Research Trainee Symposium

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POSTER ABSTRACTS

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1. Coordinated epigenetic regulation of H3K27me3 and H3.3S31p in diffuse midline glioma: Insights from CUT&RUN profiling of K27M and S31A mutants.

Bhattarai, S., *Hakkim, F.L., Grigore, F., Day, C., Langfald, A., Hinchcliffe, E., Robinson, J.*

Author Affiliation

The Hormel Institute, University of Minnesota

Abstract

Diffuse midline glioma (DMG) is a lethal pediatric brain tumor driven by the H3.3K27M oncohistone, which causes global depletion of H3K27me3. We previously showed that H3.3K27M reduces phosphorylation of the neighboring H3.3S31 residue by trapping CHK1 at pericentromeric chromatin, inducing chromosomal instability. However, the high-resolution, genome-wide relationship between H3.3K27M, H3.3S31p, and H3K27me3 restoration remains poorly defined.

We performed high-resolution CUT&RUN profiling for H3K27me3, H3.3K27M, H3.3S31p, H3K4me3, EZH2, and IgG controls. Profiling was conducted across an isogenic DMG panel: parental H3.3K27M-mutant cells, CRISPR-engineered K27WT revertant lines, and a phospho-dead H3.3S31A mutant line.

Removal of H3.3K27M restored global H3K27me3 enrichment in K27WT revertants, confirming broad recovery of PRC2-mediated repression. Clustering analysis identified a unique K27M-enriched genomic niche characterized by moderate H3.3K27M, residual H3K27me3, and focal H3K4me3, indicating an aberrant, “poised” regulatory state. EZH2 occupancy was generally low, reflecting transient binding, but showed selective re-recruitment to this poised niche upon K27M removal. Notably, H3.3S31p and H3K27me3 signals mirrored one another in a WT context, showing highly coordinated enrichment flanking pericentromeric domains (e.g., Chromosome 1) and repeat-rich regions. In contrast, parental K27M cells showed a marked reduction in both marks at these domains. Crucially, the S31A mutant lacked H3.3S31p signal, validating antibody specificity and failed to maintain normal H3K27me3 patterns, demonstrating that K27 methylation depends on stable S31 phosphorylation.

Our preliminary CUT&RUN data suggest that H3.3K27M disrupts chromatin organization beyond canonical H3K27me3 depletion by altering phosphorylation of the adjacent H3.3S31 residue at repeat-rich and pericentromeric regions. Restoration of K27WT partially re-establishes H3K27me3 and H3.3S31p landscapes, whereas S31A mutation phenocopies aspects of chromatin disruption. Together, these findings support a model in which coordinated regulation of H3.3K27 methylation and H3.3S31 phosphorylation maintains heterochromatic repeat domains, and its disruption may contribute to chromosomal instability in DMG.

2. Epitope mapping of recombinant SARS-COV-2 RBD variants for vaccine development

Boyle, J.*[1]*, *Bator, C.*[1]*, Hafenstein, S.*[1]*, McCormick, L.*[2]*, White, J.*[2]*, Estrada, M.*[2]*, Dong, S.*[2]*, Kim, J.*[2]*, Strych, U.*[3]*, Pollet, J.*[3]*, Ciciriello, A.*[3]

Author Affiliation

[1]The Hormel Institute, University of Minnesota
[2]PATH, Seattle, Washington
[3]Baylor College of Medicine, Houston, Texas

Abstract

The vaccines developed against COVID-19 infection heavily depend on inhibiting the interaction between the ACE2 receptor and the receptor binding domain (RBD) of the spike protein. COVID vaccine distribution to low-income countries is far behind high-income countries, so a more accessible alternative vaccine is necessary. Collaborators at Baylor College of Medicine have produced a recombinant RBD that binds antibodies similarly to the wild-type RBD. The recombinant RBD is purified from a yeast expression system, making it more affordable and easily scalable. The recombinant RBD is part of a COVID-19 protein subunit vaccine that was recently WHO EUL listed and has been developed into the licensed Corbevax and Indovac vaccines in India and Indonesia, respectively. My structural project aims to understand the interactions between the recombinant RBD from three SARS-CoV-2 strains (Wuhan, Delta, Omicron) and a cross-reactive SARS-CoV-2 antibody that recognizes all three RBDs. Antibody fragments (Fabs) were obtained from the cross-reactive antibody and incubated with each RBD for 1 h at 4°C. Immediately before vitrification, 8 mM CHAPSO was added to each solution to prevent aggregation to the air-water interface. Each cryoEM dataset was collected over 48 hours on the TFS Glacios 2 at the Mayo Clinic and processed using CryoSparc. The final EM densities had local resolutions ranging from 2.2-3.7 Å, with sub-3Å resolution within the binding interfaces. Atomic models were built into the EM densities and residues involved in binding were identified. Epitope mapping gives insight into the antigenic recognition and evolution of the antibodies. This allows for more targeted and efficient development of vaccines, enabling a quicker response to future virus variants.

