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Meeting Abstracts

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Keynote Presentations

The Role of DISE in Health and Disease

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Abstract

Micro(mi)RNAs are short noncoding RNAs that through their seed sequence (pos. 2 - 7/8 of the guide strand) regulate cell function by targeting complementary sequences (seed matches) located mostly in the 3′ untranslated region (3′UTR) of mRNAs. Certain short (s)RNAs when loaded into the RNA induced silencing complex (RISC) are toxic to cancer cells by targeting a network of genes that are critical for cell survival, a process we have termed as death induced by survival gene elimination (DISE). DISE involves simultaneous activation of multiple cell death mechanisms by targeting a large network of genes, that likely differs from cell to cell. These toxic sRNAs carry a G-rich 6mer seed region that targets C-rich seed matches mainly located in the 3'UTR of genes in a miRNA-like manner. G-rich seeds are part of the seed of a number of conserved tumor suppressive miRNAs and the ratio of toxic-to-nontoxic miRNAs predicts treatment outcome in ovarian cancer. We demonstrated that sRNAs delivered using multiple delivery vehicles cause tumor regression in different *in vivo* mouse and rat tumor models of ovarian, prostate and liver cancer with no side effects. DISE works on any cancer, it is effective on therapy resistant cancer cells, cancer cells cannot become resistant to treatment, there is no evidence of toxicity to treated animals, and one can keep treating and/or switch to different delivery methods without losing efficacy. Because it is an evolutionary conserved cell death mechanism, overactive DISE may lead to human diseases that involve tissue degeneration. Alzheimer's disease (AD) is characterized by progressive neurodegeneration, but the specific events that cause cell death remain poorly understood. The RISC of most cells is occupied by miRNAs with nontoxic 6mer seeds, which may protect them from DISE by blocking loading of sRNAs that carry toxic G-rich 6mer seeds, including miRNAs, tRNA or rRNA fragments. However, during aging miRNA expression decreases in the brain, and toxic sRNAs may enter the RISC more readily leaving cells primed for DISE. We found evidence that DISE contributes to neuronal loss during AD. We analyzed RISC bound sRNAs (R-sRNAs) of multiple *in vitro* models and in the brains of *in vivo* AD mouse models, aged mice, and AD patients. We found that in the mouse models that show neurodegeneration and during aging R-sRNAs shift to more toxic seeds. In contrast, in cells that survived in the post-mortem brains of AD patients and the brains of "SuperAgers", individuals over age 80 who have superior memory performance, R-sRNAs shift to more nontoxic seeds, supporting a protective function of miRNAs. Our data provides first evidence of a contribution of DISE to the neurotoxicity seen in AD. Our data suggests that toxic 6mer seed containing sRNAs can be developed into a general form of cancer therapy and ways to increase the levels of protective miRNAs in the brain may provide a novel way of treating neurodegenerative diseases such as AD.

The Art of Delivery: Labeling and Manipulating Intracellular Targets

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Abstract

Cell delivery of free molecules into the cytoplasm and nucleus using arginine-rich cell-penetrating peptides (CPPs) has been limited to small cargos, while big cargos like antibodies are taken up and trapped in endocytic vesicles. Functional antibody delivery in living cells would enable the labeling and manipulation of intracellular antigens, constituting, a long-thought goal in cell biology and medicine. We have shown that the uptake efficiency of arginine-rich CPPs can be greatly enhanced by their cyclization. We have now tested the ability of cyclic CPPs to transport full-length proteins to the cytosol of living cells. We

will present a modular strategy for creating functional cell-permeable antibodies and other proteins capable of targeted labeling and manipulation of intracellular antigens in living cells. This technology constitutes a major step in the labeling, delivery and targeted manipulation of intracellular antigens, such as the p53 tumor suppressor protein. Ultimately, this approach opens the door towards immunostaining in living cells and the expansion of immunotherapies to intracellular antigen targets.

Regulation of the Pyruvate Dehydrogenase Complex: Many Ways to Dance Around in Cancer Cells

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Abstract

Mammalian pyruvate dehydrogenase complex (PDC), a gatekeeper of glucose oxidation, is composed of three catalytic enzymes: pyruvate dehydrogenase (PDH), dihydrolipoamide acetyltransferase, and dihydrolipoamide dehydrogenase. PDC activity in normal cells is controlled by the reversible phosphorylation of three serine residues in the alpha subunit of PDH by dedicated pyruvate dehydrogenase kinases 1-4 (PDK 1-4) and phospho-pyruvate dehydrogenase phosphatase 1-2 (PDP 1-2). Mechanisms governing the regulation of PDC are markedly different in cancer cells, involving novel posttranslational modifications of the PDC proteins and transcriptional mechanisms. Aerobic glycolysis (the Warburg effect) appears to be a key metabolic switch with attenuation of mitochondrial oxidative metabolism in some types of tumors. In this switch, novel tyrosine phosphorylation of the specific residues in PDHalpha, PDK1 and PDP1 proteins by oncogenic kinases causes alterations in their catalytic rates, resulting in reduction of PDC activity. Also. increased gene expression of PDK1 enhances inactivation of PDH by serine phosphorylation. In some cancer cells, to enhance the mitochondrial oxidative metabolism to meet increased energy requirement, PDC is maintained in its active state by employing yet another novel mechanism involving AMPK-mediated phosphorylation of two different serine residues. Interestingly, impairment in PDC function as a major supplier of mitochondrial acetyl-CoA to the nuclear pool of acetyl-CoA is circumvented by the translocation of the PDC to the nucleus for histone acetylation. These unique cancer-specific PDC regulatory mechanisms represent an incredible advancement in our understanding of reprogramming of cellular metabolism in cancer cells.

Too Much is as Bad as Too Little: Autophagy Deficiency-induced Liver Tumorigenesis

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Abstract

Autophagy is a lysosomal degradation pathway that is essential for maintaining cellular homeostasis by removing damaged organelles and proteins. The liver-specific knockout of Atg5 (referred to as L-Atg5 KO), a crucial autophagy-related gene, leads to hepatomegaly, liver injury, inflammation, fibrosis, and spontaneous liver tumorigenesis. This further emphasizes the critical role of autophagy in liver physiology and its function as a tumor suppressor. L-Atg5 KO mice have increased accumulation of SQSTM1/p62, which activates non- canonical NFE2L2/NRF2 (nuclear factor, erythroid 2 like 2) and mechanistic target of rapamycin (mTOR). Although the activation of NRF2 has been widely recognized as an important mechanism in preventing tumor formation, paradoxically, persistent NRF2 activation is a primary factor in liver injury and tumorigenesis in the absence of autophagy. Deletion of Mtor or Raptor (an essential component of mTORC1) in -LAtg5 KO mice reduces liver injury in younger mice but increases the risk of liver tumorigenesis in older mice. Conversely, deleting Tsc1 (a negative regulator of mTORC1) in L-Atg5 KO mice exacerbates hepatomegaly, ductular reaction (DR), hepatocyte degeneration, and fibrosis in younger mice, but prevents liver tumorigenesis, albeit with increased mortality in aged mice. Interestingly, deleting SQSTM1/p62 alleviates DR, hepatocyte degeneration, metabolic reprogramming, and the increased mortality seen in L-Atg5 and Tsc1 double knockout mice, but unexpectedly promotes liver tumorigenesis. Overall, these findings suggest that a balanced activity of SQSTM1/p62, NRF2 and mTOR is crucial for maintaining normal liver physiological functions.

30,000 Nanotextured Orthopedic Implants in Humans: No Failures, No Infection and Still Counting

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Abstract

Nanomedicine is the use of nanomaterials to improve disease prevention, detection, and treatment which has resulted in hundreds of FDA approved medical products. While nanomedicine has been around for several decades, new technological advances are pushing its boundaries. For example, this presentation will present an over 25-year journey of commercializing nano orthopedic implants now in over 30,000 patients to date showing no signs of failure (over the past 5 years). Current orthopedic implants face a failure rate of 5 - 10% and sometimes as high as 60% for bone cancer patients. Further, artificial intelligence (AI) has revolutionized numerous industries to date. However, its use in nanomedicine has remained few and far between. One area that AI has significantly improved nanomedicine is through implantable sensors. This talk will present research in which implantable sensors, using AI, can learn from patient's response to implants and predict future outcomes. Such implantable sensors not only incorporate AI, but also communicate to a handheld device, and can reverse AI predicted adverse events. Examples will be given in which AI implantable sensors have been used in orthopedics to inhibit implant infection and promote prolonged bone growth. *In vitro* and *in vivo* experiments will be provided that demonstrate how AI can be used towards our advantage in nanomedicine, especially implantable sensors. Lastly, this talk will summarize recent advances in nanomedicine to both help human health and save the environment.

Condensate Formation, DNA Repair and Oscillations in Cell Dynamics

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Abstract

When cells are damaged or stressed, they respond by oscillating protein densities as have been observed for two famous proteins/transcription factors p53 and NF-kB. For p53 the oscillations appear after DNA damage by radiation. We show that liquid-liquid phase separations lead to condensates of repair proteins around damage sites which occur in an oscillating fashion thus preventing Oswald ripening. The period of oscillations provides an optimal time scale for the repair mechanism. By applying an external periodic protein signal, the internal oscillation can lock to the external signal and thus controls the genes. The locking occurs when the ratio between the two frequencies is a rational number leading to Arnold tongues. If tongues overlap, chaotic dynamics appear which strongly influence gene production. Our findings are supported by experimental data from our collaborative groups at Harvard Medical and Taipei.

Oral Presentations

Tunneling Nanotube-like Structures Regulate Distant Cellular Interactions During Heart Formation

Mingfu Wu*, Lianjie Miao, Yangyang Lu, Anika Nusrat, Guizhen Fan, Shaohua Zhang, Luqi Zhao, Chia-Ling Wu, Hongyan Guo, Trang Le Nu Huyen, Yi Zheng, Zhen-Chuan Fan, Weinian Shou, Robert J Schwartz, Yu Liu, Ashok Kumar, Haixin Sui, Irina I Serysheva, Alan R Burns, Leo Q. Wan, Bin Zhou and Sylvia M. Evans

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Abstract

In the developing mammalian heart, the endocardium and the myocardium are separated by so-called cardiac jelly. Communication between the endocardium and the myocardium is essential for cardiac morphogenesis. How membrane-localized receptors and ligands achieve interaction across the cardiac jelly is not understood. Working in developing mouse cardiac morphogenesis models, we used a variety of cellular, imaging, and genetic approaches to elucidate this question. We found that myocardium and endocardium interacted directly via microstructures termed Tunneling Nanotube-Like structures (TNTLs). TNTLs extended from cardiomyocytes (CMs) to contact endocardial cells (ECs) directly. TNTLs transported cytoplasmic proteins, transduced signals between CMs and ECs, and initiated myocardial growth toward the heart lumen to form ventricular trabeculae- like structures. Loss of TNTLs disturbed signalling interactions and, subsequently, ventricular patterning.

The Integrated Stress Response Engages a Cell-Autonomous, Ligand-independent, DR5-driven Apoptosis Switch

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Abstract

The integrated stress response (ISR) is a central signaling network that leverages the cell's biosynthetic capacity against different stresses to restore homeostasis. However, when homeostasis is unattainable, the ISR switches to drive cell death and eliminate irreparably damaged cells. During persistent endoplasmic reticulum (ER) stress, prolonged activity of the ISR kinase PERK induces apoptosis downstream of death receptor 5 (DR5). ER stress provides activating signals that engage the ectodomain (ED) of DR5 to drive its unconventional activation in the Golgi apparatus. Here, using a chemical-genetics-based approach that uncouples stress sensing from ISR actuation, we found that DR5 signaling from the Golgi apparatus is integral to the ISR and not specific to ER stress. Furthermore, we show that DR5 activation is driven solely by increased expression and does not require DR5's ED. These findings indicate that a general ISR kill switch eliminates irreversibly injured cells.

