified Neuroblastoma

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Abstract

Neuroblastoma is one of the most common extra-cranial tumors in children, arising within the sympathetic nervous system. MYCN amplification, a known driver for neuroblastoma, occurs in 40-50% of high-risk cases and is an unfavorable factor for survival. We have identified the aryl hydrocarbon receptor (AhR) as a transcription factor strongly contributing to MycN functions in neuroblastoma cells and with a potential oncogenic role in this tumor type. In particular, we revealed that AhR contributes to suppression of neuroblastoma cells differentiation and that AhR

pharmacological inhibition synergizes with retinoid treatment in promoting differentiation in vitro and suppressing tumor growth in vivo. Thus, AhR could represent a novel therapeutic target in MYCN-amplified neuroblastoma.

USP22 Regulates Signaling Cascades in Development and Disease

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Abstract

USP22 overexpression is observed in several human cancers and is correlated with poor patient outcomes. The molecular basis underlying this correlation is not clear. Usp22 is the catalytic subunit of the deubiquitylation module in the SAGA histone-modifying complex, which regulates gene transcription. Our previous work demonstrated that the loss of Usp22 in mice leads to decreased expression of several components of receptor tyrosine kinases like ERBB2 and TGFβ signaling pathways. To determine whether these pathways are upregulated when Usp22 is overexpressed, we first created a mouse model that expresses high levels of Usp22 in all tissues. Phenotypic characterization of these mice revealed over-branching of the mammary glands in females. Transcriptomic analyses indicate the upregulation of key pathways involved in mammary gland branching in mammary epithelial cells derived from the Usp22-overexpressing mice, including estrogen receptor, ERK/MAPK, and TGFβ signaling. However, Usp22 overexpression did not lead to increased tumorigenesis in any tissue. To determine whether changes in USP22 expression affects ERBB2-driven tumorigenesis, we introduced conditional overexpression or deletion alleles of Usp22 into mice bearing the MMTV-NIC transgene. USP22 overexpression in mammary glands did not further enhance primary tumorigenesis in MMTV-NIC female mice. However, deletion of Usp22 significantly decreased tumor burden and increased survival of MMTV-NIC mice. These effects were associated with markedly decreased levels of both Erbb2 mRNA and protein, indicating Usp22 loss impacts MMTV promoter activity. Usp22 loss had no impact on ERBB2 expression in human cancer cell lines, suggesting that the role of Usp22 loss on tumorigenesis cannot be assessed in this model due to unexpected effects on MMTV-driven Erbb2/Neu expression.

Patients with Systemic Anaplastic Large Cell Lymphoma: Potential Candidates for PD-1 Blockade Immunotherapy

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Abstract

The programmed cell death 1 (PD-1) pathway is a recently recognized mechanism of tumor immune evasion. In our study, programmed cell death ligand 1 (PD-L1) expression was evaluated in patients with systemic anaplastic large cell lymphoma: including ALK+ and ALK-negative cases. ALK+ anaplastic large cell lymphoma was more often positive for PD-L1 than ALK-negative anaplastic large cell lymphoma. ALK-negative anaplastic large cell lymphoma showed a strong correlation between PD-L1 expression and STAT3 activation. In contrast, the PD-L1/pSTAT3 correlation was weaker in ALK+ anaplastic large cell lymphoma. In ALK-negative anaplastic large cell lymphoma, the PD-L1+ subgroup was more often EMA positive and tended to be less often CD2 positive. In ALK+ anaplastic large cell lymphoma, PD-L1 was not associated with pathologic features. Negative ALK status and high IPI score (> 3) were associated with shorter overall survival. Overall survival was not different between patients with PD-L1+ versus PD-L1-negative anaplastic large cell lymphoma, regardless of ALK status and International Prognostic Index (IPI) score. We conclude that PD-L1 expression is more common in ALK+ anaplastic large cell lymphoma than ALK-negative anaplastic large cell lymphoma. In ALK-negative anaplastic large cell lymphoma, PD-L1 is strongly correlated with STAT3 activation and is associated with more frequent EMA and less frequent CD2 expression. PD-L1 has no prognostic significance in predicting the outcome of patients with systemic anaplastic large cell lymphoma, regardless of ALK status. PD-L1 expression on the anaplastic large cell lymphoma cells suggests these patients as potential candidates for PD-1 blockade immunotherapy.

Syntaphilin Regulates Mitochondrial Dynamics and Tumor Cell Invasion

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Abstract

Recent studies support the concept of spatiotemporal regulation of energy production as a driver of tumor cell motility and invasiveness. We recently identified the molecular motors and adaptors responsible for trafficking of mitochondria in tumor cells by a genome-wide shRNA screen. One of the top hits in the screen was Syntaphilin (SNPH), a molecule that arrests mitochondrial trafficking at sites of high energy demands in neurons. SNPH was expressed in brain and other tissues, and in non-transformed and cancer cell lines. Depletion of SNPH in cancer cells constitutively accelerated focal adhesion complex dynamics, stimulated 2D cell motility and increased tumor cell invasion. Furthermore, depletion of SNPH stimulated the repositioning of mitochondria from their perinuclear localization to the cortical cytoskeleton of tumor cells. Mitochondria in SNPH-depleted tumor cells exhibited faster speed of movements, higher processivity, and greater distance traveled, compared to control. Mutants of SNPH that lacked the microtubule binding domain, kinesin-binding domain, or LC8-binding domain were not able to regulate tumor cell invasion. Further, loss of SNPH was sufficient to deregulate mitochondrial trafficking of energetically active organelles. In patients, SNPH's mRNA was downregulated during tumor progression, and exogenous expression SNPH severely reduced metastatic dissemination in vivo. Finally, we describe an alternative spliced isoform

of SNPH which is the main isoform expressed in non-neuronal normal and cancer cells, with novel localization to the inner mitochondrial membrane and functions in bioenergetics. In conclusion, we provided compelling evidence that SNPH-directed mitochondrial dynamics regulates the bioenergetics requirements of tumor cell invasion.

Vimentin Regulates Colorectal Cancer Progression by Interaction with Myosin 10

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Abstract

Colorectal cancer (CRC) is one of the most common adenocarcinomas, with a progressive increase in metastasis-related mortality. Epithelial-mesenchymal transition (EMT) is an essential process in cancer cell invasion, which involves vimentin (Vim), a hallmark of EMT in epithelial cells that is increased in progressive tumors. Unconventional myosin 10 (Myo10) is an actin-based motor protein whose expression correlates with cancer metastasis and poor prognosis. However, the relationship of these two important cancer markers, Myo10 and Vim, in migrating cells is not defined. We tested the hypothesis that Vim contributes to cancer progression via regulation of the of Myo10 aggregation at cell extension tips. In cultured fibroblasts and CRC cells, we found that Myo10 tracks along actin bundles, at least in part, as dimers [1,2]. Dimerization is promoted by the strong association between Myo10 and Vim (Kd 57nM), which supports dimer stability, and in turn, enhances Myo10 tracking along actin filaments and promotes the cell extensions formation. Further, we found that the Vim-Myo10 complex, as observed by two-color STED, also incorporates MT1, which is translocated toward the plasma membrane where MT1 is secreted. In human biopsies, Vim-MT1 colocalization increases during discrete stages of CRC progression and that in cultured cells, Vim-enhanced trafficking of MT-1 contributes to collagen proteolysis. Moreover, Vim deletion reduced the velocity of Myo10 along cell extensions and Myo10 aggregation at the termini of cell extensions. Collectively our data indicate that Vim plays a central role in Myo10 dynamics and in MT-1 translocation in cancer invasion of soft connective tissues.

Identification of a CD4+ T Cell Line with Treg-like Activity

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Abstract

Regulatory T cells (Tregs) suppress adaptive immunity and inflammation. Although they play a role in suppressing anti-tumor responses, development of therapeutics that target Tregs is limited by their low abundance, heterogeneity, and lack of specific cell surface markers. Since it is not possible to functionally characterize cells after intracellular Foxp3 staining, we identified and characterized a human T cell line, MoT, as a model of human Foxp3$^+$ Tregs. Unlike the CD4+ Jurkat T cell line, MoT cells express surface markers consistent with human PBMC-derived Tregs such as: CD4, CD25, glucocorticoid induced TNF receptor (GITR), lymphocyte activation gene (LAG-3), programmed death receptor ligand 1 (PD-L1), and C-C Motif Chemokine Receptor 4 (CCR4). Human PBMC-derived Foxp3$^+$ Tregs and MoT cells, but not Jurkat cells, inhibited proliferation of human CD4$^+$ responder PBMCs in a MoT:PBMC ratio-dependent manner. Transwell membrane separation prevented suppression of stimulated CD4$^+$ PBMC proliferation by MoT cells, suggesting cell-cell contact is required for suppressive activity. Blocking antibodies against PD-L1, LAG-3, GITR, CCR4, HLA-DR, or CTLA-4 did not reverse the suppressive activity of MoT cells. Herein, we show that human PBMC-derived CD4$^+$ CD25^{high} Foxp3$^+$ Tregs and MoT cells suppress stimulated CD4$^+$ PBMCs in a cell contact-dependent manner, suggesting that a Foxp3$^+$ Treg population suppresses immune responses by an unknown cell contact-dependent mechanism.

Mechanistic Insight on how 1,4-dihydropyridines Induce TβRII Degradation

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Abstract

Transforming growth factor beta (TGFβ) is a multifunctional cytokine involved in various cellular processes. However, aberrant TGFβ signalling fuels epithelial-to-mesenchymal transition (EMT) and metastasis and is a distinctive trait of many epithelial-derived tumors, including lung carcinomas. TGFβ signals through a receptor complex composed of TGFβ receptor type I (TβRI) and type II (TβRII). Recently, a novel class of 1,4-dihydropyridines (DHPs) has been shown to specifically inhibit TβRII during cardiomyogenesis, without affecting TβRI levels. The effect of DHPs on non-small cell lung tumorigenesis has not been investigated, which led us to our overall goal to characterize how DHPs inhibit TGFβ signalling, trafficking and TGFβ-related functional outcomes in non-small cell lung cancer (NSCLC) cells. Using Western blotting we observed that DHPs significantly decreased TβRII protein levels and TGFβ-dependent signaling in NSCLC cell lines and inhibited TGFβ-

dependent events such as E-cadherin to N-cadherin shift. Using immunofluorescence microscopy, we observed decreased formation of TGFβ-induced actin stress fibers. Preliminary data suggests that TβRII endocytosis may not be affected by DHPs. Currently, most anti-TGFβ clinical trials involve compounds that target TβRI kinase activity, and this results in incomplete inhibition of TGFβ signalling pathways that induce EMT, because TβRII can continue to signal even when TβRI is inhibited. By targeting TβRII using the DHPs, TGFβ-dependent EMT inhibition may be much more effective in NSCLC cells, laying a solid foundation for the potential use of these novel compounds as an effective anti-TGFβ treatment strategy to inhibit metastasis of NSCLC cells in a clinical setting.

All Trans Retinoic Acid Modulates the Retinoic Acid Receptor Signaling Pathway in Merkel Cell Carcinoma Cells

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Abstract

The biological activity of retinoic acid/all-trans retinoic acid (ATRA) is mediated by retinoic receptors, which are ligand-dependent transcription factors that activate crucial cell differentiation genes. Dysregulations of retinoic pathway lead to carcinogenesis.[1] A strong antitumor activity of ATRA by modulating the retinoic pathway has been proved in different carcinoma histotypes. However, the effect of this molecule in Merkel cell carcinoma (MCC), a rare but aggressive skin neoplasm of viral origin in 80% of cases,[2] is unknown.[3,4] Herein, we investigated the antineoplastic effect of ATRA in Merkel cell polyomavirus (MCPyV)-positive/-negative MCC cells and in control human fibroblasts. The antineoplastic effect of ATRA was evaluated by testing MCC cell proliferation, migration and clonogenicity. Apoptosis/cell death and cell cycle were evaluated by Annexin-V/PI and TALI assays, respectively. Apoptotic and retinoic pathway genes were evaluated by RT2 Profiler PCR array and by western blot (WB) analysis. ATRA treatment led to a strong reduction in MCC cell proliferation, migration and clonogenicity, while promoting cell cycle arrest and apoptosis/cell death, with a more pronounced effect in MCPyV-positive cells. A significant overexpression of pro-apoptotic markers in ATRA-treated MCC cells compared to untreated cells was determined through gene expression array and WB. Neither phenotypic nor molecular effects were found in ATRA-treated fibroblast control cells. Numerous retinoic signaling genes, such as BMP2, FOXA1, MAFB, OLIG2, UCP1 and RBP4, resulted as differentially expressed in ATRA-treated MCC cells compared to untreated cells. Our data indicate that ATRA presents an antineoplastic activity in MCC cells by modulating retinoic receptor pathway.

Elucidation of Necroptosis Pathway Using Optogenetics

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Abstract

Necroptosis is an immunogenic cell death, featured by active secretion of proinflammatory cytokines. Due to its inflammatory nature, necroptosis has been implicated with numerous inflammatory diseases such as inflammatory bowel diseases and neurodegenerative diseases. Among many proteins that participate cellular signaling pathway of necroptosis, Receptor Interacting Protein Kinase 3 (RIPK3) has been characterized as an essential protein to induce necroptosis via formation of hetero-oligomeric protein complex called necrosome, which is downstream of tumor-necrosis-factor-receptor-1 associated protein complex, complex-I. Although the pathway of necroptosis for inducing membrane permeabilization is well-studied, the node-specific function of the necroptosis pathway for cytokine production is incompletely understood. Using optogenetic approach, I have successfully developed blue-light-inducible RIPK3 (OptoRIPK3) system to oligomerize and activate RIPK3. Upon the light treatment, OptoRIPK3 can be activated/phosphorylated which triggers necroptotic cell death and cytokine production. Interestingly, the kinetics of cell death and cytokine production seems to have different peak time, suggesting the presence of different regulation mechanisms for these two cellular responses. In addition, types of cytokines that are produced from the activated OptoRIPK3 was not identical to the those from complex-I. Moreover, OptoRIPK3 induced cytokines were categorized into RIPK3 kinase dependent and independent types. Currently we are trying to understand the role of necrosome for differential regulation of cytokine production via RNA-seq. This study will provide an additional insight into the regulation mechanism of cytokine production from the different nodes of necroptosis pathway, which could be synergistic or orthogonal cause of different type of inflammatory diseases.

Dysregulated Cellular Dynamics in Insulin Resistance

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Abstract

Insulin signaling is central to normal metabolic control and is known to be dysregulated in metabolic diseases but the underlying mechanism that produces dysfunctional cells is incompletely understood. We have found that insulin receptor (IR) is incorporated into biomolecular condensates whose dynamic properties are altered in insulin resistance. In normal cells, insulin stimulation results in accumulation of IR in clusters that display dynamic molecular characteristics expected of liquid-like condensates. In insulin-resistant cells, both IR accumulation in condensates and the normal dynamic behavior of these condensates are reduced, suggesting a physico-mechanical link between insulin response and the dynamic molecular behavior of IR condensates. The observed defects in IR condensate behaviors are caused, at least in part, by an increase in oxidative stress in insulin-resistant

cells. Treatment of insulin-resistant cells with metformin, a first-line drug used to treat type 2 diabetes, can rescue the dynamic behaviors of IR condensates, consistent with metformin's effect of reducing the levels of reactive oxygen species. The observation that IR is incorporated into biomolecular condensates during the response to insulin stimulation, and evidence that changes in the dynamic features of IR condensates contribute to insulin resistance, have implications for improved therapeutic approaches for patients.