3. Influence of Environmental Conditions and Antifungal Drugs on C. albicans

Boevers, G.K., ***Burrack, L.S.***

Author Affiliation

Gustavus Adolphus College, St. Peter, Minnesota

Abstract

Candida species are fungi commonly found in the human gastrointestinal and vaginal microbiome. Candida albicans can cause serious bloodstream infections in immunocompromised individuals, with high mortality rates due to limited treatment options and the ability of the organism to develop drug resistance or tolerance. Antifungal drug tolerance enables cells to grow at drug concentrations that would normally inhibit susceptible strains. This study investigated how specific environmental conditions that mimic aspects of the human host, including normal body temperature (37°C), acidic pH, oxidative stress induced by hydrogen peroxide, and more physiologically relevant nutrient levels (RPMI-1640), affect antifungal drug susceptibility in C. albicans. Both tetraploid and aneuploid strains of C. albicans were evaluated to determine how genome ploidy interacts with the environment to impact antifungal responses. Minimum Inhibitory Concentration (MIC) assays were used to measure resistance and Supra-MIC Growth (SMG) measurements were performed to measure tolerance. Assays were performed using fluconazole as a representative azole and micafungin as an example echninocandin drug. No significant changes to MICs were observed for any conditions. However, cultures grown at 37°C exhibited higher antifungal tolerance to fluconazole. Acidic conditions and oxidative stress resulted in lower SMG values, suggesting that these single stress conditions suppress antifungal tolerance to fluconazole. No changes to micafungin tolerance were observed. Overall, these findings show that changing single environmental conditions can significantly influence antifungal tolerance without altering antifungal resistance. Interestingly, the most physiologically relevant condition (RPMI-1640 tissue culture media with 10% fetal bovine serum at 5% CO2 at 37°C), resulted in lower fluconazole MICs as well as changes to fluconazole tolerance. These results provide insight into the physiological mechanisms underlying antifungal drug tolerance and highlight the importance of environmental conditions in shaping fungal responses to antifungal treatment.

4. Utilizing a CRISPR interference functional genomic screen to identify novel modulators of high density lipoprotein cholesterol uptake

Diaz L., *Bielczyk-Maczynska E.*

Author Affiliation

The Hormel Institute, University of Minnesota

Abstract

Cardiovascular, or heart disease, is the leading cause of death in the United States, with one person estimated to die every 34 seconds. Atherosclerosis, which is the buildup of plaques in blood vessels, is a major cause of cardiovascular disease, leading to downstream effects like heart attack, stroke, and kidney failure. Abnormal levels of high density lipoprotein cholesterol (HDL), are associated with increased risk of atherosclerotic cardiovascular disease. HDL, sometimes called the "good cholesterol," is responsible for reverse cholesterol transport, which allows it to clear excess cholesterol from the blood and divert it to the liver. Research into the genetic mechanisms behind changes in HDL levels has been sparse, but better understanding HDL has the potential to decrease cardiovascular disease and disease risk.

Here, we utilize data from genome-wide association studies that identified single nucleotide genetic variants associated with changes in blood HDL levels. Genes close to identified variants were filtered to select liver expressed genes that encode proteins that are predicted to localize at the cell surface and could therefore contribute to the clearance of cholesterol from the blood. Utilizing a clustered regularly interspaced palindromic repeat (CRISPR) interference system, we knocked down these genes in a human liver cancer cell line using a lentiviral library containing pooled single-guide RNAs (sgRNAs) targeting 272 genes. We then used fluorescence-activated cell sorting to separate these cell populations based on their degree of uptake of fluorescently tagged HDL, in order to identify genes whose expression is associated with decreases or increases in HDL uptake. Through isolation and sequencing of viral constructs present in the different cell populations, we were able to identify multiple novel genes with the potential to regulate HDL uptake. We further selected and are in the process of validating the role of these genes in HDL and lipid metabolism.