Human Induced Pluripotent Stem Cells for Brain Tumor Research and Therapy

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Abstract

Human induced pluripotent stem cells (iPSCs) have been widely used for disease modeling and cell replacement therapy, mainly for degenerative diseases. iPSCs also have many potential applications in cancer research, including modeling, cellular therapy, and immunotherapy. This talk will first discuss an iPSC-based model of MYC-driven medulloblastoma (MB), an aggressive pediatric brain tumor. We applied a synthetic mRNA-driven strategy to generate neuronal precursors from human iPSCs and transformed these neuronal precursors to establish a MYC-driven MB model. We further found that the marine compound Frondoside A effectively inhibits this MYC-driven MB model *in vitro* and *in vivo*. This talk will further present the use of iPSC-derived neural stem cells for intratumoral delivery of the immunostimulatory cytokine IL7 to glioblastoma (GBM) tumors. This cellular therapy effectively promotes the recruitment of cytotoxic T lymphocytes and potently induces anti-GBM immunity.

Enhancing Osteoarthritis Cell Therapy with Biomedical Engineering: Evaluating Fat Pad-derived Mesenchymal Stem in 3D Spheroid Culture and on Collagen-based Scaffold

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Abstract

The current clinical standard for osteoarthritis (OA) therapy involves using bone marrow-derived mesenchymal stem cells (BMMSCs) to address clinical needs and promote healing. However, infrapatellar fat-pad-derived MSCs (FPMSCs) offer a less invasive harvesting method and are a promising alternative. Since OA is an inflammatory disease, the immunosuppressive potential of MSCs is crucial for promoting cartilage repair by reducing excessive inflammation. Notably, MSCs cultured in three-dimensional (3D) systems, like spheroids, demonstrate increased stemness and can enhance immunomodulatory capabilities compared to traditional 2D cultures. Additionally, 3D biomimetic scaffolds can maximize regenerative therapies by allowing spatiotemporal control over signaling molecules at the nanoscale. Based on these considerations, we first assessed the feasibility of human FPMSCs for cell therapy by comparing their stemness and immunomodulatory capabilities to human BMMSCs. We optimized cell culture conditions to create stable 3D spheroids and evaluated for viability using CellTiter-Glo® and Live/Dead assays. Stem marker characterization was performed via flow cytometry (CD105+, CD73+, CD90+, and CD45- phenotype). Inflammatory conditions were simulated using 40 ng/mL of IFN-γ and TNF-α. Our results show that 3D FPMSC spheroids exhibit superior immunosuppressive potential compared to 2D cultures, evidenced by higher PTGES, PTGS2, and IDO1 expression. Finally, to enhance biomimicry, we used a freeze-dried collagen scaffold as a substrate for 3D spheroid culture and

determined optimal spheroid density through seeding titration. Viability and spheroid distribution throughout the scaffold were also evaluated.

Partial Reprogramming to Restore Age-associated Loss of Mitochondrial Function

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[2]*University of Colorado, USA*
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Abstract

We discovered that ectopic expression of pluripotent transcription factor, NANOG completely restored the mitochondrial function in senescent stem cells and that NANOG-driven rejuvenation led to metabolic reprogramming of senesced cells to the state of young proliferating cells. Using several models of cellular senescence, we show that senescent cells exhibited severe mitochondrial dysfunction, as evidenced by significantly reduced mitochondrial membrane potential, reduced respiratory function and excessive accumulation of reactive oxygen species (ROS). To delineate cell metabolic profile, we employed Seahorse analyzer and observed complete restoration of mitochondrial respiratory capacity in aged MSCs after NANOG overexpression. Metabolomic analysis revealed increased dependance of senescent cells on glutamine to meet their bioenergetic demand and this was confirmed by increased glutaminase (GLS) activity in aged cells. This observation also held true for our *in-vivo* study, where we observed increased GLS expression and activity in heart, aorta and skin of progeric (LAKI) as well as geriatric mice. Enhanced deamination of glutamine with senescence results in accumulation of toxic end-product, urea leading to detrimental consequences for mitochondrial function and DNA damage. Interestingly, inhibition of GLS activity in senescent cells by CB-839 decreased urea accumulation and partially restored mitochondrial function, decreased ROS and DNA damage. Furthermore, we examined the effects of CB-839 on LAKI mice and observed decreased age-associated ROS and urea accumulation in heart, skin and muscle, concomitant with improved mitochondrial function and physical performance. Hence, the inhibition of GLS activity rejuvenated mitochondrial function and led to amelioration of aging hallmarks including physical performance of aged prematurely mice.

Pharmacologic and Mechanical Force Effects on Macrophage Actin Cytoskeleton for Abrogation of Transplant Chronic Rejection

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Abstract

Around 70% of transplanted organs undergo long-term (chronic) rejection within 10 years post-transplantation. At present, there is no cure for chronic rejection. Our studies showed that chronic rejection depends on macrophages, which infiltrate grafts, causing vessel occlusion and fibrosis. The macrophage movement to the graft depends on the actin cytoskeleton regulated by small GTPase RhoA and its downstream effector ROCK kinase. We showed that inhibiting the RhoA pathway using inhibitors of ROCK disrupts the actin cytoskeleton, affects the distribution of organelles, elongates macrophages, prevents their movement to the graft, and abrogates chronic rejection in cardiac transplantation rodent model systems. Similar disruption of actin cytoskeleton and elongation of macrophages occurs after macrophage exposure to the mechanical force created by the magnetic field gradient. These findings indicate that RhoA pathway inhibitors or magnetic gradient-creating devices can inhibit chronic rejection of transplanted organs in the clinical setting. Currently, two clinical trials with the FDA-approved RhoA inhibitors Fingolimod and Rezurock repurposed from their original applications (Fingolimod for the treatment of multiple sclerosis and Rezurock for the treatment of chronic graft versus host disease) are undergoing at Houston Methodist in kidney transplanted patients.

Genetically Engineering Microbial Consortia and Non-model Microbes Using Programmable RNA Tools

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Abstract

Microbial consortia are found almost everywhere (e.g., in soils, rivers, and guts and on skin, minerals, and built materials) and underlie processes critical to sustainable agriculture, waste treatment, the longevity of materials, and health. Current microbiome engineering approaches primarily rely on domestication, whereby individual species are isolated, genetically manipulated in the lab, and then reintroduced back into microbiomes. However, this approach can be arduous and incompatible with the large fraction of uncultured microbes found in native microbiomes. The overarching goal of my group is to establish a framework to create genetic programs that are efficiently transferred into natural consortia, can be precisely controlled, and can actuate useful genetically encoded functions in these communities (e.g., biosensing, antibiotic, production of metabolites). To achieve this, we innovate RNA technologies that allow us to efficiently transfer, control, and actuate programs in native consortia and non-model microbes for diverse applications in biotechnology and biomedicine.

Strength from within: A New Binding Mode Enables Actin Filament Stabilization

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Abstract

Entry of *Salmonella* into polarized host enterocytes relies on its pathogenicity island 1 effector SipA. We found that the C-terminal fragment of SipA binds to F-actin in a unique mode in a 1:2 stoichiometry. This binding ratio is bestowed by the presence of two separate actin binding sites with distinct properties. A cryo- EM reconstruction revealed that SipA's globular core binds at the grove between actin strands, whereas the extended C-terminal arm penetrates deeply into the inter-strand space, stabilizing F-actin from within with a picomolar Kd. This unusually strong association of SipA with F-actin is achieved via a combination of fast association via the core and very slow dissociation dictated by the arm, which fails to bind actin in the absence of the core. Similarly to Pi, BeF3, and phalloidin, SipA potently inhibited actin depolymerization by ADF/cofilin, which correlated with the increased filament persistence length, supporting the hypothesis that F-actin's mechanical properties contribute to the recognition of its nucleotide state by protein partners. The remarkably strong binding to F-actin maximizes the toxin's effects at the injection site while minimizing diffusion and global influence on the cytoskeleton and preventing pathogen detection by the host cell. We speculate that the unusual binding mode of SipA deep in the cleft separating two strands of F-actin confers unique qualities that SipA shares with other bacterial and likely host proteins, whose functionality is yet to be explored and understood.

High-throughput Dissective RNA Assay to Study RNA-protein Interactions in Cells

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Abstract

RNA-binding proteins (RBPs) play a crucial role in many RNA-mediated biological processes, making it essential to understand how they interact with RNA at the molecular level. In this study, we introduce a massively parallel RNA assay combined with immunoprecipitation (MPRNA-IP) - a high-throughput assay that tests tens of thousands of RNA sequences for RBP-binding potential by immunoprecipitation. We also develop statistical models to pinpoint RNA regions and evaluate the role of RNA structure in these interactions. By using MPRNA- IP, we can determine which RNA domains, sequences, and structural features are required, and even sufficient, for binding to a given protein, all within a single experiment. MPRNA-IP was successfully applied to multiple RNAs of interest, including NORAD and human telomerase RNA, and we use it to study RNA's interaction with the DNA-looping factor CTCF. By combining systematic mutation analysis with immunoprecipita-

tion, MPRNA- IP offers a new high-throughput approach for uncovering how RNA contributes to RNA-protein interactions within cells.

Membrane Protein Chaperone Affects the Kinetics and Morphology of Protein Aggregation

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Abstract

Protein aggregation is a biological phenomenon caused by the accumulation of misfolded proteins. Amyloid beta (Aβ) peptides are derived from the cleavage of a larger membrane protein molecule and accumulate to form plaques extracellularly. According to the amyloid hypothesis, accumulation of Aβ aggregates in the brain is primarily responsible for the pathogenesis of Alzheimer's disease (AD). Therefore, the disassembly of Aβ aggregates may provide opportunities for alleviating or treating AD. Here, we show that the novel protein targeting machinery from chloroplast, chloroplast signal recognition particle 43 (cpSRP43), is an ATP- independent membrane protein chaperone that can both prevent and reverse Aβ aggregation effectively. Using of Thioflavin T dye, we obtained the aggregation kinetics of Aβ aggregation and determined that the chaperone prevents Aβ aggregation in a concentration-dependent manner. Size exclusion chromatography and sedimentation assays showed that 10-fold excess of cpSRP43 can keep Aβ in the soluble monomeric form. Electron microscopy showed that the fibril structure was disrupted in the presence of this chaperone. Importantly, cpSRP43 utilizes the binding energy to actively remodel the preformed Aβ aggregates without assistance by a cochaperone and ATP, emphasizing its unique function among protein chaperones. Our results demonstrate that the presence of cpSRP43 inhibits or retards Aβ peptide aggregation, potentially opening new avenues to strategically develop an effective treatment for AD.

RNF4 and USP7 Cooperate in Ubiquitin-regulated Steps of DNA Replication

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Abstract

DNA replication requires precise regulation achieved through post-translational modifications, including ubiquitination and SUMOylation. These modifications are linked by the SUMO-targeted E3 ubiquitin ligases (STUbLs). Ring finger protein 4 (RNF4), one of only two mammalian STUbLs, participates in double-strand break repair and resolving DNA-protein cross-links. However, its role in DNA replication has been poorly understood. Using CRISPR/Cas9 genetic screens, we discovered an unexpected dependency of RNF4 mutants on ubiquitin specific peptidase 7 (USP7) for survival in TP53-null retinal pigment epithelial cells. TP53-/-/RNF4-/-/ USP7-/- triple knockout (TKO) cells displayed defects in DNA replication that cause genomic instability. These defects were exacerbated by the proteasome inhibitor bortezomib, which limited the nuclear ubiquitin pool. A shortage of free ubiquitin suppressed the ataxia telangiectasia and Rad3-related (ATR)-mediated checkpoint response, leading to increased cell death. In conclusion, RNF4 and USP7 work cooperatively to sustain a functional level of nuclear ubiquitin to maintain the integrity of the genome.