Transcriptome Changes in Cervical Cancer Cells Due to Small Interfering RNA Induced Inhibition of Notch-3: the Enigma Unravelled

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Abstract

Background: Cervical cancer (CC) is a type of cancer that commence in woman's cervix. Previously, Notch-3 signaling was identified as a critical regulator of HPV associated CC, but their underlying mechanisms via association of hub genes are not fully understood. This study aimed to elucidate the candidate hub genes and key pathways in HPV-/+ CC cell lines, via esiRNA induced inhibition of Notch-3.

Methods: HPV-16 positive (Caski) and HPV-16 negative (C-33A) cells of CC were knocked down by Notch-3 esiRNA transfection followed by high-throughput RNA sequencing of paired-end fragments through Illumina HiSeq 10X Sequencer. The gene co-expression network analysis was performed through Cytoscape using plugin, cytoHubba. The biological pathways were predicted using ClueGO. The bioinformatics analysis of high-quality reads were done through HISAT2, StringTie, and Ballgawn. Functional enrichment analysis and network of all the DF genes were also done.

Results: A total of 1307 genes were found to be DF in HPV- CC cell line C33A (control vs test), among which SF3B1 was found to be hub gene via protein-protein network analysis. (ii) 789 genes in HPV+ CC cell line Caski (control vs test), and (iii) 1768 genes between Caski test vs C33A test, among which HSCB, CS, UBB, and PPP2R1B were found to be major hub genes.

Conclusions: Overall, above candidate hub genes and enrichment pathways shed light on the synergistic molecular mechanisms involved in the progression of Notch-3 induced pathogenesis of CC which might have important clinical implications for CC diagnosis and treatment.

The Balance Between Gasdermin D and Sting Signaling Shapes the Severity of Schistosome Immunopathology

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Abstract

There is significant disease heterogeneity among mouse strains infected with the helminth *Schistosoma mansoni*. Here, we uncover a unique balance in two critical innate pathways governing the severity of disease. In the low-pathology setting, parasite egg-stimulated dendritic cells (DCs) induce robust interferon (IFN)β production, which is dependent on the cyclic GMP-AMP synthase (cGAS)/stimulaor of interferon genes (STING) cytosolic DNA sensing pathway and results in a Th2 response with suppression of proinflammatory cytokine production and Th17 cell activation. IFNβ induces signal transducer and activator of transcription (STAT)1, which suppresses CD209a, a C-type lectin receptor associated with severe disease. In contrast, in the high-pathology setting, enhanced DC expression of the pore-forming protein gasdermin D (Gsdmd) results in reduced expression of cGAS/STING, impaired IFNβ, and enhanced pyroptosis. Our findings demonstrate that cGAS/STING signaling represents a unique mechanism inducing protective type I IFN, which is counteracted by Gsdmd.

Crosstalk Between Regulatory T Cell and Hair Follicle Stem Cell is Dependent on Glucocorticoid Signaling

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Abstract

The maintenance of tissue homeostasis in steady state or under stress is dependent on the proper communication between the stem cells and the supporting cells in their microenvironment or “niche”. In addition to promoting immune tolerance, regulatory T cells (Tregs) have recently emerged as a critical component of the stem cell niche in the hair follicle (HF), injured muscle, bone marrow, and

small intestine to support stem cell differentiation or maintain their quiescence. How Treg cells sense the dynamic signals in the niche environment and communicate with stem cells during tissue regeneration is largely unknown. Here, by using HF as a model, we uncover a hitherto unrecognized function of steroid hormone glucocorticoid that instructs skin resident Treg cells through the glucocorticoid receptor (GR) to facilitate hair follicle stem cell (HFSC) activation and HF regeneration. Ablation of GR signaling in Tregs blocked depilation-induced hair regeneration and natural hair growth without affecting Treg's immune suppressive function. Mechanistic study revealed that GR signaling induces skin-resident Tregs to produce TGF-b3, which directly activates Smad2/3 in HFSCs and facilitates HFSC activation and proliferation. Our study identifies a novel crosstalk between skin-resident Tregs and HFSCs mediated by the GR/TGF-b3 axis, highlighting a new avenue to manipulate Tregs to support tissue regeneration.

Structural Basis of DNA Polymerase Catalyzed DNA Synthesis, Error Incorporation, and Double-strand Break Repair

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Abstract

DNA polymerases catalyze template dependent DNA synthesis during DNA replication and repair. Error incorporations by DNA polymerases can result in human diseases and cancer, whereas targeting DNA polymerases have been proven effective in various cancer treatment. We have employed leading-edge structural techniques to investigate the mechanism of DNA polymerases. By observing DNA synthesis directly with time-resolved crystallography, we dissected the role of local conformational changes and metal ion cofactor binding in correct and incorrect DNA synthesis. We found that three metal ions are required for promoting substrate alignment and catalysis, in contrary to the well-established two metal ion dependent model. Moreover, the primer end alignment induced by metal ion binding play critical roles in discriminating error incorporation and nucleoside analog drug inhibition of polymerase. Our structural studies of DNA polymerases shed light on the general mechanism of DNA synthesis, error incorporation and repair, and provide valuable information for targeting polymerases for cancer treatment.

Molecular Basis of NAADP-evoked Endolysosomal Calcium Signaling

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Abstract

Nicotinic acid adenine dinucleotide phosphate (NAADP) is a potent Ca2+-mobilizing second messenger, which uniquely mobilizes Ca2+ from acidic endolysosomal organelles. However, the

molecular basis of the NAADP-evoked endolysosomal calcium signaling remains unclear. Given the necessity of the endolysosomal two-pore channel (TPC1 or TPC2) in NAADP signaling, we identified a Sm-like protein Lsm12 functioning as both a high-affinity NAADP binding protein and a TPC interacting partner via its Lsm domain. Lsm12 is essential and immediately participates in NAADP-evoked TPC activation and Ca2+ mobilization. Furthermore, we found that Lsm12 inhibited PI(3,5)P2-induced TPC2 activation and NAADP reduced Lsm12's inhibition of PI(3,5)P2-induced TPC2 activation. These findings reveal Lsm12's multifaceted function as an NAADP receptor and a TPC auxiliary protein in regulation of the TPC gating by PI(3,5)P2 and NAADP, and provide a molecular basis for understanding the mechanisms of NAADP signaling.

Utilization of tRNA as a Cofactor Beyond Ribosomal Translation

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Abstract

Transfer RNA (tRNA) is best known to function in ribosome-mediated protein synthesis. However, in a less-known role, arginyl-tRNA is essential for catalyzing a unique and poorly understood protein post-translational modification that regulates protein turnover. In this arginylation reaction, ATE1 (arginyltransferase 1) facilitates arginine transfer to protein targets using a mechanism that depends on, and is selective for, arginyl-tRNA^{Arg} as the donor cofactor. However, how ATE1 (and other tRNA-dependent aminoacyl-transferases) recognizes specific tRNA cofactors and competes with the highly efficient ribosomal machinery remains a mystery for decades. Here, we describe the three-dimensional structures of *Saccharomyces cerevisiae* ATE1 with and without its tRNA cofactor. Importantly, the putative substrate binding domain of ATE1 adopts a previously uncharacterized fold that contains an atypical zinc-binding site critical for ATE1 stability and function. The unique recognition of tRNA^{Arg} by ATE1 is coordinated through interactions with the major groove of the acceptor arm of tRNA. Binding of tRNA induces conformational changes in ATE1 that helps explain the novel mechanism of substrate arginylation.

Functional Variants in LMNA and DSP are Revealed by the Qatar Genome Programme

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Abstract

The American College of Medical Genetics and Genomics (ACMG) published a recommendation to report medically actionable pathogenic variants in 59 genes when ordering clinical genetic testing. While the prevalence of medically actionable variants is known from large population studies, these lack in Arab and other Middle Eastern populations. We aimed to understand the genetic variation present in these genes in the Qatari population. We used data from 6,045 whole genomes from the Qatar Genome Program (QGP) and integrated it with phenotypic data collected by the Qatar Biobank. We identified known pathogenic and likely pathogenic variants based on ClinVar and HGMD. Additionally, we identified novel variants and assessed their phenotypic associations. Two novel variants were functionally characterized in zebrafish, using Morpholinos targeting the corresponding orthologs and synthetic wild-type and mutant RNA injection assays. We identified a total of 64 pathogenic and likely pathogenic variants in 27 ACMG genes in 170 individuals, as 75 novel, potentially pathogenic variants. Overall, 2.8% of the QGP-sequenced participants carried a pathogenic or likely pathogenic variant and 4.5% carried a potentially pathogenic variant in one of the 59 ACMG genes. We assessed two of the novel cardiovascular variants, c.1841A>G (p.Asp614Gly) in *DSP* and c.326T>G (p.Val109Gly) in *LMNA*. Our results showed that both variants caused abnormal heart development, heart rate and rhythm. The prevalence of medically actionable variants in the Qatari population is slightly higher than in other populations. Two novel variants in *LMNA* and *DSP* caused abnormal cardiovascular phenotypes in zebrafish.

Using 3D Single-molecule Fluorescence Microscopy to Map Cellular Function

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Abstract

Cellular function is governed by the molecular organization and interactions at the nanoscale. Here, I will describe our 3D single-molecule imaging platforms that combine light sheet illumination with point spread function engineering for improved 3D single-molecule tracking of dynamics and 3D super-resolution imaging of nanoscale structures within entire mammalian cells. By optically

sectioning cells using light sheet illumination we reduce fluorescence background, photobleaching, and the risk of photodamaging sensitive samples. When combining this approach with point spread function engineering, we are able to detect the positions of single molecules in 3D with tens of nanometer precision throughout the cells. I will demonstrate applications where we have utilized these platforms to provide dynamic information about chromosomal loci in living cells and structural details of e.g., the nuclear lamina, sugars in the glycocalyx and how they are modulated during cancer progression, and of previously unknown protein structures in primary cilia. These imaging platforms are versatile and can be utilized to study molecular dynamics, nanoscale structures, and molecular mechanisms to address a wide range of biochemical, biophysical, and biomedical questions related to cellular function and pathogenesis.

Applying Information about miRNAs from Developmental Biology to Treat Diseases: miRNA106a can Reverse Cardiomyocyte Hypertrophy by Targeting Genes Known to Cause Heart Failure

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Abstract

microRNAs have entered the causal fray of developmental defects. One function of miRNAs is to control protein 'amounts' in cells by binding to the 3'UTR of target mRNAs. As a result, if miRNAs are mis-expressed during development, protein homeostasis can be compromised resulting in defects in the development of organ systems. We previously showed using CRISPR/CAS9 technology that knocking out individual miRNAs that reside in the miRNA17 family inhibit differentiation of embryonic stem cells into the cardiac lineage. More importantly, we found that cardiac differentiation was rescued by adding back each miRNA in a dose-dependent manner. Interestingly, some genes that were affected have been implicated in heart failure when misregulated. These results led to the hypothesis that if these miRNAs can rescue heart development, then they should be able to rescue cardiac identity when lost to disease such as heart failure (HF). Causes and treatments for HF have been investigated for over a century. Unfortunately, to date, HF remains a progressive disease with no therapies targeting the cardiomyocytes directly. A recent paradigm shift for treating specific diseases has been the use of antisense technology (e.g., microRNAs) to molecularly target pathologies leading to disease onset. Although this paradigm shift was postulated over a decade ago, only within

the past few years has it become feasible. Here, we show that miRNA106a targets genes that have been shown to cause hypertrophy and eventual HF. Most importantly, using a cardiac targeting peptide reversibly linked to miRNA106a, we show delivery is specific to cardiomyocytes.

Decoding Single-cell Transcriptomic Phenotypes from the Cell Images Enabled by Robotic Data Acquisition and Deep Learning

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Abstract

Molecular cell phenotype identification is important for the investigation of the role of cells and for their medical applications. For this identification, single-cell whole-transcriptome analysis is the gold standard approach. However, this method kills the target cell, which prevents further investigation of the cell such as dynamics measurements. To overcome this open problem, we first developed a multifunctional robot, the Automated Live imaging and cell Picking System (ALPS), and used it to perform single-cell RNA sequencing for microscopically observed cells, *e.g.*, peripheral blood mononuclear cells, with multiple imaging modes. Using robotically obtained data that linked cell images and the whole transcriptome, we successfully predicted transcriptome-defined cell types and states using cell image-based deep learning. This noninvasive approach opens a new window to determine the live-cell whole transcriptome in real time. Moreover, this work, which is based on a data-driven approach, is a proof-of-concept for determining the transcriptome-defined (omics-based) phenotypes (i.e., not relying on specific genes) of any cell from cell images using a model trained on linked datasets.

Simultaneous Stabilization of Actin Cytoskeleton in Multiple Nephron-specific Cells Protects Kidney from Diverse Injury

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Abstract

Chronic kidney diseases and acute kidney injury are mechanistically distinct kidney diseases. While chronic kidney diseases are associated with podocyte injury, acute kidney injury affects renal tubular epithelial cells. Despite these differences, a cardinal feature of both acute and chronic kidney diseases is dysregulated actin cytoskeleton. We have shown that pharmacological activation of GTPase dynamin ameliorates podocyte injury in murine models of chronic kidney diseases by promoting actin polymerization. Here we establish dynamin's role in modulating stiffness and polarity of renal tubular epithelial cells by crosslinking actin filaments into branched networks. Activation of dynamin's crosslinking capability by a small molecule agonist stabilizes the actomyosin cortex of the apical membrane against injury, which in turn preserves renal function in various murine models of

acute kidney injury. Notably, a dynamin agonist simultaneously attenuates podocyte and tubular injury in the genetic murine model of Alport syndrome. Our study provides evidence for the feasibility and highlights the benefits of novel holistic nephron-protective therapies.

Common Signaling Principles and Interconnectivity Between the ISR-UPR Networks

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Abstract

The integrated stress response (ISR) and the unfolded protein response (UPR) are conserved signaling networks governed by stress sensor kinases. In the ISR, four stress sensor kinases, GCN2, HRI, PERK, and PKR, phosphorylate eIF2α to arrest global protein synthesis and promote translation of selective transcripts. Through this bipartite mechanism, the ISR reprograms the transcriptome and proteome. In the UPR, ER stress activates two stress sensor kinases, IRE1 and PERK, and a transcription factor, ATF6, to induce a gene expression program that reinstates ER homeostasis. Yet, common signaling principles and interconnectivity between the ISR and UPR stress sensor kinases are largely unknown. Here, we show that (i) PKR assembles into dynamic clusters to buffer ISR signaling and (ii) the ISR unconventionally activates IRE1 without a canonical UPR. PKR clusters are strongly reminiscent of the high-order assemblies of IRE1, and PERK observed during ER stress, hinting at a common signaling principle. Strikingly, eIF2α is not recruited to PKR clusters, and PKR cluster disruption enhances eIF2α phosphorylation. These results support a model in which PKR clusters operate as enzyme reservoirs to control PKR-eIF2α encounters. Remarkably, induction of the ISR selectively activates IRE1, thus coupling the ISR and the UPR outside their common node PERK. IRE1 activation does not require IRE1's sensor domain and is controlled by protein synthesis rate. Together, our data provide new mechanistic insights into fundamental aspects of stress sensor kinase signaling.