5. Mitochondrial targeting as a differential strategy for coordinated immune enhancement and tumor suppression.

*Soni, J.[1], **Ghosh, A.[1],** Mortazavi Farsani, S.S.[1], Jin, L.[1], Hilakivi-Clarke, L.[1], Clarke, R.[1], Cheema, A.[2], Verma, V.[1]*

Author Affiliations

[1] The Hormel Institute, University of Minnesota
[2] Department of Oncology, Georgetown Lombardi Comprehensive Cancer Center, Georgetown University

Abstract

Mitochondria enhance T cell persistence and potency while simultaneously influencing the metabolic vulnerabilities and drug sensitivity of tumor cells. This raises the possibility that mitochondria may serve as a differential therapeutic target within the tumor microenvironment. However, strategies to stabilize mitochondrial function remain scarce. We find that pharmacologic activation of pyruvate kinase M2 (PKM2) markedly enhances mitochondrial integrity and oxidative metabolism in CD8 T cells. In contrast, the same mitochondrial enhancement imposes bioenergetic stress on tumor cells, reducing proliferation and triggering immunogenic cell death. This dual activity demonstrates that mitochondrial targeting is a tractable therapeutic strategy to restore immune competence while suppressing tumor growth. Supported by multiomics analyses, our findings position mitochondrial targeting as a coordinated platform for immune enhancement and tumor suppression and highlight its potential as a selective therapeutic axis in metabolically complex tumors.

6. Identifying a novel population of epithelial cells in the blood and bone marrow of normal mice and humans

Holtorf, S.M., *Morris, R.J.*

Author Affiliation

The Hormel Institute, University of Minnesota

Abstract

Using the circulatory system, cancer can spread throughout the body. As most cancers are of epithelial origin, such as skin, an epithelial marker is used to detect circulating tumor cells (CTCs) in the blood and bone marrow (BM) of cancer patients. CTCs are used clinically to diagnose cancer patients. These CTCs express epithelial markers such as keratin and epithelial cell adhesion molecule (EpCAM). Interestingly, some of the healthy human control samples also had EpCAM+ cells in their blood and BM. We wanted to study this unidentified epithelial cell population in healthy samples using EpCAM and other epithelial markers, such as E-cadherin and keratin.

7. A Python application framework for an AI assistive device

Salivia, G., Million, F., ***Imdieke, A.***

Author Affiliation

Gustavus Adolphus College

Abstract

Users who are blind or visually impaired increasingly rely on AI-powered assistive devices, but existing systems share three structural limitations: dependence on cloud services that impose recurring costs that may be inaccessible, connectivity requirements, and privacy exposure; a closed, fixed set of functions with no extension model; and voice-only interaction with no deterministic fallback when speech recognition fails. This project addresses these gaps by developing an open-source application framework powered by an on-board AI accelerator, which will be hosted on a Raspberry Pi device, designed specifically as an assistive device for these individuals.