Exercise Hormone Irisin Lowers Amyloid-β Levels by Triggering Neprilysin Release from Astrocytes

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Abstract

One of the key pathological features of Alzheimer's disease (AD) is the accumulation of amyloid-beta (Aβ) protein in the brain. Studies have shown that physical exercise can alleviate Aβ burden in various AD mouse models, although the mechanisms behind this remain unclear. Irisin is a hormone released during exercise. Utilizing a three-dimensional (3D) cell culture model

of AD, we reveal that irisin significantly mitigates Aβ pathology by enhancing astrocytic secretion of the Aβ-degrading enzyme neprilysin (NEP). This process occurs through the downregulation of ERK-STAT3 signaling. We also identify integrin αV/β5 as the receptor for irisin on astrocytes, which mediates irisin-induced astrocytic NEP releasee.

nc886, a Multi-Functional Noncoding RNA Implicated in Innate Immunity and Cancer

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Abstract

nc886 is a medium-sized non-coding RNA (ncRNA) that is transcribed by RNA polymerase III (Pol III). Although Pol III-transcribed ncRNAs, such as 5S rRNA, tRNAs and U6 small nuclear RNA, are generally thought to be constitutively expressed, nc886 is an intriguing one. The nc886 promoter region contains two CpG islands, hypermethylation of which silences nc886 expression. nc886 is a polymorphically imprinted gene; nc886 is methylated on the maternal allele in 75% of human individuals, but unmethylated on both alleles in the remaining 25%. Consequently, nc886 expression is variable among normal human individuals and is frequently silenced in cancer. Our laboratory has found that nc886 regulates gene expression by controlling the activity of target proteins, including protein kinase R and Dicer, both of which are involved in innate immunity and cancer. By controlling these, nc886 plays a critical role in viral infection, tumorigenesis, the detection and elimination of pre-cancerous cells, and the efficacy of chemotherapeutic anti-cancer drugs. Taken together with its intriguing regulation and roles in cancer, we speculate that nc886 is a critical factor in determining susceptibility to pathogens and predisposition to cancer in human individuals.

Acknowledgments

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Multi-functional Roles of UBE4B-mediated Ubiqtuitination in Neuroblastoma Cell Signaling

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Abstract

Children with high-risk neuroblastoma are unlikely to be cured despite intensive treatment, and new treatments directed against biologically relevant pathways are likely to improve survival rates. We have shown that low expression of the E3/E4 ubiquitin ligase UBE4B are associated with poor neuroblastoma patient outcomes. The functional roles of UBE4B in neuroblastoma pathogenesis, however, are not well understood. We have shown that UBE4B mediates trafficking of growth factor receptors such as RET and lysosomal receptor degradation in neuroblastoma cells, which in turn regulates neuroblastoma intracellular signaling pathway activity and tumor cell differentiation. Our recent studies have also identified UBE4B interactions with the E3 ubiquitin ligase ITCH/AIP4, and this complex serves to generate Lys48/Lys63 branched polyubiquitin chains on Ku70, c-FLIPL, and p53, leading to proteasomal degradation and induction of cell death. We have identified further interactions between UBE4B and the deubiquitinase USP7, with USP7 activity leading to reduced Ku70 and c-FLIPL ubiquitination and reduced degradation and with USP7 inhibition being effective against neuroblastoma, further emphasizing the critical role of UBE4B in neuroblastoma tumor growth and patient outcomes via effects on growth factor receptor signaling as well as effects on cell death pathways in response to therapy. Our results have therefore identified novel therapeutic targets for children with neuroblastoma by demonstrating the efficacy and mechanisms of action of USP7 inhibitors as well as activators of the UBE4B/ITCH complex in neuroblastoma tumors.

Targeting GLUT3 as an Anti-metastasis Regimen in Triple-negative Breast Cancer

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Abstract

Obesity and its associated hyperglycemia are linked to poor cancer prognosis, including breast cancer. Clinical studies show that hyperglycemia is associated with worse outcomes in breast cancer patients, although the molecular mechanisms remain to be elucidated. Cancer cells overexpress glucose transporters (GLUTs) especially GLUT1 and GLUT3 to uptake extracellular glucose. Cell mechanics, which involve cell stiffness and contractile force, play a critical role in cell invasion and metastasis. Previously we have shown that higher extracellular glucose boosts glycolysis and activates the RhoA signaling pathway, leading to increased cell stiffness and contractility in triple-negative breast cancer (TNBC) cells, promoting cell migration and invasion. This work highlighted the critical role of glucose on cell invasion. In this study, we discovered that inhibition of GLUT3 either by small molecule inhibitor, G3iA, or by gene knockout significantly reduces the motility of TNBC cells. Suppression of GLUT3 decreased glucose uptake and glycolysis, subsequently activated AMP-activated protein kinase (AMPK). Active AMPK enhances phosphorylation of yes-associated protein (YAP) and suppressed non-muscle myosin II activity. GLUT3 inhibition also increased intracellular cAMP levels, which activates protein kinase A (PKA) and inactivates VASP. Reduced VASP activity was correlated with decreased F-actin levels, leading to reduced cell stiffness. The AMPK and PKA signalling directly regulated cell invasion *in vitro*. These findings suggest that GLUT3 inhibition alters TNBC cell mechanics, decreasing cell motility through the AMPK and PKA axes. Given the elevated GLUT3 levels in TNBC, further studies are warranted to evaluate G3iA's potential as an anti-metastatic treatment in hyperglycemic TNBC models.

Long Non-coding RNA-protein Interactions in Multiple Myeloma: Impact on Therapy Resistance and Patient Outcomes

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Abstract

Multiple myeloma (MM) is an incurable malignancy of plasma cells, with over 35,000 new cases diagnosed annually in the United States. Despite therapeutic advancements, nearly all patients relapse and develop drug resistance. The standard treatment for transplant-eligible MM patients combines a proteasome inhibitor, an immunomodulatory drug, and a steroid, followed by high-dose melphalan and autologous stem cell transplantation. While novel agents have improved outcomes, high toxicity and resistance demand the development of new therapeutic strategies. The mechanisms underlying MM progression and treatment response remain unclear, particularly the role of long non-coding RNAs (lncRNAs). Thereby, we performed transcriptome sequencing on newly diagnosed MM (NDMM) patients with short (< 24 months) vs prolonged (> 24 months) progression-free survival (PFS) following the standard treatment. We identified 157 lncRNAs upregulated in patients with short PFS, focusing on LINC01432, the most highly upregulated lncRNA. Functional studies revealed that LINC01432 overexpression enhances cell viability and inhibits apoptosis, while knockdown reduces viability and increases apoptosis. In addition, decreasing expression of LINC01432 shows regulation of apoptotic markers. LINC01432 localizes to both the cytoplasm and nucleus in NDMM patient samples and MM cell lines, suggesting multifunctional regulatory roles. We next identified CELF2 as a predicted RNA- binding protein for LINC01432, which we validated via iCLIP, RT-qPCR, and multiplexed fluorescent RNA in situ hybridization. Knockdown of LINC01432 using locked nucleic acid antisense oligonucleotides significantly increased apoptosis. This study identifies lncRNAs associated with poor response to NDMM treatment and is the first to characterize LINC01432, highlighting its potential as a therapeutic target in MM.

Plant Polymeric Scaffolds for the Growth and Development of Triple-negative Breast Cancer Cells

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Abstract

Biopolymers are functional materials and among biopolymers arabinoxylans (AX) are the main non-starch polysaccharide in cereals and other plants. AX is composed of xylose and arabinose, and the ester-linkage of ferulic acid with arabinose confers its bioactive properties. The backbone of AX is similar to that of glycosaminoglycans, a major extracellular matrix of the human body. The objective was to develop plant- derived AX mixed soft scaffolds suitable for supporting the growth and proliferation of triple-negative breast cancer (TNBC) cells such as MDA MB231 and patient-derived xenograft (PDX). TNBC is a very aggressive form of breast cancer that accounts for 15% - 20% of breast cancer cases. Patients with TNBC have the worst prognosis caused by stemness of the cells and clumping of cells with hypoxia regions within the clumps. Wheat bran and sugar beet pulp are two major agricultural byproducts and feeds for livestock left over after milling and extracting sugar respectively. We have extracted AX from wheat bran (WBAX) and sugar beet pulp (SBAX) and developed various soft scaffolds with varying proportions of Na-Alginate, collagen, WBAX, and SBAX. The scaffolds were seeded with triple-negative breast cancer cell-line MDA MB231 and PDX. We observed the growth and development of cells for up to 21 days, cell migration within the hydrogels, stemness of the cells, and hypoxia regions while cells were clumped together.

Mechanoregulation of Tissue Heterogeneity and Breast Cancer Risk

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Abstract

During mammary tumor development, the ECM undergoes substantial remodeling, including changes in both biochemical composition and biomechanical properties. These ECM changes not only facilitate metastatic breast cancer progression but also appear to influence cell phenotype and fate in mammary tissues well before tumor initiation. Tissue stem/progenitor cell frequency is also implicated in tumor progression, but specific factors linking stem/progenitor cell numbers to cancer risk remain ill-defined. We observed that the breast tissue from women with high mammographic density, who exhibit increased lifetime risk for breast cancer, is both stiffer and enriched in stem/progenitor epithelial cells. Using genetically engineered mouse models with elevated integrin mechanosignaling and collagen density, along with syngeneic manipulations and spheroid models, we demonstrate that a stiff ECM and heightened integrin mechanosignaling increase stem/progenitor cell frequency and promote mammary tumor initiation *in vivo*. Mechanistically, we found that augmented tissue stiffness expands breast epithelial stem-progenitor cell number by potentiating ERK activity to foster progesterone receptor-dependent RANKL signaling. Consistent with this, elevated phosphorylated ERK and progesterone receptor and increased levels of RANKL signaling were detected in stiff breast tissues from women with high mammographic density. These findings establish a causal link between tissue fibrosis, integrin mechanosignaling, stem/progenitor cell frequency and breast cancer risk. Targeting RANK or ERK signaling may therefore mitigate the tumor-promoting effects of fibrosis in women with dense breast tissue. Current efforts are focused on uncovering how ECM stiffness and progesterone receptor signaling reprogram epithelial and stromal immune cell states in both normal and premalignant mammary environments.

Ferroptosis Defense Systems in Cancer

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Abstract

Cellular life and death dynamics play a crucial role in both the onset and treatment of cancer. While the induction of apoptosis, a programmed cell death mechanism, serves as a cornerstone in cancer therapy, tumors often develop strategies to evade this process. The emergence of ferroptosis, an iron-dependent non-apoptotic cell death pathway, holds promise in combating drug-resistant malignancies. We have spotlighted key regulators that orchestrate ferroptotic resistance in pancreatic cancer. Additionally, we've identified an underexplored role of a cytosolic complex that inhibits ferroptosis. Leveraging advanced mass spectrometry, metabolomics, and site-directed mutagenesis, we've unraveled the mechanism by which this complex impedes phospholipid peroxidation and membrane rupture, thereby inhibiting ferroptotic cell death. Through rigorous screening, we've pinpointed a potent small-molecule compound capable of interrupting the functionality of this complex. In summary, our findings provide an expanded perspective on ferroptosis defense systems, paving the way for developing precise and potent therapeutic strategies in cancer treatment.