The Ubiquitin-proteasome System Assures Glomerular Filtration Barrier Integrity

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Abstract

Functionality of the kidney filtration barrier is ensured by the interaction of glomerular podocytes, endothelial and mesangial cells. A breakdown of kidney filtration with protein accumulations at the filtration barrier is pathognomonic for glomerular injury. The mechanisms that ensure glomerular filter patency and thus functionality are unknown. Here we identify proteasome processivity to be pivotal for filter function in a glomerular cell-type specific manner. State-of-the art transgenic and inhibitor-based *in vivo* human, pig, mouse, and *Drosophila* models were combined and analyzed by proteomic, complex biochemical, histologic, and live-imaging approaches. We demonstrate that filtration barrier integrity is ensured by the proteasome in glomerular cell-specific constitutions. While degradative activity depends on the constitutive proteasome in podocytes, glomerular endothelial cells depend on the immunoproteasome. Genetic immunoproteasome deficiency in endothelial cells as well as pan-proteasome inhibition in mice functionally and morphologically disrupt the filtration barrier in glomerular cell type specific modality, resulting in immunoglobulin deposition in a podocyte- or endothelial-specific pattern. Mechanistically, pulse-chase and live-imaging approaches identify reduced endocytic activity in the setting of proteasome alteration. Proteasome processivity affects endocytic activity in part by an altered regulation and membrane expression of endocytic receptors in glomerular cell types. These findings identify the proteasome as an orchestrator of glomerular filtration and protein clearance in a cell type specific pattern and may lead to new therapeutic principles in targeting disease-associated glomerular protein accumulations.

TRIP12 Ubiquitination of Glucocerebrosidase Contributes to Neurodegeneration in Parkinson's Disease

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Abstract

Glucocerebrosidase (GCase) impairment is strongly associated with Parkinson's disease (PD); however, the regulators responsible for this impairment are still unknown. We identify the E3 ligase Thyroid Hormone Receptor Interacting Protein 12 (TRIP12) as a key regulator of GCase. TRIP12 interacts with GCase and ubiquitinates it at lysine 293 to control its degradation through the ubiquitin proteasomal system. The ubiquitination of GCase by TRIP12 results in functional impairment, leading to premature degradation and the subsequent accumulation of α-synuclein. Overexpression of TRIP12 causes mitochondrial dysfunction, which can be ameliorated by GCase overexpression. Furthermore, conditional TRIP12 knockout in vitro and knockdown in vivo promotes the expression of GCase, which blocks α-synuclein preformed fibrils (α-syn PFFs)-provoked dopaminergic neurodegeneration. Additionally, TRIP12 accumulates in human PD brains and α-synuclein-based mouse models. The identification of TRIP12 as a regulator of GCase provides a new perspective on the molecular mechanisms underlying dysfunctional GCase-driven neurodegeneration in PD.

Roles of TMEM175 in Lysosomal pH Homeostasis and Parkinson's Diseases

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Abstract

Human TMEM175, a non-canonical potassium (K+) channel in endo-lysosomes, contributes to their pH stability and is implicated in the pathogenesis of Parkinson's diseases (PD). Structurally, the TMEM175 family exhibits an architecture distinct from canonical potassium channels, as it lacks the typical TVGYG selectivity filter. Here we show that human TMEM175 not only exhibits pH-dependent structural changes that reduce K+ permeation at acidic pH, but also displays proton permeation. TMEM175 constitutively conducts K+ at pH 7.4 but displays reduced K+ permeation at lower pH. In contrast, proton current through TMEM175 increases with decreasing pH due to the increased proton gradient. Molecular dynamics simulation, structure-based mutagenesis, and electrophysiological analysis suggest that K+ ions and protons share the same permeation pathway. The M393T variant of human TMEM175 associated with PD shows reduced function in both K+ and proton permeation. Together, our structural and physiological analysis reveal the roles of TMEM175 in lysosomal pH homeostasis and PD pathogenesis.

Diurnal Variation as an Endogenous Neuroprotection Mechanism in a Rat Model of TBI

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Abstract

Traumatic brain injury is a great public health problem that needs to be studied, mainly due to the consequences for patients who suffer some trauma. For its study, it has been described that TBI induces two types of brain damage: primary and secondary. The primary damage is considered irreversible and is generated at the moment of impact. On the other hand, secondary damage initiates a series of pathophysiological processes, such as oxidative stress, excitotoxicity, and neuroinflammation. The long-term perpetuation of these processes has deleterious consequences for neuronal function and survival. Previously in our laboratory, we showed a diurnal variation in the damage caused by a brain injury; we found a lower susceptibility to damage in rats subjected to TBI during the night compared to rats subjected during the day. We evaluated the effects induced by trauma at two different points during the light–dark cycle. We evaluated behavioral tests and found that rats subjected to trauma during the dark hours had a better behavioral score than those injured during the light hours. Then, we evaluated the histopathological damage using three different stains. We found less morphological damage in the perilesional zone (cerebral cortex), and specific areas of the hippocampus in rats that were subjected to TBI during the dark hours. Our results suggest that diurnal variation is a crucial determinant of the outcome after a TBI. The time of day when an injury occurs should be considered for future research.

Neuroprotective Roles of lncRNAs in Developmental Brain Injury

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Abstract

Alcohol consumption during pregnancy can have detrimental effects on fetal development, leading to a range of deficiencies collectively known as Fetal Alcohol Spectrum Disorders (FASD). Among the consequences of FASD is alcohol-induced developmental neurotoxicity (AIDN), which can result in cognitive impairment and behavioral problems throughout life, likely due to neuronal cell loss. The mechanisms underlying AIDN remain largely unknown, and there are currently no effective neuroprotective strategies available. Although animal studies have provided some direct evidence of AIDN, there is a lack of human models that can adequately emulate the developing brain. Recent

studies have demonstrated that human induced pluripotent stem cell-derived 3D cerebral organoids can closely mimic human fetal brain development at molecular, cellular, structural, and functional levels. This model has the potential to serve as a platform for investigating the pathogenesis of various neurological disorders and developing therapeutic approaches in vitro. Furthermore, brain organoids offer a promising approach for studying alcohol-induced brain injury. This presentation will cover two main topics. First, we will discuss the use of brain organoids to model anesthetic- and alcohol-induced developmental brain injury. Second, we will investigate lncRNA-mediated neuroprotective signaling in AIDN. Our findings not only confirm the versatility of brain organoids as models for AIDN but also provide novel insights into the lncRNA-related mechanisms underlying brain injury and potential neuroprotective strategies that may prevent or mitigate AIDN.

Is the Type II TGFβ Receptor the Achilles' Heel of TGFβ Signaling?

John Di Guglielmo

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Abstract

Transforming growth factor beta (TGFβ) promotes the metastasis of late-stage NSCLC disease and results and poor patient prognosis. We previously reported that pharmacological and pathway-specific inhibition of endocytosis and autophagy inhibit TGFβ signal transduction, and our most recent results suggest that this inhibition correlates strongly with the loss of steady state TβRII levels. Based on these observations, we hypothesize that inactivating TβRII will inhibit the TGFβ signaling pathways that induce EMT in NSCLC cells. We tested this using a 1, 4 dihydropyridine compound, termed Inhibitor of Type II TGFβ receptor which induces Degradation (ITD-1) and found that it inhibits TGFβ-dependent signaling and EMT in NSCLC cells via TGFβ type II receptor degradation. Future studies in NSCLC organoids, in the context of the tumor microenvironment will assess if the use of ITD-1 will target tumor cell metastatic potential.

Impacts of Microglial IRF4 and IRF5 on Ischemic Stroke

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Abstract

Microglia are an important source of inflammatory cytokines in neuroinflammation following stroke. Microglia becomes activated and causes secondary neuronal damage after stroke （pro-inflammatory), but also help in ischemic debris clearance and tissue recovery (anti-inflammatory). The mechanisms regulating the two opposite phenotypes of microglia are not clear. We have recently

found two interferon regulatory factors (IRF5 and IRF4) are involved in microglial pro- and anti-inflammatory responses. To determine the roles of microglial IRF4/5 signaling in cerebral ischemia, we performed in vitro (oxygen-glucose deprivation; OGD) and in vivo (middle cerebral artery occlusion; MCAO) ischemia model in primary microglial cultures and young (8-12 weeks old) transgenic mice respectively. The in vitro assays (western blots) revealed that the cytosolic IRF 4 and IRF5 were phosphorylated and translocated into the nucleus to regulate the production of anti-and pro-inflammatory cytokines respectively after OGD, a process that can be interrupted by inhibition of interleukin 1 receptor associated kinase 4 (IRAK4). Flow cytometry performed on stroke brains of microglial IRF4 conditional knockout (CKO) mice showed increased expression of pro-inflammatory (CD68, IL-1β) cytokines in microglia, whereas IRF5 CKO led to increased anti-inflammatory cytokines (CD206, IL-4). Worse or improved stroke outcomes were seen in IRF4 or IRF5 CKO mice respectively after MCAO. We conclude that the IRF4-IRF5 regulatory axis is a key determinant in microglial activation. The IRF4-IRF5 regulatory axis is a potential therapeutic target for neuroinflammation and ischemic stroke.

An Axonal Signal for Dendritic Maturation: Role of Neuregulin1 Nuclear Signaling in Assembly of Hippocampal Circuits

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Abstract

The Neuregulin1(Nrg1) -ErbB4 signaling axis is an important regulator of synaptic connectivity involving bi-directional signaling by both Nrg1 and ErbB4 - acting as receptors and ligands for each other. The Type III isoform of Nrg1 is uniquely localized to presynaptic sites in axons where it interacts with ErbB4 on dendritic postsynaptic sites. Upon interaction with ErbB4, the C-terminal transmembrane domain (TMc) of TypeIII Nrg1 can be cleaved by gamma secretase to produce a cytosolic intracellular domain (ICD), which translocates to the nucleus. A psychosis-associated missense mutation was identified in the TMc (V321L) which impaired processing by gamma secretase. We generated a transgenic mouse harboring the V321L mutation. Cultured neurons showed high levels of baseline nuclear ICD at 10 days in vitro (DIV) after which the levels of nuclear Nrg1 ICD declined. Mapping the axon-dendrite growth of wild-type (WT) and mutant neurons revealed that between DIV5 and DIV10 WT neurons undergo a period of axonal elaboration and axon-dendrite contact, a period correlating with maximal Nrg1 nuclear back signaling. After this, there was extensive dendritic branching between DIV10-14. To investigate this further, we stimulated DIV10 WT neurons with ErbB4 to identify the acutely regulated transcriptome by Nrg1 ICD. Overall, the gene expression signature pointed to suppression of axonal growth and a concurrent increase in

dendritic growth. Thus, we propose a model whereby axonal Nrg1 signals successful target contact to the nucleus to induce increases in dendritic complexity to complete circuit formation and synaptogenesis on both the afferent and efferent ends.

Clinically Validated Expression Profiling Strongly Suggests Loss of Structural Integrity and Failing Regenerative Repair as Important Driving Forces for the Multidimensional Progression of Chronic Obstructive Pulmonary Disease

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Abstract

Three yet unresolved characteristics of Chronic Obstructive Pulmonary Disease (COPD) have troubled cell biologists and physicians alike: its complex pathology driven by both bronchial inflammation and environmental hazards, its substantial individual heterogeneity, and finally, the sheer duration of its progress. It was the ECLIPSE study, which first demonstrated the benefits of a sequential analysis guided by clinical manifestations of COPD. By combining this valuable approach with a systematic genome-wide transcription analysis of lung tissue, we have been able to provide a new, integrated view on COPD pathology. The data suggest an incremental pathology driven by the biophysical and metabolic consequences of failing structural integrity causing relentless airway inflammation, vulnerability towards various airborne hazards and a mounting challenge for primary pulmonary repair. Hence, the clinical characteristics of COPD will only gradually unfold, largely owing to an individual's repair capacity lessening with age. However, only if the structural basis for organ regeneration, the integrity of extracellular matrix, will fail, all clinical and functional features of COPD are present. The complete failure of regenerative pulmonary restructuring only leaves one solution: regaining structuralintegrity by mechanisms of secondary repair, even at the prize of loss of function. Future research and development will now confirm and exploit this first integrative view on COPD pathology to establish individualized diagnosis and appropriate treatment of this complex pathology.

Early Immune Responses in Acute Pancreatitis and their Role in Predicting Disease Severity in a South African Cohort

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Abstract

Pancreatic cancer is one of the most aggressive solid malignancies characterized by chemoresistance and consequently low survival rates. Due to its heterogeneous microenvironment, standard chemotherapy such as gemcitabine, is not sufficiently efficacious. Herbal medicines such as Betulinic acid (BA) have shown anti-tumour potential, although with limitations. Polymer-drug conjugation has the potential to circumvent these limitations thereby increasing drug efficacy. This study aimed to determine the effect of the conjugation of a polymer (polyethylene glycol) to betulinic acid, PEG-BA, on pancreatic cancer cells. Cytotoxic analysis of the compounds on the MIA PaCa-2 and Vero cells showed that compared to gemcitabine (7.63±2.04 μM), PEG-BA treatment resulted in a dose-dependent cytotoxic effect and higher potency (3.01±0.62 μM; p-value = 0,0044) on MIA PaCa-2 cells. Furthermore, treatment with PEG-BA resulted in increased apoptosis in MIA PaCa-2 cells and induced a shift of the cells from G1 to sub-G1 phase compared to gemcitabine where the cells populated in the S-phase. The conjugate also reduced ROS levels in MIA PaCa-2 cells compared to BA and untreated cells. In conclusion, conjugation of BA to PEG improved cytotoxicity, potency, specificity, apoptosis induction, ROS reduction, and antioxidant activity in MIA PaCa-2 cells unlike Vero cells, suggesting its potential for effective therapy.

Hsa-microRNA-1249-3p/Homeobox A13 Axis Modulates the Human Epithelial Cell Clonogenicity

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Abstract

Hsa-miR-1249-3p is dysregulated in several cancers, including hepatocellular and breast carcinomas.1,2 However, its function in human epithelial cells is unknown.3 We functionally investigated the effect of hsa-miR-1249-3p on the proliferation, migration, clonogenicity and apoptosis of human epithelial cells and explored the underlying mechanism. DdPCR was used to evaluate the hsa-miR-1249-3p expression in keratinocyte cell lines HaCaT and NCTC and in control cervical carcinoma cell lines SiHa, CaSki and HeLa. Then, the hsa-miR-1249-3p mimic, inhibitor and negative/positive controls were transfected onto HaCaT cells. Upon transfections, cell proliferation, clonogenicity, migration and apoptosis were assessed by WST, clonogenic, wound healing and western blot assays, respectively. Hsa-miR-1249-3p resulted as overexpressed in HaCaT and NCTC cells, respectively, compared to cervical carcinoma cells. Hsa-miR-1249-3p resulted as undetectable in miR-inhibitor HaCaT condition, while being strongly overexpressed miR-mimic HaCaT, compared to untreated cells. Hsa-miR-1249-3p knockdown modestly favored cell proliferation and migration potential in HaCaT cells, without perturbing apoptosis. Contrariwise, a strong clonogenic effect was detected in hsa-miR-1249-3p-inhibited HaCaT cells. Furthermore, computational analyses identified the oncogene Homeobox A13 (HOXA13) as a hsa-miR-1249-3p downstream target. Mechanistically, hsa-miR-1249-3p inhibition prompted the up-regulation of HOXA13 transcript in HaCaT cells. Our

data indicate that hsa-miR-1249-3p can target HOXA13 to regulate the clonogenic potential of HaCaT cells. These data will allow the development of further studies aimed in investigating the role of has-miR-1249-3p/HOXA13 axis4 in epithelial cell clonogenicity, such as evaluating the relationship between this miRNA/target gene axis and its downstream genes implicated in cell-cell adhesion pathways, i.e., β-catenin, c-Met and c-Jun.