8. PHGG supplementation modulates gut microbiome metabolites and breast cancer progression

Kenanoglu S., *de Oliveira Andrade F., Hilakivi-Clarke L.*

Author Affiliation

The Hormel Institute, University of Minnesota

Abstract

Short-chain fatty acids (SCFAs) are linked to improved anti-tumor immune responses and reduced breast cancer risk. Isoflavones in soybean-based (SB) high-fiber diets may block fiber's advantages by triggering immunosuppression by activating estrogen receptors in the tumor microenvironment (TME). We investigated the effect of an SB diet, combined with partially hydrolyzed guar gum (PHGG) fiber or maltodextrin (MDX) control supplementation, on E0771 triple-negative breast cancer (TNBC) growth in mice fed an isoflavone-free AIN-93G control or an AIN-93 G diet containing soybean meal (SB). We found that the SB diet increased tumor burden, and PHGG reversed the increase. Fecal microbiota transplants (FMTs) were prepared from the AIN-93G, SB, SB+PHGG, and SB+MDX fed mice, and transferred to antibiotic-treated hosts fed an AIN-93G diet. Tumor burden was similar in hosts receiving FMT from AIN-93G, SB, or SB+MDX donors, suggesting that the SB diet did not increase mammary tumorigenesis through changes in the gut microbiome. Mice receiving FMT from SB+PHGG-fed donors exhibited significantly reduced tumor burden, compared with SB+MDX-fed donors. In our earlier study, sufficient fiber content in the AIN-93G diet affected PHGG's ability to reduce mammary tumorigenesis, compared with MDX. Thus, we next fed mice fiber (cellulose) -free AIN-93G diet. In this setting, PHGG reduced tumorigenesis compared with MDX. PHGG increased fecal SCFAs both in tumor-free cellulose-containing and cellulose-free AIN-93G diet-fed mice. Our findings thus indicate that PHGG reduces mammary tumorigenesis in mice fed a low-fiber diet or a diet containing soy isoflavones.

9. Epitope mapping of polyclonal immune response against Coxsackievirus B3 with high-resolution cryoEM

Langley, C.H.[1], *Rodriguez, B.A.*[2], *Bator, C.M.*[1], *Geller, R.*[2], *Hafenstein, S.L.*[1]

Author Affiliation

[1]The Hormel Institute, University of Minnesota
[2]Universitat de Valencia-CSIC, Valencia, Spain

Abstract

Coxsackievirus B3 (CVB3) is a common human enterovirus that can cause meningitis and is the leading cause of viral myocarditis. CVB3 is a small (30nm), nonenveloped, single stranded RNA virus that contains four structural proteins: VP1, VP2, VP3, and VP4. During CVB3 infection and cell entry, CVB3 engages with the coxsackievirus adenovirus receptor (CAR), which triggers temperature dependent conformational changes resulting in the formation of a hydrophobic entry intermediate (Altered or A-particle). To understand how neutralizing antibodies recognize the CVB3 capsid, the binding site of various monoclonal antibodies has previously been determined. Here we use a cryoEM-based polyclonal epitope mapping approach to determine the binding sites of neutralizing antibodies from human serum. This approach will provide a detailed picture of the human immune response and allow for a more in-situ like analysis of the polyclonal response to a CVB3 infection. CVB3 Nancy was vitrified, the data were collected, and the virus structure was solved at 1.34Å resolution. At atomic resolution, a model of CVB3 was able to be built unambiguously. CVB3 Nancy was incubated with polyclonal antibody fragments (Fabs) in excess, the complex was vitrified, and the data were collected for single particle analysis. The resulting 3.1Å structure revealed one Fab binding slightly offset the icosahedral twofold axis. The virus had the conformation of an A-particle, including radial expansion of the capsid, genome rearrangement, no pocket factor, VP1 extrusion, and expulsion of VP4. Subparticles centered at the icosahedral twofold were extracted and reconstructed at 3.1Å resolution. 3D classification was used to improve the Fab cryoEM density and the Fab binding site was identified. Characterizing the human polyclonal response to CVB3 and understanding the methods of neutralization will allow for greater understanding of CVB3 and host interactions and allow for potential development of new therapeutics.

10. Structural characterization of HPV16 minor capsid protein L2

Orji, E.P., *Gramm, A.J., Langley, C.H., Lee, H., Messina, K.J., Bator, C.M., Swulius, M., Brendle, S., DiMaio, D., Christensen, N.D., Hafenstein, S.L., Anand, G.*

Author Affiliation

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Abstract

The human papillomavirus (HPV) capsid consists of two structural proteins (L1 and L2), but only the structure of the major capsid protein L1 has been resolved. The elusive minor capsid protein L2 plays an important role in virus entry, replication, and assembly, yet its structure, copy number, and location within the virion remain unknown. Determining the structure of L2 has been difficult due to its flexibility and asymmetric incorporation, as well as the HPV capsid's flexibility.
In this study, we combined high-resolution cryoEM and cryoET with structural mass spectrometry to visualize L2 both inside and outside the HPV capsid. Using three established antibodies that recognize the N-terminal regions of L2, we mapped L2 extensions from the capsid. Additionally, cryoEM single-particle analysis identified L2 extrusion sites within the capsid and a part of L2 outside the capsid. Subparticle extraction and 3D classification further revealed L2 copy number and orientation within the capsid. Our findings resolve long-standing questions about the HPV minor capsid protein and provide insight for biochemical and pharmaceutical studies of L2 as a potential vaccine target.