Is DUSP22-rearranged Anaplastic Large Cell Lymphoma a Potential Target 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. Anaplastic large cell lymphoma (ALCL) is a peripheral T cell lymphoma with strong and uniform CD30 expression and is divided into ALK+ and ALK-negative subtypes based on ALK rearrangement or expression. In contrast with ALK+ ALCL, ALK-negative ALCL is a genetically heterogenous entity, with 20 - 30% of the cases harboring DUSP22 rearrangement. In our study, programmed cell death ligand 1 (PD-L1) expression was evaluated in patients with systemic ALCL including ALK+ and ALK-negative cases. We found that ALK+ ALCL was more often positive for PD-L1 than ALK-negative ALCL (76% vs 42%, $p = 0.002$). ALK-negative ALCL showed a strong correlation between PD-L1 expression and STAT3 activation (pSTAT3) ($r = 0.8$, $p < 0.0001$). Interestingly, a small subset of ALK-negative ALCL cases did not show either PD-L1 or pSTAT3. Our study also showed that patients with DUSP22-rearranged ALK-negative ALCL were younger than those with DUSP22-non-rearranged neoplasms ($p = 0.049$). DUSP22-R ALK-negative ALCL cases had a unique immunophenotype: more often positivity for CD15, CD8, and less frequent expression of granzyme B, EMA, PD-L1 and pSTAT3 (all $p < 0.05$). We conclude that PD-L1 expressions are more common in ALK+ ALCL than in ALK-negative ALCL, suggesting that patients with ALK+ ALCL may be good candidates for PD-1 blockade immunotherapy. In contrast, PD- L1 expression is absent in DUSP22-R ALK-negative ALCL, suggesting these patients may not be potential candidates for PD-1 blockade therapy.

BRK-mediated Dysregulation of TGF-β/SMAD Signaling Promotes Breast Cancer Metastasis

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Abstract

Breast tumor kinase (BRK), frequently overexpressed in breast cancer, dysregulates TGF-β/SMAD signaling by phosphorylating SMAD4 at Y353 and Y412. Phosphorylation at Y412 triggers SMAD4 proteasomal degradation, while modification at Y353 disrupts SMAD2/SMAD4 association and enables the interaction of SMAD4 with the nucleosome remodeling and deacetylase (NuRD) complex. Consequently, BRK-driven SMAD4 depletion represses the tumor suppressor FRK and elevates mesenchymal markers SNAIL and SLUG, fostering an epithelial–mesenchymal transition and promoting metastatic progression. By directly impeding SMAD4 function, BRK exacerbates malignant phenotypes, highlighting the need for targeted intervention. These findings underscore a pivotal mechanism by which BRK, through distinct SMAD4 phosphorylation events, impairs canonical TGF-β signaling and advances breast cancer metastasis. Thus, inhibiting BRK while restoring SMAD4 func-

tion could yield synergistic therapeutic benefit. Altogether, this study identifies novel BRK-mediated alterations in SMAD4 regulation, pinpointing a potential therapeutic target for aggressive breast cancers characterized by elevated BRK expression and TGF-β pathway dysfunction.

Modern Kidney Cancer Classification in the Era of Molecular Pathology Practical Considerations and Clinical & Therapeutic Implications

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Abstract

With the incorporation of molecular testing techniques in clinical practice of kidney cancer management, surgical pathologists' understanding of kidney cancer classification has significantly advanced. While the new information based on enhanced molecular interrogations is exciting and revealing, tumor classification is a dynamic and complex process which requires the integration of various tumor characteristics including molecular phenotype, histopathologic and immunohistochemical features as well as clinical behavior. The newest (5th edition) WHO classification of tumors introduced a new category of molecularly defined renal tumor entities. In recent years, studies have shown that some of these molecularly defined tumors have an overly broad morphological spectrum without an unequivocal genotype–phenotype correlation. Therefore, molecular testing including next-generation sequencing is increasingly used to classify RCC, and it has especially broadened the differential diagnosis of renal tumors considered in the oncocytoma and chromophobe renal cell carcinoma (RCC) category, which now include fumarate hydratase–deficient RCC, epithelioid angiomyolipoma, TFE3- rearranged and TFEB-altered RCCs, and eosinophilic solid and cystic RCC. However, there are some criticisms that the classification system has become perhaps too complex and demanding, with some voicing concerns about the limited availability and accessibility of the molecular techniques in some parts of the globe. This presentation aims to go over the practical considerations and potential clinical and therapeutic implications of the newest classification system in clinical practice.

Sensitive Detection of Multiple Blood Biomarkers via Immunomagnetic Exosomal PCR for the Diagnosis of Alzheimer Disease

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Abstract

Blood exosomes are emerging as potential biomarkers for diagnosing brain diseases such as Alzheimer's disease (AD). There is currently a lack of ultrasensitive technology for identifying core AD biomarkers in blood exosomes to optimize the utility of biomarkers in clinical practice. Here, an immunomagnetic exosomal polymerase chain reaction (iMEP) platform was developed using DNA-conjugated antibodies for the rapid detection of amyloid-β (Aβ1–40 and Aβ1–42) and phosphorylated tau (p-tau396,404 and p-tau181) in clinical blood exosomes. The toehold shift–mediated DNA affinity pulldown eliminates the high detection background, which allows the detection of biomarkers at concentrations down to 10 femtograms per milliliter. With the iMEP assay, exosomal Aβ1–42 was more accurate in differentiating patients with AD from healthy individuals compared with exosomal p-tau181 and p-tau396,404, with a sensitivity of 95.0% and a specificity of 95.0%. The iMEP technique is also adept at quantifying the levels of different exosomal biomarkers associated with disease pathogenesis.

Neuronal Sensory Network in the Duodenal Epithelium and its Role Controlling Intestinal Absorptive and Secretory Processes, Serotonin Secretion and Mucosal Innate Immune Response

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Abstract

The communication between the lumen and the enteric nervous system is believed, in general, to be mediated by serotonin (5-HT) released from enteroendocrine cells, which would sense chemical, osmotic, and mechanical stimuli through receptors

exposed to the intestinal lumen. Then, serotonin activates specific receptors in the afferent nerve terminals that reach the sub-epithelial region of the intestinal mucosa and transmit information to the intestinal plexuses, intestinal ganglia, and spinal cord. Contrasting with the existing hypothesis, we recently reported histological and functional evidence demonstrating a complete epithelial neural network that reaches the epithelial surface and connects the duodenal lumen with the enteric nervous system (JHC 71(11) 601–630, 2023). The neuron reaches the lumen through a neuronal apical extension and the subepithelial space through axonal extensions, connecting with various sub-epithelial elements at the lamina propria. The epithelial neuron has morphological characteristics of sensory neurons. It is a polarized cell with a thin and long apical process that finishes in a knob-like terminal protrusion in contact with the luminal space. At its basal region, it presents axon extensions, connecting with glia, afferent nerve terminals, enterochromaffin cells, smooth muscle bundles, and sub-epithelial lymphoid follicles at the lamina propria. Here, we present histological evidence related to the potential implications of this epithelial, neuronal network in sensory and regulatory functions of intestinal absorption and secretion, endocrine homeostasis, and immune innate response at duodenal mucosa, offering opportunities for future research and clinical applications.

Modulating Polyamine Metabolism for Neuroprotection Against Tauopathy

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Abstract

Polyamines are small, positively charged molecules that are abundant in cells, primarily including spermidine and spermine, along with their precursor, putrescine. Precise regulation of polyamine metabolism is essential for cellular and organismal health. Mutations in the gene encoding spermine synthase (SMS) cause Snyder-Robinson syndrome (SRS), a rare X-linked intellectual disability disorder marked by spermidine accumulation and impaired autophagy in the nervous system. While polyamine metabolism has been implicated in various autophagy-related neurodegenerative diseases, including Tauopathy, the functional crosstalk between these pathways remains poorly understood. In this study, we investigated how modulating SMS expressions affect autophagy and Tau pathology using both Drosophila and human cellular models. Complete loss of Drosophila spermine synthase (dSms) impaired lysosomal function and blocked autophagic flux, mirroring SRS phenotypes. In contrast, partial reduction of dSms enhanced autophagic flux, decreased Tau protein accumulation, and led to improved motor function and extended lifespan in Tauopathy flies. Polyamine profiling revealed a mild increase in spermidine in flies with partial dSms loss. Consistent with these findings, partial knockdown of SMS in human neuronal and glial cells similarly enhanced autophagy and reduced Tau levels. Furthermore, proteomic analysis of postmortem brain tissue from Alzheimer's disease (AD) patients showed a modest but significant upregulation of SMS expression. Together, our results reveal a functional link between polyamine metabolism and autophagy in the context of neurodegeneration. We propose that moderate SMS inhibition promotes autophagic clearance of Tau and mitigates neurodegenerative phenotypes, highlighting SMS as a potential therapeutic target for Alzheimer's disease and related tauopathies.

Proteomics and Structures of Ion Channel Signaling Complexes

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Abstract

Ion-channel modulation by auxiliary subunits and binding proteins is a critical mechanism in cellular signaling, with profound implications for various physiological and pathophysiological processes. Our research aims to understand how these binding proteins influence ion-channel activity by integrating proteomics, structural biology, and electrophysiology approaches. Through the study of interactions between binding proteins and ion channels, we explore the mechanisms by which these proteins regulate channel opening, closing, and gating dynamics. Using quantitative proteomics (SILAC) and co-immunoprecipitation (Co-IP), we identified LSm12 as a receptor for NAADP in NAADP-evoked calcium release from two-pore channels (TPCs). We established that LSm12 is a shared interacting partner of NAADP, TPC1, and TPC2. LSm12 directly binds NAADP via its LSm domain, colocalizes with TPC2, and mediates the association of NAADP with purified TPC2. Our findings demonstrate that LSm12 is essential for NAADP-evoked TPC activation and Ca^{2+} mobilization, suggesting it functions as both an NAADP receptor and a TPC regulatory protein. To gain a deeper understanding of these interactions, we employ Cryo-EM and other biochemical methods to visualize, at high resolution, and characterize the molecular interactions between small regulatory proteins and ion channels, revealing conformational changes and allosteric effects that govern channel behavior.

DNA Flexibility Regulates Transcription Factor Binding to Nucleosomes

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Abstract

Cell fate decisions are controlled by sequence-specific transcription factors (TFs), referred to as 'pioneer' factors, that bind their target sites within nucleosomes (pioneer binding') and thus initiate chromatin opening. However, genomic studies have shown that pioneer factors recognize just a minority of their recognition sequences, suggesting that the sequence context may regulate pioneer binding. We have developed PIONEAR- seq, a biochemical assay for high-throughput analysis of pioneer binding to nucleosome positioning sequences, which we used to characterize 10 proposed human pioneer factors. Comparison of TF binding to nucleosomes based on synthetic versus genomic sequences revealed that the pioneer binding, while determined primarily by the presence of the known TF recognition motifs, is further modulated by the broader sequence context (~150 bp) of nucleosomes; TFs previously described as having an intrinsic preference for certain nucleosome binding modes (i.e., dyad, periodic and end binding) exhibited only end binding to nucleosomes based on genomic sequences, suggesting a nucleosome-specific positional preference for TF recruitment. We validated these findings by using a variety of structural, biochemical, genomics and computational analyses. We propose a model where the nucleosome exploits the local bendability of the DNA sequence to regulate the positional preference of pioneer binding, revealing another cis-regulatory layer in eukaryotic genomes.

Carcinogenesis and Therapy-role of Epigenetics

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Abstract

Carcinogenesis originates from cancer stem/progenitor cells. Epigenetic alterations play a significant role in generation of cancer progenitor cells and cancer progression. Both deacetylation of histones by class I HDACs and DNA methylation by DNMT1 show allosteric regulation suggesting signaling regulate these processes. Acetylation and demethylation express tumor suppressor genes and make the cancer cells more susceptible to initiate combination therapy. Combination therapy including epigenetic drugs possibly enhance the efficacy of treatment.

PHGDH-mediated De Novo Serine Synthesis Regulates Retinal Vascularization by Modulating Metabolic Balance in Neovascular Retinopathy

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Abstract

Purpose: In retinopathy of prematurity oxygen-induced retinopathy (OIR), retinal fuel utilization critical for treating pathological vascularization. Mitochondrial dysfunction contributes to hypoxia-induced retinal angiogenesis. Mutations in phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme in de novo serine synthesis, is linked to macular telangiectasia and endothelial mitochondrial defects. We previously observed elevated retinal serine in ROP mice, but its role in metabolic regulation and vascularization remains largely unexplored.