Uncovering New Functions of RNA Modifications in mRNA Processing

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Abstract

Emerging evidence indicates that eukaryotic messenger RNAs are extensively decorated with modified nucleosides that have the potential to regulate eukaryotic genes expression through effects on splicing, export, translation and decay. Our work revealed that pseudouridine is installed co-transcriptionally and thus has the potential to influence all stages in the mRNA life cycle[1]. Genetic manipulation of the pre-mRNA modifying pseudouridine synthases (PUS) induces thousands of alternative splicing and pre-mRNA 3′ end processing events in human cells. Consistent with such widespread PUS-dependent alternative splicing, pre-mRNAs are prevalently modified with pseudouridines, which are significantly enriched in introns flanking alternatively spliced regions, near splice sites and overlapping hundreds of binding sites for RNA-binding proteins. Individual pseudouridines identified in cells are sufficient to directly affect splicing outcome in nuclear extracts from human cells, demonstrating a direct mechanistic effect of endogenous pseudouridines on splicing. Pseudouridine is regulated across cell types and in response to cellular stress suggesting a broad regulatory role for this modification in controlling gene expression. This function of pseudouridine synthases in pre-mRNA processing could be implicated in the many diseases associated with human pseudouridine synthase dysregulation. Current work is focused on how PUS select sites for modification co-transcriptionally and how these mechanisms are dysregulated in diseases.

Local Activation of Focal Adhesion Kinase Orchestrates the Positioning of Presynaptic Scaffold Proteins and Ca^{2+} Signaling to Control Glucose Dependent Insulin Secretion

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Abstract

A developing understanding suggests that spatial compartmentalisation in pancreatic β cells is critical in controlling insulin secretion. To investigate the mechanisms, we have developed live-cell sub-cellular imaging methods using the mouse organotypic pancreatic slice. We demonstrate that the organotypic pancreatic slice, when compared with isolated islets, preserves intact β cell structure, and enhances glucose dependent Ca^{2+} responses and insulin secretion. Using the slice technique, we have discovered the essential role of local activation of integrins and the downstream component, focal adhesion kinase, in regulating β cells. Integrins and focal adhesion kinase are exclusively activated at the β cell capillary interface and using in situ and in vitro models we show their activation both positions presynaptic scaffold proteins, like ELKS and liprin, and regulates glucose dependent Ca2+ responses and insulin secretion. We conclude that focal adhesion kinase orchestrates the final steps of glucose dependent insulin secretion within the restricted domain where β cells contact the islet capillaries.

Skeletal Stem Cell Heterogeneity and Regulation in Bone Regeneration and Repair

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Abstract

Skeletal stem cells (SSCs) are known to be a heterogeneous population reside in periosteum and bone marrow and play an essential role in lifelong bone regeneration and bone injury repair. However, little is known about the functional difference and selective regulatory mechanisms of discrete SSCs in different locations. Here, by using a series of animal models that can selectively label periosteal SSCs (P-SSCs) and bone marrow SSCs (BM-SSCs), we recently identified that endogenous P-SSCs are present in the cambium layer of the periosteum and can be transiently labeled by *myxovirus resistance-1* (*Mx1*) with αSMA^{GFP} expression. These $Mx1^{+}\alpha SMA^{GFP+}$ periosteal cells are a subset of long-term repopulating stem cells that are responsible for periosteal osteoblasts and bone repair. Upon injury, P-SSCs, rather than BM-SSCs, rapidly relocate toward the injury site and supply the majority of osteoblasts in the injury callus. Conversely, local ablation of P-SSCs results in reduced osteoblasts at the injury site and delayed healing. Single cell RNA sequencing of human and mouse periosteal tissues revealed that periosteal cells display cell heterogeneity and are assigned to distinct clusters compared to bone marrow stromal cells. Further, P-SSCs are enriched in periosteal clusters that are distinct from bone marrow clusters and previously reported $Ctsk^{+}$ periosteal progenitor cells. These P-SSC clusters show higher expression of unique progenitor markers and injury responsive genes. In addition, human and mouse P-SSC clusters have a selective expression of a new marker, LRP1. These $LRP1^{+}$ human P-SSCs are perivascular cells with highly osteochondrogenic but little adipogenic differentiation abilities. Notably, they can maintain themselves *in vitro* and contribute to bone healing *in vivo* upon transplantation into mouse calvarial injury. Thus, our study demonstrates

that human and mouse periosteum contains distinct SSC subsets with a unique regulatory mechanism required for injury repair and may be a therapeutic source for fracture healing.

SSTR2 as an Anatomical Imaging Marker and a Safety Switch to Monitor and Manage CAR T Cell Toxicity: Clinical and Preclinical Study

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Abstract

Inability to determine the distribution and activity of adoptively transferred T cells in the body presents a great barrier to advancement of adoptive T cell therapy in cancer. We recently developed affinity-tuned chimeric antigen receptor (CAR) T cells against ICAM-1, and this CAR T cells are being tested against advanced thyroid cancer in a phase I clinical study (NCT04420754). To monitor CAR T cells in the body, we introduced a PET imaging marker, somatostatin receptor-2 (SSTR2) into CAR T cells, which has shown its ability to detect CAR T localization at tumors in patients with tumor response to therapy. To further demonstrate the utility of SSTR2 as a safety switch, we have shown that SSTR2 specific drug conjugate, PEN-221 (octreotate-maytansinoid) can rapidly eliminate CAR T cells, which concurred with rapid reversal of systemic toxicity induced by aberrant activation and expansion of CAR T cells. Our study demonstrate the first example of a genetic reporter SSTR2 that provides spatiotemporal activity of CAR T cells and serves as an imaging-guided safety switch.

Photobiomodulation at 660 nm and Its Effect on Inflammation and Apoptosis in Wounded Diabetic Fibroblast Models

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Abstract

Recalcitrant diabetic wounds occur due to prolonged inflammation and excess apoptosis of cells important to tissue repair. Cells exposed to light at various wavelengths (nm) and fluencies (J/cm2) undergo physiological processes that may enhance or impede tissue repair. We investigated the effect of photobiomodulation (PBM) (660 nm; 5 J/cm2) in the following fibroblast models: normal (N), normal wounded (NW), diabetic (D), diabetic wounded (DW) and D-galactose wounded (DGW).

Cellular morphology (light microscopy), levels of IL-6, cox-2 and sRAGE (ELISA), and the percentage apoptosis (flow cytometry) were determined at 0, 24 and 48h post-PBM. Control cells remained unirradiated. No morphological changes were observed between the control and experimental models, but PBM exposure accelerated wound closure in wounded models after 48h. IL-6 levels were significantly lower in the D and DW groups at 24h ($P \leq 0.05$ and $P \leq 0.001$, respectively) and 48h ($P \leq 0.05$), while cox-2 levels increased in the D and DW groups at 48h ($P \leq 0.05$ and $P \leq 0.01$, respectively) post-PBM. In the DW models, there were no significant differences in the percentage apoptosis between the control and PBM models across all three time periods. sRAGE levels were significantly elevated in the D ($P=0.044$) and DW ($P=0.003$) models at 48h post-PBM. Overall and irrespective of model, PBM significantly decreased sRAGE levels at 0 and 24h ($P \leq 0.001$ and $P=0.015$, respectively) followed by a significant increase in sRAGE at 48h ($P=0.005$). PBM may thus influence inflammation and apoptosis in wound repair through its effect on sRAGE, a decoy AGE receptor, and further investigation is recommended.

Ethnopharmacological Assessment of Medicinal Plant Extract in Ameliorating Cardiotoxicity and Nephrotoxicity in Experimental Animals

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Abstract

Natural therapeutics are widely investigated for their biological and pharmacological properties such as antioxidant, blood lipid reduction and anticancer. This study investigated the cardioprotective and nephroprotective effects of Azadirachta indica (AI) on isoproterenol induced cardiotoxicity and nephrotoxicity together with its possible molecular mechanism of action. There were five experimental groups, corn oil was administered orally for 14 days to Group A. 100mg/kg Isoproterenol (ISO) was administered intraperitoneally on days 15 & 16 respectively to Group B. Groups C and D received AI at 100 and 200mg/kg for 14 days followed by ISO at 100mg/kg intraperitoneally on days 15 & 16 respectively. Group E received Clofibrate orally at 300mg/kg for 14 days and ISO intraperitoneally on days 15 & 16 respectively. Blood pressure parameters, markers of oxidative stress, cardiac and renal damage were measured. The immunohistochemistry of cardiac and renal injury was also determined. Results showed significant elevation in the cardiac and renal oxidative stress markers and reduction in antioxidant status in ISO intoxicated rats. Immunohistochemistry revealed reduced expressions of PPARα and BCL2 in the cardiac and renal tissues of ISO-only treated rats. However, pre-treatment with AI mitigated both cardiac and renal oxidative stress. Reduced serum NO level and blood pressure were normalized in rats pre-treated with AI with higher expressions of PPARα and BCL2 in the cardiac and renal tissues respectively. Findings from this study suggest that pre-treatment with AI (100 and 200mg/kg) offered

cardioprotective and nephroprotective effects through reduction of oxidative stress, and up-regulation of PPARα and BCL2 signaling.

Targeting CFTR Post-translational Modifications for Cystic Fibrosis Therapy

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Abstract

Cystic fibrosis (CF) is a recessive disease caused by mutations on *CFTR*, which encodes the cystic fibrosis transmembrane conductance regulator, an ATP-gated anion channel mediating Cl^-/HCO_3^- exchange across the luminal surface of epithelial cells. The most common F508del-CFTR mutation is responsible for protein misfolding and aberrant trafficking to the plasma membrane, leading CFTR retention in the endoplasmic reticulum and degradation via the ubiquitin-proteasome system. Although the use of Trikafta represents the breakthrough therapy for CF patients carrying at least one F508del-CFTR allele, an improvement of its effectiveness and an extension of its use to CF patient harboring different *CFTR* variants are needed. Since CFTR is subjected to various post-translational modifications (PTMs), we evaluated the possibility to modulate these PTMs to enhance Trikafta efficacy for the functional recovery of diverse disease-associated CFTR mutants. We found that targeting specific E1/E2 ubiquitin-activating enzymes with inhibitors/siRNAs significantly increases the rescue of F508del and other rare misfolded CFTR mutants induced by Trikafta. Moreover, to test if favoring methylation over ubiquitination at CFTR lysine residues could increase the stability of the channel, we used a siRNA library against all the human demethylases and we identified specific demethylases whose downregulation enhances the stability of the F508del-CFTR and boosts its functional rescue induced by Trikafta. Our results show that counteracting the CFTR ubiquitination by targeting either specific enzymes of the ubiquitination pathway or specific demethylases leads to improve Trikafta therapy and this approach may smooth the path for an extension of Trikafta to low/non-responding rare misfolded CFTR mutants.

CFTR Correctors from Cystic Fibrosis to Muscular Dystrophy

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Abstract

Sarcoglycanopathies are muscular dystrophies caused by mutations in sarcoglycan (SG) coding genes. More than 65% of such mutations are missense mutations impacting folding and trafficking of the defective SGs, which although potentially functional, are recognized and discarded by the Endoplasmic reticulum associated degradation (ERAD) (2,3). We have proved that the application of small molecules developed to rescue folding and trafficking of the ΔF508-CFTR, also improved the maturation of several SG mutants that were consequently rescued at the plasma membrane (4-6). It is conceivable that CFTR correctors help the process of maturation of SG mutants by modulating the activity, composition and/or concentration of elements of the proteostasis network, eventually affecting the balance of the biosynthetic/degradative pathways and fostering the assembly of the SG-complex. Key step in drug discovery is the identification of the molecular target(s) and the mechanism(s) of action of the pharmacological compound. Therefore, we aim to unveil the mechanism of action of CFTR correctors and to identify their interactor(s) in sarcoglycanopathy. Starting from the structure of our most promising CFTR corrector, C17, we synthesized immobilized "baits" that were used, in a fishing for target approach, to recover the compound target(s) which will be identified by mass spectrometry and validated by biochemical assays. The results obtained indicate that corrector C17 directly or indirectly interacts to components of the ERAD pathway. These data may provide valuable insights into the mechanism of action of CFTR correctors fostering the identification of new therapeutic targets for protein misfolding diseases.

Targeting the Gut Microbiota-endocannabinoid System Crosstalk to Treat Rare and Untreatable Skeletal Muscle Disorders

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Abstract

Nothing is known about the potential implication of gut microbiota in skeletal muscle disorders. In this study, we provide evidence that faecal microbiota composition along with circulating levels of short-chain fatty acids (SCFAs, primarily Acetate, propionate and butyrate) and related metabolites

(ketone bodies, KB) are altered in the mdx mouse model of Duchenne muscular dystrophy (DMD) compared to healthy controls. Supplementation with sodium butyrate (NaB) in mdx mice rescued muscle strength and autophagy and prevented inflammation associated with excessive endocannabinoid signalling at CB1 receptors to the same extent as deflazacort (DFZ), the standard palliative care for DMD. In LPS-stimulated C2C12 myoblasts, NaB reduces inflammation, promotes autophagy and prevents dysregulation of microRNAs targeting the endocannabinoid CB1 receptor gene, in a manner depending on the activation of GPR109A and PPARγ receptors. In sum, we propose a novel disease-modifying approach in DMD that may have benefits also in other muscular dystrophies.

Arbovirus Morphogenesis and Pathogenesis: What does Transmission Electron Microscopy Teach Us?