11. Foldavirus, a knowledge-based icosahedral capsid builder using AlphaFold

Rojas Labra, O.*[1]*, *Montoya-Munoz, D.S., Santoyo-Rivera, N., McDonald, J., Montiel-Garcia, D., Case, D.A., Reddy, V.S.*[1]

Author Affiliation

[1] The Hormel Institute, University of Minnesota

Abstract

Coat protein (CP) structures and their capsid architectures of icosahedral viruses are highly conserved within each virus family. While AlphaFold successfully predicts the tertiary structures of individual CPs, their association to form accurate quaternary assemblies cannot be accomplished currently, even for simple T=1 capsids composed of 60 subunits. We describe here a generalized method and ensuing web utility (https://foldavirus.org) that combines AlphaFold predictions of CPs with the information on icosahedral architectures (e.g., T=1, 3…) that they are likely to form based on the other members in the same virus family to generate corresponding capsids. Given the amino acid sequence of the CP(s), we successfully generated capsids up to T=9 icosahedral symmetry, including those with pseudo-T=3 symmetry comprising different CPs. Moreover, these assemblies are relaxed by Amber energy minimization to relieve steric clashes at the inter-subunit interfaces and validated using the residue annotations categorized as interface, core, and surface amino acids and by comparing with those observed in the experimentally determined capsids. As the number of available CP sequences are 3-4 orders of magnitude larger than the experimentally determined 3D-structures, this approach bridges the huge gap that exists between the sequence and structure space. The above webtool will soon be freely available to the greater virology community.

12. Characterizing the relationship among biological sex, reproductive hormones, and sucrose-seeking behaviors

Syed, Z.B., *Austin, K., Said, S., and Nett, K.E.*

Author Affiliation

Gustavus Adolphus College

Abstract

Circulating gonadal hormones interact with neural pathways to influence learning and motivated behaviors. In humans, fluctuating levels of ovarian hormones across the phases of the menstrual cycle have been associated with changes in cognition and reward processing. In rodents, a similar hormonal fluctuation occurs across the four-phase estrous cycle, providing a compelling model for investigating how sex hormones affect learning and behavior. Despite increasing recognition of sex as a biological variable, existing preclinical research relies heavily on male models, resulting in a limited understanding of the interactions between reproductive hormones and motivated behaviors. To address this gap, we examined the relationship among biological sex, estrous stage, and sucrose seeking in Sprague-Dawley rats. Female (n=10) and male (n=6) rats were trained to press a lever to receive a sucrose pellet reward before undergoing extinction and cue-induced reinstatement testing. Estrous stage was determined prior to each daily session using vaginal lavage and cell suspension. An ancillary goal of the present experiment was to better establish cell-suspension protocols for accessible and efficient estrous tracking. Mapping the relationship among sex, reproductive hormones, and sucrose-seeking behaviors will allow us to better characterize the underlying biological mechanisms of reward-seeking behaviors, improve the generalizability of preclinical models, and inform strategies for optimizing learning across hormonal states.

13. Shared Resources at The Hormel Institute: How Can We Help You?

Terranova, S.

Author Affiliation

The Hormel Institute, University of Minnesota

Abstract

Come learn about the series of services and equipment we have at The Hormel Institute, University of Minnesota.

14. NALCN regulates ‘normal’ stem cell escape and dissemination from gastric epithelium

Van de Kaa, D.[1], *Hu, L.[1], Papadopoulos, P.[1], Rahrmann, E.P.[2], Gilbertson, R.J.[1]*

Author Affiliations

[1] Cancer Research UK Cambridge Institute, University of Cambridge
[2] The Hormel Institute, University of Minnesota