Methods: Tamoxifen-induced Müller glia knockdown of PHGDH (GlastCreER: Phgdhflox/flox) mice were exposed to hyperoxia at postnatal day (P)7 and returned to room air at P12 in OIR mouse model. Retinal vasculature was assessed. Proteomics of OIR vs normoxia retinas, PHGDH inhibitor (CBR-5884) vs vehicle treated rat Müller glial cells (rMC-1), and metabolomic analysis of Cre+ vs Cre- retinas were performed. Statistical analysis was conducted using unpaired t-tests, Mann-Whitney tests, or two-way ANOVA, and data are presented as Mean ± SEM.

Results: In OIR, compared to Cre- littermates, tamoxifen-induced GlastCreER+:Phgdhflox/flox pups exhibited a 16.5% increase ($p = 0.001$, n = 10 - 13 retinas per group) at P12 in vaso-obliteration (VO). Proteomics revealed decreased glycolytic proteins and increased oxidative phosphorylation (OXPHOS) proteins in P12 wild- type (WT) OIR retinas compared to normoxia retinas. Loss of Müller glial Phgdh led to reduced glycolytic and OXPHOS protein expression in OIR vs normoxia

retinas. CBR-5884 treatment decreased mitochondrial protein expression in rMC-1 cells.

Conclusion: In WT OIR, hyperoxia induced a metabolic shift from glycolysis to OXPHOS. Loss of Müller glial Phgdh exacerbated hyperoxia-induced retinal VO, potentially due to impaired OXPHOS, possibly compensating for the metabolic deficit.

Imaging Organelles and Neuronal Architecture Using New Fluorescent Probes for Live STORM Imaging

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Abstract

Elucidating molecular organization in cell biology requires precisely localize single or aggregated molecules and analyzing quantitatively their spatial distributions. We previously developed a statistical method SODA (Statistical object distance analysis plugin, Nature Comm 2018) that uses either micro- or nanoscopy to significantly improve standard co-localization techniques. Our method considers cellular geometry and densities of molecules to provide statistical maps of isolated and associated (coupled) molecules. SODA can be used to detect indirect molecular association in conventional microscopy or even in super resolution microscopy (SIM, STED or STORM) where higher resolution prevents the use of conventional overlay methods. We will show how SODA method can be used to detect the alignment of synaptic proteins in STORM. To unravel cellular shape 3D organization, we previously presented membrane probes call the MemBright family which are six fluorescent probes compatible with long-term live-cell imaging both in cultures and tissue without any use of transfection or transgenic animals. MemBright can be used in 3D multicolor dSTORM in combination with immunostaining to labels receptors clustered at cell surface at single molecule level. We are now working on how to extend MemBright capabilities to Live STORM imaging. Live SMLM imaging is a challenging task as it requires fluorescent probes able to spontaneously blink upon irradiation. We propose here to show how new bright photoconverters that were further targeted to cell compartments and can be used during SMLM imaging to reveal lipid droplets, plasma membrane, or mitochondria on live samples.

Understanding and Modulating Regulators of Enhancers: Implications for Normal and Malignant Myelopoiesis

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Abstract

Enhancers are abundant regulatory elements that modulate gene expression in a cell-type-specific and spatiotemporal manner, often acting at considerable distances from their target promoters. The mechanisms underlying enhancer-promoter communication have remained elusive for decades. Enhancer dysfunction is implicated in various diseases, including acute myeloid leukemia (AML). During hematopoiesis, lineage- specific transcription factors such as PU.1 and CEBPA are regulated by enhancers, which are themselves influenced by these same factors. Ubiquitous transcription factors like RUNX1 further modulate enhancer activity, coordinating with lineage-specific regulators. Our team discovered that RUNX1 regulates myeloid- specific transcription via LOUP, an enhancer RNA (eRNA) that mediates enhancer-promoter interactions. We demonstrated that PU.1, a master myeloid transcription factor, is a strong inducer of LOUP, establishing a feedforward loop wherein PU.1 and LOUP mutually enhance each other's expression to drive myeloid differentiation. In AML, this regulatory axis is disrupted. The oncogenic fusion protein RUNX1-ETO impairs the interplay between transcription factors and eRNAs, leading to enhancer dysfunction, differentiation arrest, and malignant proliferation. To address this, we have developed platforms to perturb eRNA expression and are applying chemoinformatic tools to identify modulators of enhancer function. These strategies aim to restore proper enhancer activity, presenting new therapeutic opportunities for AML and other diseases linked to enhancer dysregulation.

Spermatogonia Differentiation: New Insights Gained from Studying the Cannabinoid Receptor Type 1 (CB1) Knockout Mouse Model

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Abstract

Spermatogonia (SPGs) represent a heterogeneous population of germ cells consisting of undifferentiated- SPGs, including spermatogonial stem cells (SSCs) and early progenitors, as well as differentiated-SPGs. The presence of SSC niches in seminiferous epithelium of testis guarantees a life-long production of spermatozoa. The neurotrophic factor GDNF is a critical component for the SSC niche and overall, for undifferentiated-SPGs pool homeostasis acting via the GFRα1/RET receptor complex expressed on SSC and early progenitor cell surface. The cannabinoid receptor CB1 is involved in stem cell biology in several tissues but its implications in testis have not yet been investigated. Here, using wild type (WT) vs CB1 knock out (CB1-/-) mice we characterize the involvement of CB1 activity in undifferentiated-SPGs homeostasis regulated by GDNF. We demonstrate that Cb1-deletion negatively affected GDNF production and the expression of pluripotency- associated genes (Oct4; Nanog; Nanos2; Etv5), downstream impairing the number of SSC-ID4+ and undSPG- GFRα1+ cells. In addition, the expression of commitment-associated genes (Eomes; Lin28a; Sohlh1) and molecular markers related to committed early progenitors (SOX3, NGN3; RARɣ), significantly increased in CB1-/- testis revealing a higher proneness of undifferentiated-SPGs to undergo the commitment/differentiation phase. Interestingly, an inverse relationship between GDNF and MAGL hydrolase protein levels was observed in CB1-/- testis, suggesting that CB1 regulates MAGL, and in turn testicular GDNF levels. In confirmation, testes pharmacologically stimulated *in vitro* with selective CB1 agonist/antagonist or MAGL inhibitor revealed that 2-AG is the endogenous CB1 ligand modulating GDNF levels. Collectively, our results define new implications of CB1 receptor in signaling pathways regulating SPG homeostasis.

Mitochondrial Transplantation Promotes Corneal Epithelial Wound Healing

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Abstract

The corneal epithelium, the outermost layer of the eye, serves as a critical interface that protects the eye while maintaining corneal transparency and vision. Corneal epithelial injuries and defects are among the most common ocular pathologies. Corneal recovery is slow and often incomplete, leading to scarring, opacification, and loss of vision. Therefore, treatments which improve corneal wound healing are warranted. Mitochondria, the main source of cellular energy production, plays a critical role in wound healing and mitochondrial dysfunction has been shown to be associated with corneal epithelial defects. We sought to investigate the potential effect of augmenting mitochondria levels via mitochondrial transplantation in injured corneas to promote wound healing. We incubated human corneal epithelial cells (hCECs), harvested from human corneas, with isolated mitochondria and found that mitochondrial transplantation induced the proliferation and wound healing of primary hCECs. *In-vivo*, we inflicted corneal chemical injury in mice and applied isolated mitochondria to the ocular surface. We found that mice receiving mitochondria recovered twice as fast as control mice following injury, presenting both enhanced and improved repair. Corneas which were treated with mitochondria demonstrated the re- epithelization of the wound area to a multi-layer appearance, compared to thinning and complete loss of the epithelium in control mice. Mitochondrial transplantation also prevented the thickening and disorganization of the corneal stromal lamella, restoring normal corneal dehydration. In conclusion, mitochondrial transplantation promotes corneal re-epithelization and wound healing. Augmentation of mitochondria levels via mitochondrial transplantation may serve as an effective treatment for inducing the rapid repair of corneal epithelial defects.

The Role of Paraoxonase 2 in Lung Tumorigenesis

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Abstract

Paraoxonase 2 (PON2) is a multifunctional intracellular enzyme that has received growing attention for its ability to modulate various aspects of normal and malignant cellular physiology. Recent research has revealed that PON2 is upregulated in tissues from patients with various types of solid tumors and hematologic cancers, likely due to its ability to suppress oxidative stress and evade apoptosis. However, the effects of PON2 on pulmonary oncogenesis are unknown. We first conducted studies to explore how PON2 influences lung cancer cell proliferation *in vitro*. It was found that PON2 expression deficiency hampered the proliferation of cultured lung cancer cells with concomitant cell cycle arrest at the G1 phase. In addition, the loss of endogenous PON2 expression impaired key aspects of oxidative metabolism in lung adenocarcinoma cells. Moreover, the involvement of PON2 in lung tumorigenesis was investigated using a variety of animal models. Our research reveals how the interplay between PON2 expression in lung tumors and host mice influences lung tumor initiation and progression.

The Effect of Betulinic Acid Polymer-drug Conjugation on Pancreatic Cancer Cells

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Abstract

Pancreatic cancer (PC) is one of the most aggressive solid malignancies characterized by poor response to treatment and consequently low survival rates. Herbal medicines such as betulinic acid (BA) have shown potential in treating various solid tumours, although with limitations. Polymer-drug conjugation can circumvent these limitations thereby increasing drug efficacy. This study aimed to determine the effect of the polyethylene glycol-betulinic acid polymer conjugate, PEG-BA, on PC cells. In a tetrazolium dye assay to evaluate the cytotoxic effect of PEG-BA and BA on MIA PaCa-2 and Vero cells, PEG-BA treatment resulted in selective cytotoxicity against the MIA PaCa-2 cells with an IC_{50} of 3.01 ± 0.62μM compared to 40.29 ± 3.60μM for BA. Flow cytometry assays to determine the mode of cell death showed that PEG-BA induced apoptotic cell death through the arrest of MIA-PaCa-2 cells in the Sub-G1 phase of the cell cycle compared to BA and untreated cells (39.50 ± 5.32% > 19.63 ± 4.49% > 4.57 ± 0.82%). An ELISA assay showed moderate expression of NFκB/p65 protein. However, significant ($p < 0.05$) overexpression of the pro-apoptotic genes TNF (23.72 ± 1.03), BID (3.55 ± 0.93), BAX (14.01 ± 3.55), and CASPASE 8 (27.69 ± 0.99) compared to untreated cells was notable. The antioxidant potential of PEG-BA was better (IC_{50} = 15.59 ± 0.64 μM) compared to ascorbic acid (25.58 ± 0.44 μM) and BA-only (> 100 μm) in the DPPH assay. In conclusion, conjugation of PEG to BA resulted in more specific cytotoxicity, apoptotic induction of cell death and antioxidant activity in MIA PaCa-2 cells compared to free BA suggesting its potential for effective PC treatment.

LC3-associated Phagocytosis Drives Neutrophil Granule Transfer and Necrosis Expansion in Glioblastoma

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Abstract

Necrosis is a hallmark of aggressive solid tumors and correlates with poor prognosis, particularly in glioblastoma. While ischemia can initiate necrotic lesions, the mechanisms driving necrosis expansion remain poorly understood. Emerging evidence links neutrophils to necrosis development via the induction of tumor cell ferroptosis through the transfer of myeloperoxidase (MPO)-containing granules. However, how neutrophils deliver these granules to tumor cells has been unclear. Using an unbiased small-molecule screen, we identified statins as inhibitors of neutrophil-induced tumor cell death through blockade of neutrophilic granule transfer. Mechanistically, we discovered a novel process in which tumor cells engulf neutrophils prior to MPO granule release. This uptake is initiated by integrin-dependent adhesion and mediated by LC3-associated phagocytosis (LAP), requiring the Vps34–UVRAG–RUBCN PI3K complex. Genetic depletion of Vps34 or pharmacologic inhibition of

MPO significantly reduced necrosis and extended survival in an orthotopic glioblastoma model. Our findings reveal a critical role for LAP-mediated neutrophil internalization in driving MPO granule transfer and necrosis propagation. Targeting this process may offer a new therapeutic avenue for improving glioblastoma outcomes.