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Abstract

Electron microscopy is the only technique that allows direct visualization of cellular structures at the ultrastructural level. Despite the advancement of molecular biology techniques, morphological studies using transmission electron microscopy (TEM) are still being of great importance to elucidate some aspects of viral structures, morphogenesis, and pathogenesis. In addition to cell cultures and cell suspensions, organ and tissue fragments, both from animal models and human patients, can be analyzed using TEM techniques when infected. Arboviruses (arthropod-borne viruses), such as Yellow fever virus, Zika virus, Dengue virus and Chikungunya virus have high importance in the context of global public health, especially considering climate change and uncontrolled urbanization. In this context, several studies report the use of TEM to obtain a clearer definition of viral morphology and the events involved in its morphogenesis. Tissue samples from infected animals or biopsies from fatal cases offer valuable information to elucidate several aspects of the pathogenesis and tropism of these viruses. The preparation protocol for TEM varies depending upon the type of sample to be analyzed. Basically, the sample preparation method consists of chemical fixation using glutaraldehyde or diluted Karnovsky fixative, post-fixation in osmium tetroxide, dehydration in acetone, and inclusion in epoxy resin. The samples are sliced by ultramicrotomy to ultrathin sections that are contrasted with heavy metals, uranyl acetate and lead citrate being the most commonly used. Viral suspensions can also be viewed using the negative contrast technique

The Role of BRD7 in the Alternative Insulin Signaling Pathway

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Abstract

Bromodomain-containing protein 7 (BRD7) has been identified as a regulator of glucose homeostasis. In mice with obesity, BRD7 levels are decreased in the liver, and restoring hepatic BRD7 levels improves glucose homeostasis and establishes euglycemia. Of interest, increasing BRD7 levels in the liver activates AKT as determined by phosphorylation at Thr308 and Ser473 residues in response to insulin, but it does not increase the sensitivity of the insulin receptor-insulin receptor substrate (IRS) axis. Additionally, BRD7 increases the phosphorylation of GSK3β even in the absence of AKT. These observations led to the hypothesis that BRD7 might have a dual role in the putative alternative insulin receptor signaling pathways. To investigate this, liver-specific insulin receptor knockout mice were used to understand whether BRD7 is a downstream molecule of the insulin receptor, and liver-specific IRS1/2 knockout mice were used to determine if the presence of IRS1/2 proteins is necessary for BRD7's action. The results suggest that BRD7 responds downstream of insulin receptors and that there is an alternative insulin signaling pathway that operates independently of IRS1/2, providing new insight into this previously unknown pathway.

Nutrient Sensing and Hormonal Control of Metabolism

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Abstract

Proteolytic processing of secreted peptides is a critical physiological process that regulates a wide range of fundamental biological functions. However, detecting and characterizing these peptides are challenging. Here, we introduce a computational method that identifies 2,600+ uncharacterized proteolytic cleavage fragments in the human secretome. This approach led to the discovery of BRINP2-Related Peptide (BRP), a 12-mer peptide that circulates endogenously in humans and centrally modulates food intake and obesity without impacting insulin release, energy expenditure, locomotor activity, or anxiety-like behavior in both rodent and non-rodent models. BRP induces CREB phosphorylation and *cfos* expression, with signaling abrogated by C-terminal α-amidation absence and an L-A substitution at residue 8. Notably, BRP functions independently of known hypophagic peptides such as leptin, glucagon-like receptor-1, and melanocortin-4 receptor, suggesting a non-canonical pathway for the fine-tuned regulation of satiety. The discovery of BRP and its unique mode of action offer exciting new opportunities for targeted therapeutic obesity interventions.

Dynamic Regulation of Cap-independent Translation upon Pathogen Infection

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Abstract

Protein translation is the final step of the central dogma of biology, and regulation at this step can immediately change protein levels and allows for a rapid response to physiological changes, such as plant immune responses to pathogen infection. Following stress, eukaryotes typically inhibit canonical translation initiation while selectively translating essential stress regulators through GCN2-mediated phosphorylation of the eukaryotic translation initiation factor eIF2α to reprogram their translatome. However, completely blocking eIF2α phosphorylation by knocking out GCN2 did not significantly affect plant translatome and pattern-triggered immunity (PTI), indicating a new translatome reprogramming mechanism in the plant PTI signaling pathway. My talk will focus on how plants shift their translatome from cap-dependent translation to purine rich element (R-motif)-mediated cap-independent translation to fend off pathogen infection by MPK3/6-mediated phosphorylation of PABP, eIF4G, and eIFiso4G.

Armed Oncolytic Viruses Activate Tumor-reactive Cytotoxic T Cells by Converting Tumors to Artificial Antigen-presenting Cells *In Situ*

Hongkai Zhang

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Abstract

The full potential of tumor-infiltrating lymphocyte (TIL) therapy has been hampered by the inadequate activation and low persistence of TILs, as well as inefficient neoantigen presentation by tumors. We transformed tumor cells into artificial antigen-presenting cells (aAPCs) by infecting tumor cells with a herpes simplex virus 1 (HSV-1)-based oncolytic virus encoding OX40L and IL12 (OV-OX40L/IL12) to provide local signals for optimum T cell activation. Combining OV-OX40L/IL12 and TIL therapy induced complete tumor regression in patient-derived xenograft and syngeneic mouse tumor models and elicited an antitumor immunological memory. In addition, a comparative study of GM-CSF-armed oncolytic virus , CD40L-armed oncolytic virus and OX40L/IL12-armed oncolytic virus was performed and the mechanism of actions of these oncolytic viruses were comprehensively studied using scRNA-seq of immune cells infiltrating tumor and tumor draining lymph nodes. The data demonstrates that the strategy of converting tumors to aAPC is highly efficient to reprogram tumor microenvironment and unleash the full potential of TIL therapy, which warrants further evaluation in clinical study.

Deciphering Transcription Factor Binding Dynamics for an Endogenous Gene Locus in a Single Cell

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Abstract

Gene transcription is controlled by the binding of transcription factors (TFs) to specific DNA regulatory sequences. However, the kinetic mechanism underlying this process remains elusive due to the challenge of detecting TF binding signals of individual gene loci in a single cell. Here, we develop a single-molecule fluorescent imaging method to quantify the absolute molecule number of bound TFs, epigenetically modified histones, and nascent mRNAs at individual *hunchback* (*hb*) gene loci in early *Drosophila* embryos. We find that key TFs involved in *hb* regulation create a broad distribution of binding states through nonequilibrium cooperative binding, which is associated with the deprivation of specific histone modifications. Using theoretical analysis, we decipher how different TF binding states modulate the stochastic kinetics of *hb* transcription. These results provide a general framework for uncovering the mechanisms of complex gene regulation at the single-cell and single-molecule level.

High-density Lipoprotein Regulates Angiogenesis by Affecting Autophagy via MiRNA-181a-5p

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Abstract

Normal high-density lipoprotein (nHDL) can promote angiogenesis, whereas HDL from patients with coronary artery disease (dHDL) is dysfunctional and impairs angiogenesis. Autophagy plays a critical role in angiogenesis, and HDL regulates autophagy. Whether nHDL and dHDL regulate angiogenesis by affecting autophagy remains unknown. Endothelial cells (ECs) were treated with nHDL and dHDL with or without an autophagy inhibitor. Autophagy, endothelial nitric oxide synthase (eNOS) expression, miRNA expression, nitric oxide (NO) production, superoxide anion ($O_2^{\bullet-}$) generation, angiogenesis were evaluated. nHDL suppressed miR-181a-5p expression, which promotes autophagy and eNOS expression, resulting in NO production and the inhibition of $O_2^{\bullet-}$ generation, and ultimately increasing angiogenesis. dHDL showed opposite effects comparing to nHDL and ultimately inhibiting angiogenesis. We found that autophagy-related protein 5(ATG5) was a direct target of miR-181a-5p. ATG5 silencing or miR-181a-5p mimic inhibited nHDL-induced autophagy, eNOS expression, NO production, angiogenesis, and enhanced $O_2^{\bullet-}$ generation, whereas overexpression of ATG5 or miR-181a-5p inhibitor reversed the above effects of dHDL. ATG5 expression and angiogenesis were decreased in the ischemic lower limbs of hypercholesterolemic low-density lipoprotein receptor null ($LDLr^{-/-}$) mice when compared to C57BL/6 mice. ATG5 overexpression improved angiogenesis in ischemic hypercholesterolemic $LDLr^{-/-}$ mice. Taken together, nHDL was able to stimulate autophagy by suppressing miR-181a-5p, subsequently increasing eNOS expression, which generated NO and promoted angiogenesis. In contrast, dHDL inhibited angiogenesis by increasing miR-181a-5p expression, which decreased autophagy and eNOS expression, resulting in a decrease in NO production and an increase in $O_2^{\bullet-}$generation. Our findings reveal a novel mechanism by which HDL affects angiogenesis by regulating autophagy.

Alternative Mammalian Strategies Leading Toward Gastrulation: Losing Polar Trophoblast or Gaining an Epiblast Cavity

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Abstract

In mouse embryos reciprocal interactions between the epiblast and the extraembryonic trophoblast are necessary for the induction of gastrulation. Evidence from cattle will be presented that such inductive interactions have to be restricted to the edge of the epiblast to avoid ectopic induction of gastrulation. This will be interpreted according to the central epiblast shielding hypothesis which posits that all eutherian mammals developed varied strategies for shielding the central epiblast from inductive interactions from extraembryonic tissues. One such strategy, seen in Rauber's layer mammals, is the loss of the polar trophoblast, thus precluding ectopic signalling to the central epiblast. A second strategy, seen in mammals retaining the polar trophoblast, is the formation of a cavity within the epiblast. The roof of the cavity (the amniotic ectoderm) as well as the (amniotic) cavity per se are suggested to prevent reciprocal signalling between the trophoblast and the epiblast. Thus, the ancestral function of epiblast cavitation may lie in central epiblast shielding, as opposed to formation

of the amnion which is a secondarily acquired function. Indeed, all mammals retaining the polar trophoblast exhibit epiblast cavitation while Rauber's layer animals do not. Lastly it is argued that some mammals have adapted to use the roof of the epiblast cavity to establish the gastrulation-inductive reciprocal interactions normally provided by the polar trophoblast.

Selection Pressure Behind the PfHRP2/3 Deletions and Drug Resistance in Antimalarial: Are They Correlated

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Abstract

The WHO recommends Plasmodium parasite surveillance to eliminate malaria in malaria-endemic areas. A recent study found that Peru's parasite lineages have gained resistance to sulfadoxine-pyrimethamine (sextuple dhfr/dhps mutant) and chloroquine (SVMNT in CRT and NDFCDY in mdr1) and could evade HRP2-based RDTs after 2008. In 2006, BV1 lineage cases (3/62) from Peru were reported with sextuple dhfr/dhps, CRT SVMNT, mdr1 NDFCDY, and both hrp2 and hrp3 deleted. After 2008, the BV1 lineage and hybrids dominate Peru's parasite population. PfHRP2-based RDTs may potentially promote parasite lineages without the gene.Peruvian health facilities had microscopic analysis, hence RDTs were rarely used. The limited usage of PfHRP2-based RDTs in Peru between 1998 and 2006 did not select a parasite population without pfhrp2.Drug and diagnostic resistance is rising in Peruvian *P. falciparum*. This suggests that the parasite population is under drug pressure. Our recent work discovered *P. falciparum* in 85 of 203 samples by Microscopy and PCR. Three Pf-HRP2 RDT-negative samples had Pfhrp2/3 gene deletions. Another investigation in Pf positive RDTs (n=240) demonstrated Pfhrp2 gene (exon2) deletion in a single sample with Pfhrp3 exon2. Pfhrp3 gene deletion was 4.2%. Pfhrp2 (exon2–0.4%, upstream 25.8%, downstream 9.1%) and Pfhrp3 genes have partial deletions (exon2–18.75 %, upstream - 22.08 % and downstream 13.3 %). This study found many causes for pfhrp2 deletion, although it is unclear if a selective pressure promotes parasites without it. Pfhrp2-deleted parasites may be spreading for numerous reasons. Drug resistance and pfhrp2 deletion must be addressed. It could help end malaria.

Using Model Organisms to Approach Heart Regeneration

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Abstract

Zebrafish and neonatal mice have become powerful animal models for regenerative studies because of their regenerative capacity after injury. Understanding the cellular and molecular mechanisms on their heart regeneration holds great potential for priming adult mammalian heart for regeneration. By RNA-Seq and single-cell RNA-Seq analyses, we identified several new signaling molecules for

promoting heart regeneration in zebrafish, such as Dusp6 as a regenerative repressor in zebrafish (Han et al., 2014 Cell Research); and our recent work reveals that the rat Dusp6-MAPK pathway fine-tunes neutrophil- and macrophage-mediated cardiac damage and fibrosis (Zhou et al., 2022 Nature Communications; Zhang et al., 2023 Disease Models & Mechanisms). By performing a chemical screening with neonatal rat cardiomyocytes for pro-regenerative small molecules, we identify a chemical cocktail 5SM that promotes mammalian cardiomyocyte proliferation and heart regeneration (Du et al., 2022 Cell Stem Cell). Together, our published works and ongoing research will shed light on both fundamental mechanisms of heart regeneration and translational implication on heart disease.

An Immunomodulatory Polypeptide Hydrogel for Osteochondral Defect Repair

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Abstract

Osteochondral defect is a one of the major causes for osteoarthristis, a leading disease affecting more than 32.5 million populations in United States. Owing to the limited ability in self-healing of cartilage tissues, stem cell-based tissue engineering holds great promise for osteochondral defect repairing. However, there still lacks hydrogel materials that can meet the high standard of clinical demand in terms of biodegradability, biocompatibility, and immunogenicity. The commercially available and most widely used biopolymer-based scaffolds such as GelMA suffer from heterogeneous compositions, inconsistent performances due to batch variations, and inherent or impurity-caused immunogenicity. Synthetic polymer hydrogel materials such as PEG, however, are often nondegradable, worrisomely, can stimulate immune response and foreign body reactions (FBR). By employing a concise synthetic approach, we facilely prepared a novel polypept(o)ide-based hydrogel (named PAA-RGD) with suitable biodegradability, excellent biocompatibility, and minimized immunogenicity. The implanted PAA-RGD hydrogel led to outstanding performances for osteochondral repairing in New Zealand white rabbits that outperformed both GelMA and PEG-RGD hydrogels. Remarkably, the repairing effect of PAA-RGD was even prominent at early stage of implantation. Detailed in vitro and in vivo mechanistic investigations together revealed the least FBR responses and the most polarization of macrophages into the immunosuppressive M2 subtypes for the PAA-RGD hydrogels compared to GelMA and PEG-RGD groups. Those findings demonstrate the

promising potential of PAA-RGD hydrogel for osteochondral regeneration and highlight the importance of immunomodulation. The results facilitate the development of PAA-based materials for osteochondral defect repair and other PAA-based materials for various tissue engineering and bio-implantation applications.

Bone Marrow-derived Nestin-GFP+ Progenitor Cells Persist in Immunologically Complete Recipients Immunized Against GFP

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Abstract

The ability of cells and tissues of an organism to evade systematically immune surveillance under various circumstances is called immune privileges (IPs). IPs are a major concern in cancer therapy and particularly IPs of cancer stem cells (CSCs). Multiple approaches are developed to overcome different mechanisms of CSCs' IPs. There are also reports demonstrating IPs of variable strength for non-pathological stem cells (SCs) (doi: 10.3389/fcell.2022.993056). We strengthen previous results demonstrating strong immune privileges of MSCs. We report that IPs of MSCs and other Nestin-GFP+ progenitor cells from bone marrow (BM) of transgenic mice are strong enough to provide protection from uncompromised immune system of immunized by GFP non-transgenic recipient for over 6 weeks in a model of BM implantation to the subcapsular region of the kidney. We report retention of the ability of the primary transgeneic ectopic foci to form and support the secondary hematopoietic territory after retransplantation, which is associated with preserved MSCs functionality. We hypothesize that nestin could be a marker for a wide range of quiescent SCs with strong IPs, including CSCs.