Abstract

Metastasis remains the leading cause of cancer-related deaths, yet the earliest cellular events that initiate dissemination are poorly understood. Increasing evidence suggests that epithelial cells can disseminate independently of malignant transformation, implicating disrupted tissue homeostasis as a critical early step toward metastatic competence. The sodium leak channel NALCN has emerged as a regulator of epithelial retention: it is downregulated in gastric cancer stem cells, and its genetic deletion promotes dissemination of both malignant and non-malignant epithelial cells. We therefore hypothesised that NALCN maintains gastric stem cell niche retention and epithelial organisation under homeostatic conditions. To test this, we conditionally deleted Nalcn in Prom1^{+} gastric stem cells in mice. Loss of NALCN resulted in fewer gastric glands, which presented dysmorphic and disrupted epithelial organisation. Live tissue explant imaging further revealed aberrant retrograde migration of Nalcn-deficient stem cells, which bypassed canonical upward movement along the gland axis and instead migrated basally toward the submucosa. Consistent with niche escape, glands isolated from Nalcn-deficient tissue formed fewer and smaller organoids, suggesting depletion of the resident stem cell pool. To assess conservation in human tissue, we depleted NALCN in human gastric epithelial spheroids, where loss drove invasive protrusion into the extracellular matrix. Together, these findings demonstrate that NALCN may maintain epithelial homeostasis by coupling membrane potential to adhesion and niche retention. Its disruption allows gastric stem cells to escape their niche and engage dissemination-permissive migration programmes. Mapping these early dissemination routes may reveal how cancer co-opts normal stem cell behaviour and identify potential targets for intercepting metastasis at its earliest stages.

15. TNFRSF12A Signaling in Hepatic Stellate Cells Promotes a Tumor-Permissive Liver Microenvironment in Colorectal Cancer Metastasis

Wang, Q.S., *Kang, N.L., Zhang, T.J.*

Author Affiliation

The Hormel Institute, University of Minnesota

Abstract

Colorectal cancer (CRC) liver metastasis remains a major cause of cancer-related mortality, yet the stromal mechanisms that establish a tumor-permissive hepatic microenvironment are incompletely understood. Hepatic stellate cells (HSCs) are central regulators of liver fibrosis, inflammatory remodeling, and immune signaling during chronic liver injury and tumor progression. However, the contribution of HSC-intrinsic TWEAK-TNFRSF12A/FN14 signaling to metastatic niche formation has not been defined. Here, we investigated the role of TNFRSF12A signaling in activated HSCs during CRC liver metastasis. Our preliminary studies demonstrate that TGF-β robustly induces TNFRSF12A expression in primary human HSCs, accompanied by activation of inflammatory and cytokine-associated pathways. Mechanistically, we identified EGR2 as a candidate transcriptional regulator of TNFRSF12A induction in HSCs. Functionally, activation of the TWEAK-TNFRSF12A axis enhanced expression of tumor-promoting cytokines and macrophage-associated factors, including CCL2, CSF1, and IL-6. Importantly, TWEAK stimulation of HSCs significantly enhanced colorectal cancer cell growth and migration in 3D culture and transwell systems, supporting a paracrine role for HSC-derived signaling in metastatic progression. In contrast, genetic deletion or pharmacologic inhibition of TNFRSF12A using a selective antagonist markedly suppressed these tumor-promoting effects. Furthermore, the TWEAK-TNFRSF12A axis remodels macrophage phenotypes by promoting macrophage polarization and suppressing phagocytic activity within the metastatic liver microenvironment. Collectively, these findings identify HSC-specific TWEAK-TNFRSF12A signaling as a previously unrecognized regulator of the metastatic liver niche and suggest that stromal TNFRSF12A signaling may represent a promising therapeutic target for colorectal cancer liver metastasis.

16. Human monoclonal antibodies to poliovirus bind at the three-fold symmetry axis and inhibit particle uncoating

Waddey, B.T.*[1,2]*, *Charnesky, A.J.*[3]*, Faust, J.E.*[3]*, Cho, S.H.*[4]*, Bator, C.*[1]*, Conway, J.F.*[5]*, Mahood, K.*[5]*, Hafenstein, S.L.*[1,2,6]

Author Affiliations

[1] The Hormel Institute, University of Minnesota
[2] College of Biological Sciences, University of Minnesota, Minneapolis, MN
[3] Eberly College of Science, The Pennsylvania State University, State College, PA
[4] The Huck Institutes of Life Sciences, The Pennsylvania State University, State College, PA
[5] Department of Structural Biology, University of Pittsburgh, Pittsburgh, PA
[6] Department of Infectious Diseases, Mayo Clinic, Rochester, MN