The Beta3-AR Agonist BRL37344 Ameliorates the Main Symptoms of X-linked Nephrogenic Diabetes Insipidus in the Mouse Model of the Disease

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Abstract

X-linked nephrogenic diabetes insipidus (X-NDI) is a rare genetic disorder caused by mutations that inactivate the vasopressin type-2 receptor (AVPR2). This condition is characterized by the kidneys' inability to concentrate urine, leading to severe polyuria, polydipsia and a high risk of dehydration. To date, a real cure remains elusive. A potential therapeutic strategy to bypass AVPR2 mutations could be the activation of other GPCR which share intracellular signaling pathway and localization with AVPR2. Based on previous research, we hypothesized that the β3-adrenergic receptor (β3-AR) could be an ideal target. To test this hypothesis, we investigated the effects of continuous 24 hr stimulation of β3-AR using the agonist BRL37344 in a mouse model of X-NDI. We assessed changes in urine output, urine osmolarity, water intake, and the activation of key renal transporters involved in water and electrolyte balance. Our findings demonstrate that β3-AR agonism exerts a strong antidiuretic effect. While a single intraperitoneal injection of BRL37344 (1 mg/kg) improved symptoms for only 3 hr, repeated administrations over 24 hr, mimicking a slow-release formulation, produced sustained benefits. These included a 27% reduction in urine output, a 25% increase in urine osmolarity, and a 20% decrease in water intake. At the molecular level, BRL37344 enhanced the phosphorylation of NKCC2, NCC, and AQP2 transporters in renal cell membranes, thereby improving water and electrolyte reabsorption within kidney tubules. Taken together, these results suggest that β3-AR agonists could represent a promising therapeutic strategy for managing X-NDI symptoms in humans.

The Protective Side of Immune Defenses: DNA Sensing Pathway Through the cGAS/STING/Type I Interferon in Schistosomiasis

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Abstract

There is significant disease heterogeneity among mouse strains infected with the helminth Schistosoma mansoni. 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)/stimulator 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. Additionally, STING agonist reduces liver inflammation, and induces robust IFNβ production, while IL-1β and IL-17 were impaired in the high pathology mouse model of schistosomiasis. Our findings demonstrate that cGAS/STING signaling represents a unique mechanism inducing protective type I IFN, whic Chi Lih is counteracted by Gsdmd.

Trained Immunity Affecting Dendritic Cell Differentiation and Function in Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis affects ~0.5 - 1% of the adult population and results in joint inflammation, chronic pain, and many systemic comorbidities. Immune and inflammatory tissue damage is the main pathogenic mechanism in rheumatoid arthritis and involves the hyperactivation of both innate and adaptive immune systems. Trained immunity has become well established as an important feature of the innate immune system that allows the host to mount functionally altered immune responses based on their previous history of immune exposures, independently of the classical adaptive immunological memory. However, the role of trained immunity in systemic autoimmune and inflammatory disorders remains poorly understood. In the current work, we demonstrate that emergency myelopoiesis is induced in chronic rheumatoid arthritis in murine models and acts not only to enhance innate immune cell numbers but also to produce functionally altered innate immune cells. Such effects are cell-intrinsic to hematopoietic stem and progenitor cells (HSPCs) and persist independently of the inflammatory disease milieu. Importantly, these trained immunity mechanisms impact not only macrophages but also dendritic cells, which are the major antigen presenting cells that bridge the innate and adaptive immune responses. 'Trained' dendritic cells show changes in global gene expression profiles and significantly altered responses to recall immune stimulation and capacity for T cell activation. This study therefore represents the first demonstration of 'dendritic cell trained immunity' in rheumatoid arthritis.

The Melanocortin 4 Receptor (MC4R) and Kir7.1 Interaction: A Novel Target for Obesity Management

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Abstract

The inward rectifier potassium channel Kir7.1 has emerged as a promising target for obesity management due to its unique interaction with the melanocortin-4 receptor (MC4R) in hypothalamic circuits controlling energy balance. The MC4R, expressed in the hypothalamus, plays a central role in regulating appetite and energy expenditure. Its activation by α-melanocyte stimulating hormone (α-MSH) leads to decreased food intake and increased energy expenditure. Conversely, the inverse agonist Agouti-related peptide (AgRP) promotes feeding. Recent studies have unveiled a novel G-protein-independent signaling paradigm wherein MC4R directly modulates Kir7.1 activity. Our research demonstrates that pharmacological inhibition of Kir7.1 by the small molecule ML418 activates MC4R neurons in the paraventricular nucleus of the hypothalamus, resulting in decreased food intake and weight loss in mice. These effects were found to be dependent on Kir7.1 expression in MC4R neurons, as confirmed through experiments using mice with selective deletion of Kcnj13 (the gene encoding Kir7.1) from MC4R-expressing cells. This study presents the first structures of human Kir7.1 in various conformational states, providing crucial insights into its gating mechanism and pharmacological modulation. These structural data reveal that ML418 acts as a potent blocker of Kir7.1 by binding in the inner vestibule beneath the selectivity filter. The structural and functional insights gained from this study highlight Kir7.1 as a novel and promising target for obesity management. By directly targeting Kir7.1, it may be possible to modulate MC4R signaling without the need for traditional G-protein-coupled receptor agonists or antagonists. These structural insights can guide the optimization of compounds like ML418 and the discovery of novel therapeutic agents targeting the MC4R-Kir7.1 signaling axis. This research not only elucidates a unique signaling mechanism in hypothalamic energy balance regulation but also presents Kir7.1 as a compelling target for obesity treatment. The structural and functional characterization of Kir7.1 opens new avenues for therapeutic intervention and provides a strong rationale for further exploration of Kir7.1-targeted approaches in obesity management.

The Impact of SLC39A14 Dysfunction on Manganese Metabolism

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Abstract

Manganese is an essential trace element involved in numerous physiological processes, but both deficiency and excess can be detrimental to health. ZIP14, encoded by the SLC39A14 gene, is a manganese importer highly expressed in the liver and small intestine—two key organs for manganese regulation. Loss-of-function mutations in SLC39A14 result in manganese accumulation in the brain and lead to severe neurological disorders, emphasizing the importance of ZIP14 in maintaining systemic manganese balance. Consistently, whole-body Zip14 knockout mice exhibit elevated manganese levels in the blood and brain. To understand the tissue-specific contributions of ZIP14 to manganese regulation, we generated mice with liver-specific, intestine-specific, and combined liver/intestine-specific Zip14 deletions. Immunoblotting confirmed targeted ZIP14 loss, and manganese concentrations in various tissues were measured using inductively coupled plasma mass spectrometry (ICP-MS). Our findings revealed that liver-specific knockout mice had significantly reduced hepatic manganese, but brain manganese levels remained comparable to those in control mice. This suggests that hepatic ZIP14 is critical for liver manganese uptake but not essential for overall systemic homeostasis. In contrast, mice lacking intestinal ZIP14—either alone or in combination with liver-specific deletion—displayed markedly elevated brain manganese levels. These results highlight the central role of intestinal ZIP14 in protecting against manganese overload. Mechanistic studies showed that ZIP14 is localized to the basolateral membrane of enterocytes, where it limits manganese absorption by reabsorbing freshly absorbed manganese from the portal blood. In the absence of ZIP14, manganese transport is redirected toward systemic circulation, increasing the risk of overload. Thus, ZIP14 regulates manganese levels through two complementary mechanisms: limiting intestinal absorption and promoting hepatic clearance from the portal circulation. In conclusion, our study identifies intestinal ZIP14 as a key gatekeeper of systemic manganese homeostasis and provides mechanistic insight into how SLC39A14 mutations lead to manganese toxicity.

Knockdown of USP10 with *in vivo* Self-delivering siRNA Reduces Corneal Epithelial Defects

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Abstract

Persistent corneal epithelial defect (PCED) occurs when the corneal epithelium fails to heal properly after injury, leading to visual impairment and vision loss. Corneal wound healing involves parallel repair pathways including inflammatory cell influx, myofibroblast differentiation, and scarring. The deubiquitinase USP10 has been implicated in regulating wound healing, inflammation, and fibrosis in the cornea. To test whether USP10 knockdown promotes wound healing, we established a scrape-induced PCED mouse model and applied *in vivo* self-delivering siRNA targeting USP10. We characterized open wound area, overall corneal integrity, and immune cell infiltrating the stroma. USP10 knockdown prevented wound re-opening at day 28 post- debridement compared to control. Optical coherence tomography (OCT) and hematoxylin and eosin staining showed improved corneal morphology with intact epithelium and endothelium, along with significantly reduced mean pixel intensity (scarring). Percent decrease in Fibronectin, α-SMA and Collagen III expression was 89.45%, 85.15%, and 85%, respectively. Intraocular pressure (IOP) remained unchanged between groups, and *in vivo* confocal imaging confirmed that endothelial cell counts were equivalent, indicating no siRNA- induced toxicity. Immune profiling revealed a significant decrease in CD45-positive immune cells within the corneal stroma upon USP10 knockdown. These findings demonstrate that a one-time application of USP10- targeting siRNA enhances wound healing, prevents epithelial wound re-opening, and reduces fibrosis without compromising corneal integrity or safety. Our data suggests that the cornea can serve as a model system to study fibrosis and that USP10 knockdown may represent a novel therapeutic strategy to promote wound closure and reduce scarring in ocular and non-ocular tissues.

Poster Presentations

The Regulation of Germline Proteostasis by Insulin Signaling in *Caenorhabditis elegans*

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Abstract

Protein homeostasis (proteostasis) is critical for cellular function and overall organismal health, requiring the coordinated efforts of protein synthesis, folding, transport, and turnover. Gametogenesis, which involves active protein synthesis, is highly dependent on the integrity of the proteome. Our recently published research in *Caenorhabditis elegans* highlighted the role of insulin/insulin-like signaling (IIS) during gametogenesis through controlling dietary peptide uptake in the intestine. However, the non-cell-autonomous regulation of germline proteostasis under protein toxic stress remains poorly understood. Our ongoing project has provided initial data suggesting that reducing IIS in intestinal and neuronal tissues decreases embryonic lethality during heat shock (HS). Interestingly, maternal reduction of IIS in the intestine directly affects oocyte quality, while IIS reduction in neuronal tissue confers heat shock resistance to the embryos. Furthermore, the level of misfolded proteins in germ cells is notably lower upon reducing maternal IIS from neurons. Our preliminary findings also indicate that germ cell translation is decreased when IIS is reduced in the intestine, but this reduction is not necessary for neuronal tissue, suggesting that distinct mechanisms may be involved.

Deciphering and Targeting MUSK Alternative Splicing in Neuromuscular Signal Transmission

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Abstract

Alternative splicing in genes associated with neuromuscular junction (NMJ) often compromises neuromuscular signal transmission and provokes pathological consequences. Muscle-specific receptor tyrosine kinase (MuSK) is an essential molecule in the NMJ. MUSK exon 10 encodes an important part of the frizzled-like cysteine- rich domain, which is necessary for Wnt-mediated acetylcholine receptors clustering at NMJ. MUSK exon 10 is alternatively skipped in humans but not in mice. We reported that humans acquired a unique exonic splicing silencer in exon 10 compared to mice, which promotes exon skipping coordinated by hnRNP C, YB-1, and hnRNP L. Here, we have dissected the underlying mechanisms of exon inclusion. We precisely characterized the exonic splicing enhancer (ESE) elements and determined the functional motifs. We demonstrated that SRSF6 and SRSF1 co-ordinately enhance exon inclusion through multiple functional motifs in the ESE. Remarkably, SRSF6 exerts a stronger effect than SRSF1, and SRSF6 alone can compensate the function of SRSF1. Interestingly, differentiated muscle reduces the expression of splicing suppressors, rather than enhancers, to generate a functional Wnt-sensitive MuSK isoform to promote neuromuscular signal transmission. Finally, we developed splice-switching antisense oligonucleotides, which could be used to selectively modulate the expression of MUSK isoforms toward a beneficial outcome for therapeutic intervention.