Rad1-depedent and Independent Role of the Rad9-rad1-hus1 Complex in Drosophila Meiosis

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Abstract

The 9-1-1 complex, comprising the Rad9, Hus1 and Rad1 proteins, is thought to act as part of a DNA damage checkpoint pathway. Our initial analysis on the *hus1* gene, showed that Hus1 has dual role in meiosis by regulating both meiotic DNA damage checkpoint and homologous recombination repair. In this study we further analyze the role of the other two proteins in the complex, namely

Rad9 and Rad1, in meiosis. Using CRISPR/Cas9, we generated flies mutants for *rad9* and *rad1*. We found that in similarly to *hus1*, mutations in *rad9* and *rad1* lead to female sterility. Also, double-strand DNA break (DSB) that form during meiosis are not processed efficiently and the DNA within the oocyte nucleus fail to form its characterized shape. On the other hand, in difference from the ability of mutation in *hus1* to activate the meiotic checkpoint elicited in DSB repair enzyme mutants, mutation in both *rad9* and *rad1* fail to do so. Interestingly, eliminating checkpoint activity through mutations in DmChk2, suppress the oocyte nucleus defects in *rad9* but not in *rad1* mutants. Our results suggest that the Drosophila 9-1-1 complex is required for meiosis homologous recombination repair. On the other hand, only Hus1 regulate the meiotic DNA damage checkpoint, and un-repaired DSB in both in hus1 and Rad9 but not in Rad1, activate a DmChk2 dependent DNA damage checkpoint. Thus, our results showed that the 9-1-1 complex may function together in DSB repair but also suggest that each of the proteins might have complex-independent role.

Homophilic ATP1A1 Binding Induces Activin A Secretion to Promote EMT of Tumor Cells and Myofibroblast Activation

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Abstract

Tumor cells with diverse phenotypes and biological behaviors are influenced by stromal cells through secretory factors or direct cell-cell contact. Pancreatic ductal adenocarcinoma (PDAC) is characterized by extensive desmoplasia with fibroblasts as the major cell type. In the present study, we observed enrichment of myofibroblasts in a juxta-tumoral position with tumor cells undergoing epithelial-mesenchymal transition (EMT) that facilitated invasion and correlated with a worse clinical prognosis in PDAC patients. Direct cell-cell contacts forming heterocellular aggregates between fibroblasts and tumor cells were detected in primary pancreatic tumors and circulating tumor microemboli (CTM). Mechanistically, the overexpressed ATP1A1 of tumor cells binds to and reorganizes ATP1A1 of fibroblasts inducing calcium oscillations, NF-κB activation, and activin A secretion. Silencing ATP1A1 expression or neutralizing activin A secretion suppressed tumor

invasion and colonization. Taken together, these results elucidate the direct interplay between tumor cells and bound fibroblasts in PDAC progression, thereby providing potential therapeutic opportunities for inhibiting metastasis by interfering with these cell-cell interactions.

Role of Anoctamin 7 in Prostate Cancer

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Abstract

Prostate cancer (PrCa) is the most common cancer and the third most common cause of death among European men. PrCa has a wide spectrum of clinical behavior that ranges from decades of indolence to rapid metastatic progression and lethality – with 57% of the disease risk being attributed to genetic factors. Since the majority of PrCa cases do not progress to lethality, biological markers that can distinguish between potentially lethal and non-aggressive cases would greatly improve prognostication and guide treatment decisions. The genomic position at 2q37 is a known risk locus for PrCa, originally identified by us using genetic linkage analysisin high-risk PrCa families. More recently it has given highly positive signals in several vast genome wide association studies, in all of which the hit has been in the prostate specific Anoctamin 7 (ANO7) gene. We have identified two SNPs, rs77559646 and rs148609049, in ANO7 that are associated with an increased risk for aggressive PrCa, biochemical relapse and poor survival, making them good causal candidates. Most recently, we showed that rs77559646 severely disrupts mRNA splicing, and immunohistochemical analysis of prostate samples from patients homozygous for the variant allele demonstrated a striking loss of apically localized ANO7 protein. Based on the findings, we suggest that loss of the ANO7 gene contributes to PrCa progression.

Genetic Interactions in Lymphoma Evolution

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Abstract

The majority of sequencing of clinical tumor samples is analysis of a single biopsy at a single timepoint, and differentiating between early and late mutations is achieved through quantifying the variant allele frequencies i.e., the relative clonal outgrowth of mutations. We and others have seen evidence that early clonal mutations modify selection of subsequent sub clonal mutations at other loci, but quantifying these effects and proving causal relationships requires new methodologies. Our model data for these studies is mutations isolated from insertional mutagenesis screens in mice. Mouse lymphomas generated by murine leukemia virus infection accumulate virus integrations that deregulate the expression of adjacent genes. In sufficient numbers these integration mutations cause lymphoid malignancies with 100% penetrance. Integration mutations are readily quantified over three orders of magnitude, and this permits sensitive detection of highly sub clonal mutations from small populations of cells. Associations between mutations (co-operativity and mutual exclusivity) are traditionally identified by contingency table tests. These tests have limitations in creating false positives when finding associations between clonal and sub clonal mutations. To address this, we develop alternative methods where mutations are shuffled between tumor samples whilst constraining for both mutation number and clonality in each sample. Significance of associations is identified by comparing real data to permutations and this avoids false positives and is more conservative than traditional contingency table test approaches. Shuffling constraints can also be placed on subtle tumor phenotype differences such that larger cohorts can be analyzed in concert without creating false associations due to unrecognized heterogeneity.

Melanoma Differentiation Associated Gene-9/Syndecan Binding Protein (MDA-9/SDCBP) Promotes Hepatocellular Carcinoma (HCC)

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Abstract

The oncogene Melanoma differentiation associated gene-9/syndecan binding protein (MDA-9/SDCBP) is overexpressed in many cancers, promoting aggressive, metastatic disease. However, the role of MDA-9 in regulating hepatocellular carcinoma (HCC) has not been well studied. To unravel the function of MDA-9 in HCC, we generated and characterized a transgenic mouse with hepatocyte-specific overexpression of MDA-9 (Alb/MDA-9). Compared with wild-type (WT) littermates, Alb/MDA-9 mice demonstrated significantly higher incidence of N-nitrosodiethylamine/phenobarbital-induced HCC, with marked activation and infiltration of macrophages. RNA sequencing (RNA-seq) in naive WT and Alb/MDA-9 hepatocytes identified activation of signaling pathways associated with invasion, angiogenesis, and inflammation, especially NF-κB and integrin-linked kinase signaling pathways. In nonparenchymal cells purified from naive livers, single-cell RNA-seq showed activation of Kupffer cells and macrophages in Alb/MDA-9 mice versus WT mice. A robust increase in the expression of Secreted phosphoprotein 1

(Spp1/osteopontin) was observed upon overexpression of MDA-9. Inhibition of NF-κB pathway blocked MDA-9-induced Spp1 induction and knock down of Spp1 resulted in inhibition of MDA-9-induced macrophage migration, as well as angiogenesis. Alb/MDA-9 is a novel mouse model with MDA-9 overexpression in any tissue type. Our findings unravel an HCC-promoting role of MDA-9 mediated by NF-κB and Spp1 and support the rationale of using MDA-9 inhibitors as a potential treatment for aggressive HCC.

Crizotinib Improves the Therapeutic Efficacy of PARP Inhibitors in Ovarian Cancer

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Abstract

Purpose: Autophagy is induced by multiple anti-cancer agents including poly (ADP-ribose) polymerase inhibitors (PARPi). The ALK-inhibitor crizotinib can inhibit growth of autophagic cancer cells and we have asked whether crizotinib could enhance the activity of PARPi by targeting drug-induced autophagic ovarian cancer cells.

Experimental Design: The effects of olaparib, crizotinib and their combination was measured on growth of ovarian cancer cells in cultures and xenografts. Autophagy was examined by measuring LC3 positive puncta using immunofluorescence staining and transmission electron microscopy. LC3BI/II conversion as well as phosphorylation of AKT and mTOR were measured by Western Blot analysis. Cellular oxidative stress and DNA damage were determined by assessing ROS and γ-H2AX lesions, respectively.

Results: Crizotinib enhanced the anti-tumor activity of PARPi in multiple ovarian cancer cell lines, including those resistant to olaparib. This effect was also observed in patient-derived ovarian cancer organoids and in xenograft models. Mechanistically, the combination of olaparib and crizotinib increased levels of reactive oxygen species (ROS), induced DNA damage, decreased the phosphorylation of AKT and mTOR, leading to increased olaparib-induced autophagy and apoptosis. Pharmacolgic and genetic inhibition of autophagy decreased the sensitivity of ovarian cancer cells to treatment with olaparib and crizotinib, indicating that autophagy plays a role in cell death. Blocking ROS production reduced olaparib/crizotinib-induced autophagy and cell death while restoring levels of phosphorylated AKT.

Conclusions: Crizotinib can improve the therapeutic efficacy of olaparib in ovarian cancer by enhancing autophagy, potentially offering a novel treatment approach for improving outcomes of the patients with ovarian cancer.

Molecular and Biochemical Characterization of the Most Prevalent Missense Mutations Causing SLC13A5 Epilepsy

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Abstract

The sodium-coupled citrate transporter (NaCT) is a plasma membrane transporter, which mediates the symport of sodium and the carboxylate citrate into cells. NaCT is expressed in the liver, testis, brain, bone, and teeth, where citrate plays key roles in the synthesis of neurotransmitters, cholesterol, and fatty acids, the generation of energy, and teeth/bone mineralization. In humans, loss-of-function mutations in SLC13A5, the NaCT gene, cause early infantile epileptic encephalopathy type-25 (SLC13A5-Epilepsy), which leads to epilepsy, impaired speech, limited motor skills, developmental delay, and tooth defects. Currently, there is no treatment for SLC13A5-Epilepsy. Recently, the cryo-electron microscopy structure of the human NaCT was solved in an inward-facing conformation. We classified 22 NaCT missense disease-causing mutations based on their localizations in the 3D structure. Class I mutations interfere with the transport function by blocking the elevator-type mechanism for substrate translocation. Class II cause defects in protein folding and protein trafficking to the cell surface. As there are not NaCT-specific antibodies, we expressed WT and the mutants with specific epitopes to facilitate detection, which didn't interfere with the presentation of the mutant phenotypes. The Class I mutations C50R, T142M, and T227M displayed protein and surface expression levels similar to WT. Class II mutants G219R, S427L, and L488P showed significantly decreased protein expression and no plasma membrane expression. Both classes displayed diminished transport activity. These experiments have brought us one step closer to understanding the defects of disease-causing mutations at the molecular level, allowing us to begin dissecting NaCT trafficking pathway(s).

Bromodomain Inhibition of EP300/CBP as a Therapeutic Strategy to Target MYC in Group 3 Medulloblastoma

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Abstract

EP300/CBP are paralogous, multidomain histone acetyltransferases (HATs) that regulate transcription by establishing histome marks at enhancer and promoter. EP300 and CBP also function to promote the expression of tumor-selective oncogenes such as the transcription factor *c-MYC*. This activity makes these proteins attractive targets for preclinical therapeutic development. EP300/CBP proteins contain highly homologous domains, including catalytic HAT domains and bromodomains (BRDs). However, the relative contribution of these distinct domains to tumor cell proliferation remains undetermined. To identify the role of the EP300/CBP BRD and HAT domains in cancer, we performed a cell viability-based high-throughput screen of the potent EP300/CBP BRD- and HAT-specific inhibitor, CCS1477 and A-485 respectively in 454 barcoded cancer cell lines. While most tumor lineages were nearly equivalently suppressed by either BRD or HAT domain inhibition, surprisingly, Group 3 medulloblastoma (G3MB) cell lines were the most sensitive to EP300/CBP BRD inhibition, compared to HAT inhibition. Using biochemical and structural assays, we identified that CCS1477 was highly specific for the EP300/CBP bromodomain, with limited off-target effects on other bromodomain-containing proteins in G3MB. Mechanistically, treatment of MB cells with CCS1477, but not A-485 or the BRD4 inhibitor JQ1, caused rapid early loss of mRNA expression of specific G3MB dependency networks including the driver oncogene c-*MYC*. These studies identify a selective role for the EP300/CBP bromodomain in maintaining genetic dependency networks in G3MB cells and provide new chemical approaches to disrupting malignant transcription in Group 3 medulloblastoma.

The Longevity Response to Warm Temperature is Neurally Controlled via the Regulation of Collagen Genes

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Abstract

Studies in diverse species have associated higher temperatures with shorter lifespan and lower temperatures with longer lifespan. These inverse effects of temperature on longevity are traditionally explained using the rate of living theory, which posits that higher temperatures increase chemical reaction rates, thus speeding up the aging process. Recent studies have identified specific molecules and cells that affect the longevity response to temperature, indicating that this response is regulated, not simply thermodynamic. Here, we demonstrate that in *Caenorhabditis elegans*, functional loss of NPR-8, a G protein-coupled receptor related to mammalian neuropeptide Y receptors, increases worm lifespan at 25°C but not at 20°C or 15°C, and that the lifespan extension at 25°C is regulated by the NPR-8-expressing AWB and AWC chemosensory neurons as well as AFD thermosensory neurons. Integrative transcriptomic analyses revealed that both warm temperature and old age profoundly alter gene expression and that genes involved in the metabolic and biosynthetic processes increase expression at 25°C relative to 20°C, indicating elevated metabolism at warm temperature. These data demonstrate that the temperature-induced longevity response is neurally regulated and also provide a partial molecular basis for the rate of living theory, suggesting that these two views are not mutually exclusive. Genetic manipulation and functional assays further uncovered that the NPR-8-dependent longevity response to warm temperature is achieved by regulating the expression of a subset of collagen genes. As increased collagen expression is a common feature of many lifespan-extending interventions and enhanced stress resistance, collagen expression could be critical for healthy aging.

Neuronal GPCR NMUR-1 Regulates Distinct Immune Responses to Different Pathogens

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Abstract

The pathogen-triggered host immune response is a multilayered process programmed to fight invading microorganisms and maintain healthy homeostasis. Using the *C. elegans* model system, we have demonstrated that functional loss of NMUR-1, a G protein-coupled receptor homolog to mammalian receptors for Neuromedin U, has distinct effects on *C. elegans* innate immunity to different pathogens. By focusing on two pathogens *Enterococcus faecalis* and *Salmonella enterica* which have opposite effects on *nmur-1* animal survival, we have shown that NMUR-1 mediates distinct immune gene expression in response to different pathogens. Overall, our study uncovered a molecular basis for the specificity of *C. elegans* innate immunity that could potentially provide mechanistic insights into understanding the specificity of innate immune responses in vertebrates.

Huntington's Disease is a Matter of Recycling

Xueyi Li

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Abstract

Huntington's disease (HD) is a hereditary but slowly progressive neurodegenerative disease characterized by uncontrolled dancing-like movements and early preferential degeneration of neurons in the striatum. Persons bearing the causative mutation of HD usually manifest physical symptoms in midlife and die around 20 years after. At present, no treatment is available to slow the progression of HD. Exactly how the mutation leads to HD is still under investigation but is thought to involve defects in multiple cellular pathways including gene transcription deregulation, impaired protein clearance, oxidative stress, defective energy metabolism, and vesicular trafficking perturbation. We have found that the HD mutation interferes with the vesicle dynamics at the recycling endosome by constraining a factor that activates Rab11, the gatekeeper of the recycling endosome, and shown that Rab11 dysfunction is a cause of homeostatic disturbance of HD neurons. We and other investigators have demonstrated in different HD models that approaches that enhance Rab11 are neuroprotective. In this presentation, I report our characterization of the compromised Rab11 activator as the multiprotein complex transport protein particle II and our identification of the McLeod syndrome causal protein XK as a link between Rab11 dysfunction and the preferential vulnerability of striatal neurons in HD. Altogether, our studies support the idea that decline in Rab11 function is a driving force of the progression of Huntington's disease.

QDs' Trafficking and Its Impact on Yeast Polarization and Actin Filament

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Abstract

Quantum Dots are nanoparticles (2-10 nm) that emit strong and tunable fluorescence. Quantum dots have been heavily used in high-demand commercialized products, research, and medical purposes. Emerging concerns have demonstrated the negative impact of quantum dots on living cells, however, the intracellular trafficking of QDs in yeast cells and the effect of this interaction remains unclear. The primary goal of our research is to investigate the trafficking path of red cadmium selenide zinc sulfide quantum dots (CdSe/ZnS QDs) in *Saccharomyces cerevisiae* and the impact QDs have on yeast cellular dynamics. Using cells with GFP-tagged reference organelle markers and confocal microscopy, we were able to track the internalization of QDs. We found that QDs initially aggregate at the exterior of yeast cells, enter the cell using clathrin receptor-mediated endocytosis, and distribute at the late Golgi/ Trans Golgi Network. We also found that the treatment of red CdSe/ZnS QDs resulted in growth rate reduction and loss of polarized growth in yeast cells. Our RNA sequence analysis revealed

many altered genes. Particularly we found an upregulation of *DID2*, which has previously been associated with cell cycle arrest when overexpressed, and a downregulation of *APS2*, a gene that codes for a subunit of AP2 protein important for the recruitment of proteins to clathrin-mediated endocytosis vesicle. Furthermore, CdSe/ZnS QDs treatment resulted in a slightly delayed endocytosis and altered the actin dynamics in yeast cells. We found that QDs caused an increased level of F-actin and a significant reduction in profilin protein expression. In addition, there was a significant elevation in the amount of coronin protein expressed while the level of cofilin was unchanged. Altogether, this suggests that QDs favor the assembly of actin filaments. Overall, this study provides a novel toxicity mechanism of red CdSe/ZnS QDs on yeast actin dynamics and cellular processes including endocytosis.

A Novel Signaling Pathway Delays Abscission to Prevent Chromatin Breakage

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Abstract

Chromatin bridges are strands of missegregated chromatin connecting the anaphase poles or daughter nuclei and have been linked to tumourigenesis. In response to chromatin bridges in cytokinesis, cells delay abscission, the severing of the narrow cytoplasmic canal that connects the two daughter cells, to prevent chromatin breakage or tetraploidization by regression of the cleavage furrow that are associated with genomic instability and cancer predisposition. In mammalian cells, this abscission-delay is called “the abscission checkpoint” and is dependent on the localization of the Chromosomal Passenger Complex (CPC) at the midbody. The CPC comprises the catalytic subunit Aurora B kinase, the scaffolding protein INCENP and the non-enzymatic subunits Survivin and Borealin; however, the molecular mechanisms that signal chromatin bridges to the CPC are incompletely understood. In the present study, we show that inhibition of the DNA damage kinases ATM or Chk2 impairs CPC-localization to the midbody and correlates with premature abscission and chromatin breakage in cytokinesis with trapped chromatin in human carcinoma cell lines. ATM phosphorylates Chk2-threonine 68 (T68) to activate Chk2 at the midbody. In turn, active Chk2 phosphorylates INCENP at the newly identified site serine 91 (S91) to promote CPC-localization to the midbody, to delay abscission. Expression of siRNA-resistant phosphomimetic mutant INCENP-S91D, but not the wild-type protein, rescues CPC-midbody-localization and prevents chromatin breakage in Chk2-deficient or ATM-deficient cells. These results identify an ATM-Chk2-INCENP pathway that prevents chromosome breakage in cytokinesis with chromatin bridges, by promoting CPC-midbody localization through Chk2-mediated INCENP-S91 phosphorylation.

Poster Presentations

Proteomic Profiling of Fatty Acid Amide Binding Proteins in *Drosophila melanogaster*

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Abstract

Fatty acid amides (FAAs) are a class of cell signaling lipids whose integral roles in the biological system have been substantiated by the discovery of endogenous FAAs such as anandamide and oleamide in the central and peripheral nervous system as cell signaling molecules. However, studies have been limited to only a select few FAAs, and much of this class of compounds have remained unexplored with little to no information on their metabolic pathway and functions. To elucidate the biological roles of some of these FAAs, we have employed a flexible two-fold strategy listed as follows.

a) Synthesis and characterization of FAA-targeted binding based proteomic profiling (BBPP) probe with photoreactive diazirine group and a clickable alkyne handle
b) Use of FAA-targeted BBPP probe for labelling, identification, and validation of FAA binding proteins in *Drosophila melanogaster*.

This approach has facilitated selective labelling and convenient enrichment of labelled proteomes from *D. melanogaster*. Proteome labelling was verified by tagging with either rhodamine azide for fluorescent imaging, or with biotin azide for Western blot. Biotin tagged proteomes were subjected to avidin column followed by their subsequent identification by high resolution LC-MS/MS.

Network Enhanced Similarity Search Increases the Predictive Power of a Highly Heterogenous Network

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Abstract

Data sources characterizing genetic disorders are often sparse and limited to the disease studied, species studied, and experimental procedure. This problem limits the use of data sources to study new diseases. Researchers vying to draw inferences outside of the data's original context have few options. Hence, we have developed a program, Network Enhanced Similarity Search (NESS), that

utilizes a random walk with restart over a heterogenous network in order to harmonize multi-species and multi-experimental data. We show that NESS outperforms widely used baseline algorithms. Further, due to the difficulties in studying psychological disorders in model organisms, an in-silico approach may create novel findings and drive new analyses. We utilize NESS in predicting model, transgenic mice for the study of schizophrenia. We find that neurotensin knockout mice, which are already used in metabolic studies, would be an optimal model organism.

The Effect of CerS6 on Melanoma Cells B16F10 and BP Cells

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Abstract
Introduction: Ceramides are bioactive lipids that can promote cell death. C16-ceramide is generated by ceramide synthase 6 (CerS6), and we previously demonstrated that exercise increases CerS6, C16-ceramide and apoptosis in B16F10 melanoma in mice. We investigated whether exercise similarly increases apoptosis in BrafV600E/WT and Pten-/- (BP) melanoma in mice, and the role of CerS6 in melanoma cell growth in vitro.
Methods: 6-week-old male C57BL/6J mice were injected with BP melanoma cells subcutaneously. When tumors reached ~30 mm3, the mice were assigned to nonintervention control (No Ex) or exercise (Ex) groups. Ex (treadmill running, 12 m/min for 45 min for 5 days per week for 2 weeks). Mice were euthanized 48h after final exercise. Immunofluorescent staining on tumor sections for TUNEL and caspase-3 was used to assess apoptosis.
To evaluate the role of CerS6 on cell survival in vitro, B16F10 and BP melanoma cells were treated with the CerS6 inhibitor Fumonisin B1 and tumor cell proliferation over 72 hours was quantified.
Results: Exercise did not significantly increase TUNEL or caspase-3 staining in BP tumors. In vitro, CerS6 inhibition by Fumonisin B1 significantly increased the growth of B16F10 but not BP cells compared to DMSO control.
Conclusions: In contrast to earlier findings in B16F10 tumors, exercise did not increase apoptosis in BP tumors in mice. In vitro, Fumonisin B1 treatment increased cell proliferation in B16F10 but not in BP, which indicates CerS6 restrains B16F10 cell growth.

Time-dependent Activation of Molecular Signatures is Associated with Trimethylamine-N-oxide Induced Endothelial Dysfunction in Human Microvascular Endothelial Cells

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Abstract

Cardiovascular diseases (CVD) are the principal cause of death worldwide. Endothelial dysfunction is recognized as a crucial initiating contributing factor for CVD. Trimethylamine N-oxide (TMAO), a gut microbiome-derived metabolite is involved in CVD progression. However, the underlying time-dependent molecular mechanisms resulting in the pathogenesis of TMAO in endothelial cells remain unclear. To investigate the time-dependent molecular signatures of TMAO-induced endothelial dysfunction in human microvascular endothelial cells (HMEC-1), we performed transcriptomics and metabolomics analysis on TMAO (50 μM) treated cells for 24H or 48H. Differentially expressed genes were used for overrepresentation analysis of pathways to determine the various molecular signatures involved. These were validated with quantitative real-time PCR, cell viability (prestoblue), and reactive oxygen species generation (DCFDA). TMAO treatment for 24H and 48H induced endothelial dysfunction, demonstrated by lowered cell viability, and elevated oxidative stress. Interestingly, time-dependent modifications in the molecular signatures were unique between time points. Indicatively, there were fewer GO biological processes and KEGG pathways suppressed, after 24H TMAO treatment. They were involved in cellular response and developmental processes. Compared to 48H treatment, a greater number of molecular signatures were activated and suppressed for GO biological processes and KEGG pathways. Upregulated DEG was enriched in pathways like oxidative stress and the production of inflammatory phenotypes, while suppressed pathways were associated with the structural organization of the ECM, endothelial cell proliferation, and collagen metabolism. This study indicates that TMAO-induced endothelial dysfunction is regulated through the mitigation of molecular gene signatures involved in oxidative stress and inflammation, activating endothelial cell remodelling.

Natterin-like Depletion by CRISPR/Cas9 Impairs Zebrafish (*Danio rerio*) Embryonic Development

Carla Lima da Silva, Ana Carolina Seni-Silva, Adolfo Luis Almeida Maleski, Milena Marcolino Souza, Maria Alice Pimentel Falcao, Geonildo Rodrigo Disner, Monica Lopes-Ferreira

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Abstract

Over the last 15 years, significant progress has been made in expanding the knowledge of the Natterin family, since the first discovery of the five founder members in *Thalassophryne nattereri* venom. We identified 331 species that have 859 natterin or natterin-like genes, distributed across all kingdoms of life, including plants, fungi and sessile marine animals. No homologue has been described in prokaryotes, protists, amphibians and mammals to date. Interestingly, although fish, including zebrafish (*Danio rerio*) with 10 copies of the natterin-like gene encoding 11 proteins, represent the majority of species (109 species with 598 sequences), only four species are venomous. The presence

of Natterin-like in widely divergent non-venomous species shows important adaptive value consistent with continued plurality of functions, including development. Herein, employing a combination of the CRISPR/Cas9 depletion system, phenotype-based screening, and morphometric methods, we evaluated the role of one family member, LOC795232, in the embryonic development of zebrafish. CRISPR/Cas9 depletion of the LOC7958232 gene present on chromosome 7 generated mutants with abnormal phenotypes that worsened over time and died prematurely. Severe phenotypic abnormalities of KOs included curved body axis with small bodies, head and eye deformities, with absent or reduced swim bladder, often accompanied by edema of the pericardium and yolk sac. These abnormalities affected physiologically relevant zebrafish functions, leading to severe cardiac dysfunction and a pattern of locomotor hyperactivity suggestive of high levels of stress/anxiety. The present work provided the first demonstration of the natterin-like role in embryonic development using zebrafish a standard model for loss-and-gain-of-function studies.

Evaluating the Toxicological Impacts of Nanoparticles Extracted from Sunscreens Using Human Keratinocytes

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Abstract

Inorganic ultraviolet filters such as titanium dioxide nanoparticles are commonly utilized in sunscreens. Numerous toxicological studies have been conducted *in vitro* and *in vivo*, using pristine versions of these inorganic filters. While convenient, this approach is not realistic as the nanoparticles tend to transform their physicochemical properties in the presence of a complex sunscreen formulation environment, forming a corona on the surfaces of the nanoparticles which could result in vastly different toxicological outcomes. Therefore, this study focused on characterizing real sunscreen extracted nanoparticles and evaluating their associated toxicological impacts upon exposure to human keratinocytes and human skin explants. Titanium dioxide nanoparticles were extracted from commercial sunscreens, and their properties were thoroughly characterized. The identity of the associated corona on the extracted nanoparticles was also evaluated. Cell metabolic, mitochondrial superoxide activities and reactive oxygen species level of human keratinocytes treated with the extracted nanoparticles were significantly higher than those treated with pristine nanoparticles. However, preliminary evaluation suggests that the heightened cell responses were not associated with any increase in phosphorylated γH_2AX. Similar findings were recorded in 3D wounded human skin explant cultures. This study shows that it is possible to extract nanoparticles

from sunscreens in a form that closely resembles real life exposure scenarios, so that hazard identification can be more realistically carried out using 2D and 3D cell/tissue cultures. Our results highlight the importance of using environment-specific nanoparticles, that have been transformed in a relevant product matrix, to derive physiologically relevant toxicological outcomes.

Role of DUF647 Containing Proteins in Vitamin B6 Homoeostasis

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Abstract

Vitamin B6 (vitB6) is essential to all organisms, serving as a critical cofactor for many enzymes required in cellular metabolism and signaling. The active form of vitB6, pyridoxal 5' phosphate (PLP), can be cytotoxic if left unbound in excess levels. Molecular and genetics studies in our laboratory have identified three genes that play key roles in regulating the PLP homeostasis: ROOT UV-B SENSITIVE 1 (RUS1), RUS2, and ASPARTATE AMINOTRANSFERASE 2 (ASP2). Mutant plants missing either the RUS1 or RUS2 gene are developmentally arrested, suggesting RUS1 and RUS2 play important roles in early seedling development. Genetic suppressor screens have identified ASP2 as a key player interacting with both RUS1 and RUS2. ASP2, a PLP-binding enzyme, can form a complex with RUS1 and RUS2 in regulating PLP homeostasis. From bioinformatic and amino acid sequence studies, RUS1 was found to contain bends that suggest hydrophobicity. I hypothesize that RUS1 functions as a membrane protein, interacting with both RUS2 and ASP2. To test this hypothesis, microsomal pellets will be prepared via ultracentrifugation, and western blots will be used to detect RUS1 association with the microsome presence. A RUS1-GFP-complemented RUS1 knockout mutant will be used to prepare the microsomes. An anti-GFP antibody will be used to detect the RUS1-GFP expression in the microsomes. Furthermore, antibodies against ASP2 and RUS2-GFP will be used to detect the potential co-existence of RUS1, RUS2, and ASP2. We expect to gain important insights into the interactions of RUS1, RUS2, and ASP2, and the localization and structures of these aforementioned proteins.

Impact of MKP-2 Deficiency on Bleeding Times and Protein Expression in Platelets

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Abstract

MAP kinase phosphatases (MKPs) inactivate the activity of MAPKs by direct dephosphorylation, and have been implicated in platelet activation. MKP-2 has been the subject of renewed biomedical relevance due to its role in multiple types of cancers, brain disorders and metabolic disorders. The

objective of this study is to elucidate the role of MKP-2 in platelet function and how this contributes to coagulation disorders. Tail bleed and western blot assays were performed to measure platelet activity and protein expression in global MKP-2 knockout mouse model and wild type controls. Bleeding time was significantly reduced in 12 week old female MKP-2 deficient mice compared with their wild type female counterparts. However, the bleeding time of male MKP-2 deficient mice was comparable with wild type controls. Previous studies in the lab showed that male MKP-2 KO mice exhibited enhanced serum levels of stromal cell-derived factor 1 (SDF-1), a cytokine implicated in platelet activation. In this study, we observed significantly increased protein levels of SDF-1 in platelets derived from male MKP-2 knockout mice compared to wild type controls. Furthermore, the phosphorylation of ERK and p38 MAPKs in male MKP-2 deficient platelets were also significantly increased compared to the wild type control male platelets. Overall, these results suggest MKP-2 may have a role in platelet function and coagulation.