Targeting RNA Processing Defects in Splicing Factor-mutated Cancer

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Abstract

Recurrent mutations in splicing factors (most frequent in SRSF2, SF3B1, and U2AF1) are critical drivers of oncogenesis. These mutations are prevalent in myelodysplastic syndromes and leukemia and are common in solid tumors. Studies in the past decade revealed that these mutations promote genome-wide aberrant alternative splicing. Several mRNAs generate a premature termination codon and are degraded by nonsense- mediated mRNA decay. These subsequently affect critical protein expressions, such as tumor suppressors or oncoproteins. Therapeutic approaches to modulate aberrant splicing in several monogenic genetic diseases are reaching the clinic but remain unsuccessful in cancer therapy. Here, we develop gene-specific targeted therapeutic strategies. As proof-of-concept, we included an oncogenic target, EZH2, which is aberrantly spliced in SRSF2-mutated cancer. Using minigene reporters, model cell lines, and biochemical and functional approaches, we dissected

the underlying mechanisms promoted by mutant SRSF2. We defined critical binding motifs of mutant SRSF2 in EZH2 and characterized transcript-specific interacting proteins in splicing and NMD pathways. Exploiting these mechanistic insights, we developed two promising approaches to modulate aberrant splicing using antisense and decoy oligonucleotide pharmacology, respectively. These oligonucleotides efficiently corrected the aberrant splicing of EZH2 and restored the expression of EZH2. Our approaches are promising for future therapeutic development in splicing factor-mutated cancer.

Exercise-induced Expression of Genes Associated with Aging in the Hippocampus of Rats

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Abstract

Recent research has underscored the influence of aging and exercise on brain function. In this study, we aimed to explore alterations in the expression of novel molecular factors and gain insight into underlying molecular mechanisms in the hippocampus of rats engaged in voluntary wheel running. We assessed the expression of aging-related genes in the hippocampus using a high-throughput whole genome DNA microarray approach in rats engaged in voluntary running for four weeks. The results indicated that compared to the control group, wheel running significantly altered the expressions of aging-related genes in the hippocampus. Functional categorization, utilizing pathway-focused gene classifications and disease state-focused gene classifications, along with ingenuity pathway analysis (IPA), revealed changes in expression pattern in major categories of cell death and survival, renal necrosis/cell death, and cardiovascular disease genes. These findings suggest that exercise may mitigate the risk of age-related cognitive decline by regulating of aging-related genes in the hippocampus. Further research is warranted to elucidate the mechanisms driving changes in gene expression and to determine the long-term effects of exercise on brain function.

Extracellular Vesicles Mediate Intercellular Signaling in Exercise

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Abstract

The physiological responses to exercise are numerous and diverse, encompassing adaptations in a wide range of tissues and systems. Among the numerous mechanisms that regulate these responses, emerging evidence underscores the complex role of extracellular vesicles (EVs). These nano-sized membrane-bound particles, including exosomes, are secreted by virtually all cell types and serve as vehicles for bioactive cargo, including proteins, lipids, and nucleic acids. In the context of exercise, EVs have been implicated in intercellular communication, tissue repair, metabolic regulation, and immune modulation. The aim of this presentation is to present the results of our laboratory's recent experiments on whether EVs can mediate the various effects of exercise. The objective of our initial study was to ascertain whether EVs could serve as a biomarker for fatigue resulting from exercise. Additionally, we examine the diagnostic implications of EVs as circulating biomarkers in exercise-related pathologies, such as overtraining syndrome. Moreover, we demonstrate the functional consequences of exercise-induced EV release, including the potential roles of EVs in mood-related dysfunction. To ascertain its potential as a marker of fatigue, exosomes were isolated from the subject's urine following the shuttle run test and analysed using NTA and ExoView. A proteomic analysis was employed to ascertain alterations in EV content resulting from overtraining. A miRNA sequencing and bioinformatics analysis was conducted on mouse plasma EVs to investigate the effects of exercise on mood-related processes. The results of the study demonstrated a correlation between the level of urinary EVs and the amount of blood lactate, heart rate, and rating of perceived exertion, which are used as existing fatigue markers. Additionally, the content of brain-derived EVs from excessively trained mice was found to have undergone changes, with the presence of several substances that are known to be harmful to the brain. Finally, the functional consequences of these findings were elucidated.

Host Peroxisome Proliferator-activated Receptors Play an Important Role in *Coxiella burnetii* Infection

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Abstract

The obligate intracellular bacterium *Coxiella burnetii* is transmitted to humans via inhalation and causes Q fever. Chronic Q fever typically results in endocarditis, which can develop years are the initial infection, indicating the bacterium's ability to survive for extended periods of time. Although the host cell pathways targeted by *Coxiella* are unknown, our lab has demonstrated that *Coxiella* manipulates lipid storage organelles referred to as lipid droplets (LDs) in alveolar macrophages. LD breakdown is crucial for bacterial growth, which releases precursors for immunosuppressive prostaglandin E2 (PGE2). LD breakdown-mediated PGE2 production is essential for *Coxiella* intracellular survival but is it not yet clear whether it uses host transcriptional regulation to mediate its effects. Peroxisome proliferator-activated receptors (PPARs) are transcription factors that are vital for macrophage LD metabolism and immune modulation and are involved in the pathogenesis of intracellular microbes. Hence, we hypothesize that host PPARs play a significant role in the intracellular survival of the bacterium *C. burnetii*. To identify the importance of the PPAR subtypes - α, β/δ, and γ - during *Coxiella* infection, we measured bacterial growth in cells treated with subtype-specific PPAR agonists and antagonists. Notably, altering PPARα and β/δ acclivity decreased *Coxiella* growth. Blocking PPARα activity reduced host LD numbers and PGE2 concentrations, suggesting the importance of PPARα in promoting *Coxiella* survival. Our study establishes the role of host PPARs, particularly PPARα, in *Coxiella* infection. Future studies will explore PPARs as potential therapeutic targets to inhibit *Coxiella* intracellular growth.

Bi-directional Crosstalk Between Adipocytes and African American Mammary Cancer Stem Cells: Role of Hypoxia Induced Metabolic Reprogramming

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Abstract

African American triple-negative breast cancer (AATNBC) tumors are enriched in self-renewing, metastasis- promoting mammary cancer stem cells (MCSC), which evade current treatment options. While the interactions between breast tumors and adipocytes are well established, the key players in biologically distinct AATNBCs remain unidentified. In this study, we aim to examine the specific crosstalk between AATNBC, MCSCs, and differentiated adipocytes. 3T3-L1 mouse adipocytes were co-cultured with AATNBC HCC1806 cancer stem cells enriched as mammospheres *in vitro* for 48 hr. RNA sequencing was performed; pathway enrichment identified signaling pathways. Hypoxia-induced gene signatures ($p < 0.000$) were upregulated in MCSCs when incubated with 3T3- L1 differentiated adipocytes. Metabolic reprogramming, including glycolysis ($p = 0.023$) and epithelial to mesenchymal (EMT) gene signatures ($p = 0.001$), were prominent. LOX, Serpine1, MXI1, ENO2, and P4HA1 were found upregulated in both hypoxia-induced metabolic reprogramming and EMT. EMT gene signatures ($p < 0.000$) were upregulated in the 3T3-L1 differentiated adipocytes incubated with MCSCs. We have identified receptors upregulated in the MCSCs (CAV1, ITGA5), and the corresponding ligands in the differentiated adipocytes (CCN1, COL1A1, SPP1, FN1), that may interact via the FAK pathway, stimulating cell adhesion and migration. Our data indicates early crosstalk between HCC1806 MCSCs and 3T3-L1 differentiated adipocytes that could induce aggressive gene signatures to remodel the extracellular matrix and promote metastasis. Further studies are in progress to validate this unique gene signature in additional AATNBC cell lines, Caucasian American TNBC, and estrogen receptor-positive BCs incubated with human adipocytes. Knock-in and knock- out experiments will confirm the interactions in receptor-ligand pairs.

The Importance of Protists and Viruses in Bacterial Mortality Across Anaerobic and Aerobic Environments in Siders Pond

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Abstract

Microbial interactions are fundamental to nutrient cycling, ecosystem stability, and population dynamics in aquatic environments. Bacteria drive key ecological processes, including organic matter decomposition, nutrient release, and food web dynamics. They also interact with viruses and protists, shaping biogeochemical cycles and influencing overall ecosystem productivity. This study examines the roles of protists and viruses in regulating bacterial mortality across aerobic (oxygen-rich) and anaerobic (oxygen-depleted) zones in Siders Pond, a salt-stratified meromictic pond in Falmouth, Massachusetts. We hypothesize that

protists dominate bacterial mortality in the aerobic zone due to their oxygen-dependent grazing activity, while viruses play a more prominent role in the anaerobic zone, which limits protist activity. This research aims to clarify these interactions and their significance for nutrient cycling and ecosystem health. Field and laboratory methodologies were applied to assess microbial dynamics along the oxygen gradient. Water samples were collected at varying depths and measured for dissolved oxygen, temperature, salinity, pH, dissolved organic carbon (DOC), and nutrients (PO_4, NO_3/NO_2) to determine how they affect microbial activity. Bacterial and grazer production quantified using C14-leucine incorporation. Protist abundances were determined using DAPI staining, while bacterial and viral abundances were measured using SYBR gold staining. Preliminary findings suggest that bacterial and protist production decreases when transitioning from aerobic to anaerobic zones, while viral activity remains consistent. This study contributes to understanding how microbial interactions respond to environmental stressors such as eutrophication, pollution, and climate change, offering a model for future research and strategies for preserving aquatic ecosystem health amidst global environmental challenges.

Cell Cycle Dynamics as a Framework to Study Blood-brain Barrier Disruption Under Stress Conditions

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Abstract

The blood-brain barrier (BBB) is a specialized structure critical for maintaining central nervous system (CNS) homeostasis. Composed of endothelial cells, pericytes, and astrocytes, it selectively regulates the exchange of substances between the bloodstream and the brain, ensuring protection against harmful agents while permitting nutrient transport. BBB disruption is linked to neurological disorders such as Alzheimer's disease underscoring the need to understand mechanisms that impair its integrity. This study explores the effects of hypoxia and the neurotoxic agent mercuric chloride (HgCl2) on BBB-associated cells, including human brain microvascular endothelial cells (HBMEC), human brain vascular pericytes (HBVP), and human astrocytes (HA) grown individually and in co-cultures. Cell cycle changes were analyzed after 24 hr exposure, with RT-PCR and Western blot used to assess gene and protein expression as a functional analysis of the BBB model disruption. Under hypoxia, HBMEC and HA displayed an accumulation of cells in the G2 phase, suggesting a block in mitotic progression that may impair cell proliferation and compromise BBB functionality. Similarly, HgCl2 exposure in co-culture systems reduced S phase populations and increased G2 phase cells, disrupting normal cell cycle progression and potentially destabilizing the barrier's integrity. Gene and protein expression analysis confirmed these disruptions. In conclusion, hypoxia and HgCl2 significantly affect cell cycle dynamics and protein expression in BBB- associated cells, particularly the G2/M phase. These findings provide valuable insights into how environmental and toxicological factors compromise BBB integrity and suggest studying cell cycle dynamics as a framework for investigating BBB disruption.