The Role of MKP-2 in Cellular Senescence and Metabolic Function

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Abstract

Senescence is a state of permanent cell cycle arrest which stops proliferation. It is hallmarked by changes in proteins such as p21, p53, and p16, which are regulated by mitogen-activated protein kinases (MAPKs) which are regulated by MAPK Phosphatases (MKPs). One such MKP, MKP-2, has a paucity of information on its physiological function. To study MKP-2 in senescence, we used mouse embryonic fibroblasts (MEFs) derived from novel MKP-2 whole-body knockout mice. These MEFs were analyzed using senescence-associated β-galactosidase assays and immunoblotting approaches. It was found that there were reduced β-Galactosidase positive cells and reduced senescence phenotypes in the *Mkp2*$^{-/-}$ MEFs compared with *Mkp2*$^{+/+}$ MEFs. Furthermore, in UV-stimulated MEFs phosphorylated p53 was increased in the *Mkp2* $^{-/-}$ MEFs compared with *Mkp2* $^{+/+}$ MEFs, along with alterations in phosphorylated ERK and p38 MAPK. It was also found that there was a decrease in p21 and p16 mRNA expression in the *Mkp2*$^{-/-}$ MEFs compared with *Mkp2* $^{+/+}$ MEFs. These results show that MKP-2 deficient MEFs are resistant to the development of cellular senescence.

The Effects of Dictyostelium PP2A/B56 and Superoxide Dismutase C (SodC) on the Ingestion and the Killing of Live Bacteria *Klebsiella aerogenes*

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Abstract

Nowadays, multidrug-resistant bacteria are a critical risk to public health. An effective innate immune response for the elimination of pathogens is phagocytosis. Dictyostelium discoideum is an excellent model organism to study this response. In Dictyostelium, the receptor fAR1 mediates the chemotaxis, phagocytosis of bacteria, and the activation of PKB and ErkB proteins. These proteins are regulated by phosphatase 2A (PP2A) subunit B56. Our laboratory has investigated two pathways that affect PKB: SodC/Ras/PI3K/PKB and PP2A/B56/PKB. The present study aims to determine the effect of SodC/Ras/PI3K/PKB, and PP2A/B56/PKB pathways and PKB and ErkB proteins in the bacterial ingestion and inactivation of live bacteria Klebsiella aerogenes. In this study JH10 (wild type), sodC-, Ax3 (wild type), and psrA- (knockout B56) cells were used. Phagocytosis assays and killings assays were performed in the presence or absence of LY294002 using 2 x 106 Dictyostelium cells and 2 x 104 Bacteria. Further western blots in the presence or absence of the PI3K inhibitor LY294002 were performed for cell lines Ax3 and psrA- using Anti-MAPK and Anti-pPKB antibodies. psrA- significantly increased its engulfment and bactericidal activity compared with WT cells (AX3) ($P<0.01$). After the LY294002 treatment, psrA- cells significantly decrease their bactericidal activity ($P<0.05$). The level of phospho-PKBA in psrA- after an LY294002 treatment was statistically significant ($P<0.009$). The phospho-Erk2 activity after LY294002 treatment psrA- and Ax3 cells showed an insignificant increase ($P>0.36$ and $P>0.1$). In conclusion, PP2A/B56 is essential for properly regulating the uptake and elimination of Klebsiella aerogenes.

Effects of Water from Greater Houston Watersheds on Colorectal Adenocarcinoma Cell (HT-29) Viability: Time-dependent Toxicity and Insensitivity to Tyrphostin Treatment

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Abstract

Greater Houston includes Houston, the fourth most populous city in the country, as well as its surrounding areas, which are home to over 7 million people. The area is known for its diverse economy, which includes industries such as energy, healthcare, aerospace, and technology. However, like many urban areas, Greater Houston faces environmental challenges, including air and water pollution. The region is prone to flooding due to its low elevation and proximity to the Gulf of Mexico. Given these environmental challenges, it is important to understand the potential health effects of exposure to surface water from Greater Houston watersheds. This study seeks to investigate the potential toxicity of water from Greater Houston watersheds on HT-29 cells. HT-29 cells were exposed to different dilutions of the water for 24 and 48 hours, with or without co-treatment with tyrphostin. The results showed that cell viability decreased with increasing concentration of the water after 24 hours. However, viability increased after 48 hours, when compared to the 24-hour time point. Co-treatment with tyrphostin also resulted in increased cell viability compared to treatment with the water alone. These findings suggest that the toxic effects of the sampled water on HT-29 cells was

time-dependent and was not affected by the presence tyrphostin. The reason behind non effectiveness of tyrphostin in mitigating growth in this study is unknown, however, further investigations are warranted to identify the specific toxicants present in the water and to elucidate the mechanisms underlying the proliferative effects of tyrphostin.

Cytotoxic and Apoptotic Effects of Vardhamana Pippali Rasayana (VPR) in Human Breast Cancer Cells

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Abstract

Background: Serious concerns include delayed diagnosis and adverse side-effects of conventional treatment leading to residual morbidity in Breast cancer (BC) globally. Traditional Indian medicines/ herbs via combination of conventional cancer are gaining increasing acceptance worldwide, playing a pivotal role as proven by their efficacy evaluation studies. Many plants are known to reduce the cell proliferation and tumor development after their treatment. Vardhamana Pippali Rasayana (VPR), an Ayurvedic herbal preparation of pippali (Piper longum) in gradually increasing and tapering dose is used to treat many cancers without any adverse side-effects. However, its anti-cancer role in breast cancer is not known yet. Methods: The present study has explored the cytotoxic and apoptotic effects of VPR in triplicates on human breast cancer (MCF7) and normal breast epithelial (MCF10A) cell lines at different concentrations after extracting piperine in aqueous and hydro-alcoholic soxhlet extract (0.25 to 50 µg/µl). Results: The cytotoxic activity was carried out by MTT assay and results showed that a low IC50 was observed at 3.75 µg/µl of VPR against MCF-7 as compared with normal breast epithelial cells. Detection of apoptotic cells using suitable Annexin V was done and there was a time-dependent increase in percentage of annexin V positive cells upon treatment. Conclusions: The above results indicate that VPR may have a strong anti-cancerous potential. But other functional assays are warranted to validate the efficacy of the drug against breast cancer which are under progress in our laboratory.

Circulating Exosomal MicroRNAome from Newly Identified Essential Hypertensive Adults

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Abstract

Hypertension is a leading risk factor for cardiovascular disease (CVD) and premature death globally. Circulating exosomal microRNA's are actively involved in the pathogenesis of hypertension. Therefore, our goal was to identify circulating exosomal miRNA profile from newly diagnosed essential hypertensive adults compared to normotensive adults. Total plasma exosomal RNA was extracted from stage I hypertensive and normotensive adults (30-50 years old). Samples were subjected to whole-genome small RNA sequencing, differential expression analysis, target prediction, and pathway enrichment analysis. Post-sequencing analyses identified 12 upregulated and 5 downregulated miRNAs in hypertensive adults ($n = 6$, $p < 0.05$). *In silico* analyses revealed that dysregulated exosomal miRNAs were heavily involved in regulating signal transduction and signalling pathways, associated with axon guidance, vascular and cardiac remodelling pathways. Upregulated miRNAs also regulated cellular senescence, and aldosterone synthesis and secretion. Notably, upregulated miR-206, miR-365a-5p and miR-34a-5p were predicted to regulate vascular and cardiac remodelling pathways by targeting Rho GTPases, Notch signalling or the TGFβ/SMAD pathway. Intriguingly, dysregulated miRNAs including the upregulated miR-206, and downregulated miR-34c-5p highlighted the potential involvement of axonal guidance via Notch, Roundabout and Ephrin signalling in hypertension. This is the first study to provide the complete exosomal miRNAome in essential hypertensive young adults. Results of this study provide leads for further validation and may provide unbiased insights into mechanisms involved in early stages of hypertension for novel biomarker discovery or therapy development to reduce the CVD burden.

In Silico Study of Benzothiazole Guanidino Derivates as Possible Inhibitors of AChE, BACE1 Activity and Amyloid Peptide Aggregation

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Abstract

Alzheimer's Disease (AD) is a neurodegenerative disease among older adults, presenting serious symptoms such as memory loss, language problems, mood and/or behavioral changes due to neurodegeneration caused mainly by the production of amyloid beta peptide (Aβ). The enzyme

BACE1 participates in the production of Aβ, therefore, the inhibition of BACE1 is of great importance, as well as preventing the aggregation of the Aβ peptide in its early conformations. In addition, the enzyme AChE is responsible for the hydrolysis of ACh, which is a neurotransmitter that is reduced in patients with AD. Guanidine derivatives act mainly as inhibitors of the enzyme BACE1 and benzothiazoles as inhibitors of Aβ aggregation and AChE, showing neuroprotective effects. The in-silico study was done employing 26 guanidinobenzothiazole derivatives on three Aβ conformations and on AChE and BACE1. The 3D structures were obtained from the Protein Data Bank for AChE (PDB ID 4PQE), BACE1 (PDB ID 2QP8), and Aβ peptide in alpha helix (PDB ID 1z0q) and beta-sheet (PDB ID 2BEG). The conformation of Aβ in random coil was obtained from previous results. The structure of the ligands was drawn in ChemSketch and molecular docking was performed in Autodock. To visualize the interactions, PYMOL and the BIOVIA Discovery Studio software were used. The best affinity and interactions were for the compounds 20 and 26 which acts on Aβ, AChE and BACE1, having the compound 26 better affinity than the compounds used as controls on BACE1, such as verubecestat. Therefore, these guanidinobenzothiazole derivatives could have multitarget activity and then could be useful in the treatment of AD.

Navitoclax Suppresses IL-3 Induced Human Umbilical Vein Endothelial Cells Migration and Angiogenesis Through PI3-AKT Pathway

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Abstract

Navitoclax causes cancer cell death in solid and non-solid tumors. Pathological angiogenesis in cancer is comparable to intraplaque neovascularization which largely contributed by endothelial cells activation. Pro-apoptotic effect of navitoclax on tumor cells allows cancer metastasis inhibition; therefore, it is expected to have an effect on endothelial cells' activities and may develop as a novel therapeutic agent for advanced atherosclerosis. Nevertheless, navitoclax action on endothelial cells' motility is yet to be determined. This study demonstrates the navitoclax effect in regulating IL-3 induced endothelial cells migration and angiogenesis. In-vitro study was conducted using primary

endothelial cells isolated from human umbilical veins. Three groups were included in this study; i) control; ii) 25 ng/ml IL-3; iii) 25 ng/ml IL-3 with 0.9μM navitoclax. Scratch wound assay was conducted for 24 hours and images at 0, 12 and 24 hours were captured. Next, tube formation assay on matrigel was carried out for 8 hours and the images were saved. The expression of p-AKT and CXCL8 after the treatment were detected using western blot and ELISA respectively. The images were analysed by imageJ-Angiogenesis analyzer and the statistical analysis was performed using graphpad prism. Navitoclax had a significant ($p<0.05$) large wound area after 24 hours treatment as compared to the IL-3 group. Furthermore, navitoclax treatment had reduced the tube formation induced by IL-3 significantly ($p<0.05$) within 8 hours. Next, p-AKT expressions and CXCL8 released were decreased in navitoclax group. In conclusion, navitoclax inhibits endothelial cells motility and angiogenesis through PI3K/AKT pathway.

Effect of Glycyrrhizic Acid with and without Low Level Laser Therapy on Squamous Cell Carcinoma and Oral Microbiome

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Abstract

Cancer is a leading cause of death worldwide. Low-level laser therapy (LLLT) is a promising non-invasive treatment option with controversial reports on the possible stimulatory effect of LLLT on head and neck cancer (HNSCC) cells in patients treated with radiotherapy. Glycyrrhizic acid (GA) is the active ingredient of licorice and is reported for antitumor activities in many cancers. In this study we used GA in combination with LLLT in on head and neck cell line (HEP2) to test the cytotoxic effect in vitro, and the Hamster check pouch oral squamous cell carcinoma model (OSCC) was used to test the DNA changes (PARP1 and Phospho H) in the buccal pouches and oral microbiome (16s) of the hamster in addition to liver and kidney samples. GA in combination with LLLT ameliorated the dysplastic changes in buccal and liver samples, DNA changes and slightly changed the phylogenic microbial composition.

Mouse Embryonic Stem Cell-derived Motor Neurons are Susceptible to Ferroptosis

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Abstract

Ferroptosis is a regulated form of cell death driven by the lethal accumulation of lipid peroxides in cell membranes. Several regulators of ferroptosis have been identified using cancer cell lines. However, the cellular pathways of ferroptosis in neurons remain poorly characterized. In this study, we used a mouse embryonic stem cell-derived motor neuron model to investigate how motor neurons respond to ferroptosis inducers. Pharmacological and genetic inhibition of glutathione peroxidase 4 (GPx4) induced ferroptosis in the motor neurons while system x_c^- inhibition by erastin was neutral. RNA-seq analysis showed that many genes changed their expression levels during RSL3-induced ferroptosis. Subsequent bioinformatics analysis revealed several altered biological pathways during ferroptosis, such as synaptogenesis and calcium signaling. Finally, we found that edaravone, an FDA-approved drug for treating amyotrophic lateral sclerosis (ALS) disease, rescued motor neurons from RSL3-induced ferroptosis. Our data highlight the crucial role of GPx4 in ferroptosis regulation and demonstrate that the stem cell-derived motor neuron culture is a valuable model to study ferroptosis at the single-cell level in a neuronal context.

Factors Affecting Outcomes of Bone Marrow Stem Cell Therapy for Acute Myocardial Infarction

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Abstract

Myocardial infarction (MI) poses a significant burden to both patients and the health care system. The irreversible loss of functional cardiomyocytes due to ischemia threatens both patients' immediate survival and quality of life over their lifespan. Stem cell therapy has been proposed as a solution to salvage cardiac contractility through the regeneration of cardiomyocytes, and bone marrow–derived stem cells (BMSc) are among the category of stem cells most extensively studied. Despite the promising theoretical potential of BMSc in tissue regeneration, several key aspects remain to be better understood to enable large-scale clinical application, including safety and efficacy. Our current work in synthesizing and evaluating both preclinical and clinical studies using stem cell applications in acute MI has demonstrated that BMSc transplantation is a safe therapy for MI. Although this therapy's efficacy is not consistently proven, we have significantly improved our understanding of factors contributing to its success, such as the stem cell type, patients' baseline left ventricular ejection fraction, individual hemodynamic factors, and differential expressions of specific genes. In future investigations, researchers should focus on the cellular and individual attributes of BMSc treatment to achieve maximal efficacy and outcomes for patients receiving this therapy after acute MI.

* Young Researchers

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