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BRK-mediated Dysregulation of TGF-β/SMAD Signaling Promotes Metastatic Breast Cancer

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Abstract

Metastatic breast cancer remains a leading cause of cancer-related mortality, with a pressing need to elucidate molecular mechanisms underlying its progression. Breast tumor kinase (BRK), a non-receptor tyrosine kinase, is overexpressed in approximately 80% of breast tumors, and its high expression is linked to enhanced metastatic potential and poor prognosis. We previously reported that BRK phosphorylates SMAD4 at two specific sites, Y353 and Y412, promoting metastatic potential in breast cancer cells. However, the distinct roles of these phosphorylation sites remained elusive. In this study, we demonstrated that BRK-mediated phosphorylation at Y412 leads to the proteasomal degradation of SMAD4, while phosphorylation at Y353

facilitates its interaction with the nucleosome remodeling and deacetylase (NuRD) complex. Our phosphomimetic study confirmed that phosphorylation of SMAD4 impedes its interaction with SMAD2 and SMAD3 and induces SMAD4- NuRD complex formation. These findings suggest that BRK-induced SMAD4-NuRD complex formation may reprogram epigenetic gene expression, alter TGFβ/SMAD signaling, and potentially contribute to metastatic progression. Thus, we hypothesize that silencing BRK or pharmacologically inhibiting its activity restores TGF-β/SMAD homeostasis, reducing metastatic behaviors such as epithelial-to-mesenchymal transition (EMT) and invasive capacity. To test our hypothesis, we plan to inhibit the kinase activities of BRK to restore the tumor-suppressive role of SMAD4, offering a new avenue to combat this aggressive disease.

Immune Biomarkers of SARS-CoV-2 Infection upon 2021-2022 COVID-19 Pandemics in Poland

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Abstract

We determined T-lymphocyte immunophenotype correlating with COVID-19 severity. We analysed 52 hospitalized adult patients with COVID-19 from Warsaw, Poland during 2021-2022. Patients were divided into 2 groups with: 1. mild/moderate symptoms – 44 patients, 2. severe respiratory failure – 8 patients. 26 individuals without COVID-19 served as a control. All COVID-19 patients and healthy individuals were immunophenotyping of peripheral blood cells for T and T regulatory (Treg) lymphocytes using CytoFLEX flow cytometer. COVID-19 patients had decreased level of total T-lymphocyte population in peripheral blood. No significant differences appeared for T helper cells (CD4+) and T cytotoxic cells (CD8+) both in all patients and in two groups. A significant decrease in double-positive lymphocytes (CD4+CD8+) appeared for all patients and the mild group. For Treg lymphocytes CD4+CD25HiCD127Lo and CD4+CD25HiCD127LoFoxP3+ a significant decrease was observed for all patients and for mild/acute groups. However, an important decrease in CD4+CD25HiCD127Lo lymphocytes was observed in the mild and acute groups. A significant decrease was observed for CD4+CD25+/-, CD4+CD45RA+/- and CD8+CD45RA+/- subpopulations for both all patients and the two groups. We observed an increase in IL6, IL10, and INFγ levels in all COVID-19 patients compared to the control group. Especially, IL6 level was increased in in patients with mild and acute COVID-19 compared to the control group. For serum chemokines, CCL2, CCL5, CXCL9, and CXCL10 increased in all COVID-19 patients. Only CXCL10 was increased in patients with the severe course of COVID-19 compared to the ones with the mild course. These data indicate biomarkers for severe vs mild COVID19.

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A Novel 3D *in-vivo* CAM model for Investigating Connexin Dynamics in Human Skin Wound Healing

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Abstract

Skin integrity is regulated by cell-cell contacts and is crucial for protection against environmental stress. However, the prevalence of skin diseases is increasing, but due to the lack of adequate models our understanding of human wound healing mechanisms remains limited. To address this issue, we adapted the 3D *in-vivo* chorion allantois membrane (CAM) model by applying skin biopsies after inducing defined cuts, thereby creating a suitable wound healing model to explore the wound healing mechanisms of human skin. Using this model the impact of various skin pathologies and treatment options on the dynamics of connexins (Cx), the basic components of gap junctions, during the process of wound healing can be analyzed. The experimental approach involves methods like mRNA scope, immunofluorescence staining, dPCR, and western blots. The efficacy of this model is evidenced by the successful maintenance of human skin viability on the CAM for a period of seven days, as confirmed through histological analysis. Molecular biological analysis revealed an upregulation of Cx26 after one day of wounding and cultivation on the CAM and a decrease of Cx43. Consequently, a reliable model facilitating the analysis of wound healing processes, including proliferation, and neo angiogenesis, as well as connexin expression changes can be analyzed directly in human skin tissue. The objective is to expand the scope of tests beyond the CAM model to ascertain whether connexin expression changes are attributable to CAM effects or wound healing mechanisms. Future approaches include testing modulators and identifying dysregulated pathways in diseased or irradiated skin.

Preconditioning Enhances the Anti-cancer Properties of Placenta-derived Human Mesenchymal Stem Cells

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Abstract

Stem cell therapy represents a promising avenue for cancer treatment, leveraging the unique properties of stem cells, such as their ability to secrete anti-cancer factors, home to tumor sites, induce apoptosis, and modulate immune responses. Mesenchymal stem cells (MSCs) have shown particular potential in inhibiting tumor progression, angiogenesis, and cancer cell apoptosis. In this study, we isolated and characterized placenta-derived MSCs (pMSCs) from three distinct regions of the human term placenta: the decidua basalis (DB), decidua parietalis (DP), and chorionic villus (CV). We then evaluated the impact of pMSCs on the functional behavior of human breast cancer cell lines. Breast cancer cells were treated with either the cellular component or secretome of naïve pMSCs or preconditioned pMSCs—the latter being pMSCs cultured in conditioned media from human cancer cells. Preconditioning did not alter the functional properties of pMSCs, including proliferation, migration, and invasion potential. Moreover, preconditioned pMSCs retained their stemness, comparable to naïve pMSCs. However, preconditioned pMSCs exhibited upregulated expression of pro-migratory and anti-apoptotic genes and proteins, indicating enhanced anti-cancer potential. Our results demonstrated that preconditioned pMSCs significantly reduced the functional activity of breast cancer cells compared to naïve pMSCs and untreated controls. Specifically, preconditioned pMSCs, but not naïve pMSCs, markedly decreased the levels of epithelial-to-mesenchymal transition (EMT)-related proteins and modulated the expression of other cancer-associated proteins. These findings suggest that preconditioned pMSCs can effectively suppress the invasive characteristics of breast cancer cells, highlighting their potential as a promising candidate for cell-based cancer therapies.

MARCKS Enriched in Urinary Extracellular Vesicles from COVID-19 Patients

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) remains a public health challenge as long-term complications are being investigated. Urinary extracellular vesicles (uEVs) are nanosized vesicles that are released by cells of urinary organs, including the kidney, bladder, urethra, and prostate. uEVs allow for cross-talk between cells, cellular differentiation, and changes in intracellular signaling. To identify proteins enriched in EVs that may contribute to the pathophysiology of kidney disease after COVID infection, we took a proteomic approach to identify differentially expressed proteins enriched in uEVs from COVID-19 patients compared to non-COVID-19

patients. uEVs from both groups were characterized by nanoparticle tracking analysis, transmission electron microscopy, and Western blotting. Proteomic analysis showed the myristoylated alanine-rich c-kinase substrate (MARCKS) was strongly enriched in uEVs from the COVID-19 group. Western blotting showed the density of an immunoreactive band corresponding to MARCKS like protein 1 (MLP1) normalized to CD9 was augmented in uEVs from the COVID-19 group. Current studies are aimed at sorting the uEVs into subpopulations to determine the cell types that release the MARCKS and MLP1 enriched EVs. Also, the potential of the MARCKS/ MLP-1 enriched EVs from the COVID-19 patients to alter signaling cascades in healthy human proximal tubules cells is being investigated. This work was funded in part by a CICMD pilot award.

Transfer: Programmable, Cell-based Macromolecule Delivery via Engineered Trogocytosis

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Abstract

Cell-type-specific delivery remains a critical bottleneck in realizing the full potential of macromolecule therapies. Traditional delivery systems, such as viral vectors and nanoparticles, often lack specificity and were not capable of delivering therapeutics in a durable and reversible manner, calling for novel macromolecule delivery modalities. Cells have evolved intricate mechanisms for direct material transfer for cell-cell communication. Trogocytosis, a natural process in which cells exchange membrane patches and associated proteins through direct contact, represents an understudied process with promise for macromolecular delivery. Inspired by trogocytosis, we developed TRANSFER (Trogocytosis-based transfer and functional effector release), a programmable cell-based delivery platform that harnesses engineered cells for cell-type-specific delivery of functional macromolecules. Our approach employed engineered donor cells equipped with cell surface receptors to transfer cargos to recipient cells expressing a specific surface marker. By co-transferring pH-sensitive transmembrane proteins known as fusogens, the delivered cargos could escape the endosome through endosome membrane fusion. Furthermore, through pH-sensitive protein linker engineering, we enabled translocation of the trogocytosed membrane-bound cargo to other cellular compartments to perform its function. Our cell-based, membrane-mediated delivery platform enabled delivery of functional proteins including CRISPR-Cas9 nuclease, zinc finger nucleases (ZFNs), recombinase, and prodrug-converting enzymes to various cell types, with high specificity and efficiency. We achieved successful Cas9 delivery and genome editing *in vivo*, as validated by activation of a reporter system in transplanted recipient cells. In conclusion, TRANSFER represents a versatile and programmable cell-based macromolecular delivery platform, laying the foundation for broad applications in research and therapeutics.

SNX17-Retriever and SNX27-Retromer Mediate Distinct Recycling Pathways Essential for Synaptic Maintenance and Plasticity

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Abstract

Integrins play a crucial role in synaptic function by regulating adhesion, signaling, and plasticity at excitatory synapses. However, the mechanisms governing their trafficking and recycling in neurons are not completely understood. Here, we investigate the roles of two endosomal recycling pathways, SNX17-Retriever and SNX27-Retromer, in maintaining synaptic integrity and function. Using a combination of electrophysiology, cell biology, and biochemical approaches, we demonstrate that SNX17-Retriever and SNX27-Retromer mediate the recycling of distinct cargoes at excitatory synapses. Loss of SNX17-Retriever impairs β1 integrin trafficking, leading to deficits in synaptic maintenance, while disruption of SNX27-Retromer alters the recycling of AMPA- type glutamate receptors, affecting synaptic plasticity. Our findings highlight the necessity of these specialized recycling pathways in sustaining excitatory synapse function and reveal distinct molecular mechanisms that govern integrin

homeostasis in neuronal circuits. These results provide new insights into how dysregulated endosomal trafficking can contribute to synaptic dysfunction in neurological disorders.

YTHDF2 Facilitates Aggresome Formation via UPF1 in an m6A-independent Manner

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Abstract

YTHDF2 has been extensively studied and typified as an RNA-binding protein that specifically recognizes and destabilizes RNAs harboring N6-methyladenosine (m6A), the most prevalent internal modification found in eukaryotic RNAs. In this study, we unravel the m6A-independent role of YTHDF2 in the formation of an aggresome, where cytoplasmic protein aggregates are selectively sequestered upon failure of protein homeostasis mediated by the ubiquitin-proteasome system. Downregulation of YTHDF2 in HeLa cells reduces the circularity of aggresomes and the rate of movement of misfolded polypeptides, inhibits aggresome formation, and thereby promotes cellular apoptosis. Mechanistically, YTHDF2 is recruited to a misfolded polypeptide-associated complex composed of UPF1, CTIF, eEF1A1, and DCTN1 through its interaction with UPF1. Subsequently, YTHDF2 increases the interaction between the dynein motor protein and the misfolded polypeptide-associated complex, facilitating the diffusion dynamics of the movement of misfolded polypeptides toward aggresomes. Therefore, our data reveals that YTHDF2 is a cellular factor involved in protein quality control.

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