Abstract

Poliovirus remains a serious threat to human health. Complete eradication of wild-type poliovirus has not yet succeeded, making the development of successful antivirals critical. Through cryoEM single particle analysis, we solved high resolution structures of four distinct human IgG FAbs complexed with poliovirus. These antibodies bind to capsids at the three-fold symmetry axis at novel epitopes and one of these antibodies, 6A1, neutralizes all three PV serotypes. Through biochemical assays and single particle cryoEM, we determined that 6A1 can bind at the same time as poliovirus receptor on the capsid and mildly inhibits PV genome release. Through structural analyses of ternary PV-6A1-PVR complexes, we hypothesize that 6A1's mechanism of neutralization involves both steric blocking of PVR binding and inhibition of conformational changes on the PV capsid leading to particle maturation and genome release. These studies highlight novel epitopes and mechanisms of neutralization of human antibodies towards poliovirus and may facilitate development of therapeutics for the ongoing efforts in global eradication of poliovirus.

17. Prebiotic Fiber Supplementation Mitigates the Effects of Maternal Obesity on Offspring's Gut Microbiome and Mammary Tumorigenesis

Yadav, S., *Andrade, F.O., Ozgul Onal, M., Shirk, S., Staley, C., and Hilakivi-Clarke, L.*

Author Affiliation

The Hormel Institute, University of Minnesota

Abstract

The prevalence of maternal obesity has gradually increased over successive decades, with over 50% of pre-pregnant women in the United States at present time classified as overweight or obese. Maternal obesity exerts deleterious effects on numerous pregnancy outcomes and imparts enduring negative health consequences on offspring, including elevating female offspring's susceptibility to and mortality from breast cancer. Since the gut microbiome contributes to obesity and its adverse effects, and influences breast carcinogenesis, we investigated here whether gut dysbiosis, characterized by reduced fecal short-chain fatty acid (SCFA) levels, in mouse offspring born to diet-induced obese C57BL/6 dams (OB offspring) contributed to their higher E0771 mammary tumor burden, compared with control offspring. Fecal microbiota transplant (FMT) from OB offspring increased E0771 mammary tumor burden in control offspring hosts, suggesting a causality between gut dysbiosis and increased mammary tumorigenesis in OB offspring. Further, levels of SCFA receptor FFAR2 in CD4+ T cells, CD8+ T cells, and Foxp3+ regulatory T cells in Peyer's patches in the gut and in CD8+ T cells in tumors were significantly lower in control offspring receiving FMT from an OB offspring donor than from a control offspring donor. To determine if increased mammary tumorigenesis in OB offspring can be reversed by increasing fecal SCFA production by dietary fiber, we employed the MMTV-PyMT mammary tumor model, in which mammary tumors develop "spontaneously" by 4 months of age. Our results indicated that MMTV-PyMT OB offspring exhibited significantly increased mammary tumor burden compared with controls. Supplementing offspring via drinking water with partially hydrolyzed guar gum (PHGG), a prebiotic fiber, significantly reduced mammary tumorigenesis and increased fecal levels of SCFA propionic acid compared with OB offspring receiving maltodextrin (MDX) control. In contrast, control offspring receiving PHGG did not show changes in mammary tumorigenesis, although PHGG supplementation increased their fecal butyric acid levels. These results suggest that increased mammary tumorigenesis in OB offspring is linked to their gut dysbiosis. In addition, prebiotic fiber PHGG, consumed by adult OB offspring, reverses adverse effects of maternal obesity on both their gut microbiome and mammary tumorigenesis.

18. SNAI1 drives smooth muscle cell proliferation in pulmonary hypertension

Zhong, Z.R.[1], *Elmadbouh, I.[1], Chikoore, S.[1], Babicheva, A.[1], Thompson, M.[2], Prakash, Y.S.[2], Pabelick, C.[2]*

Author Affiliations

[1] The Hormel Institute, University of Minnesota
[2] Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Rochester

Abstract

Rationale: Pulmonary hypertension (PH) is a fatal disease characterized by a sustained elevated pulmonary vascular resistance that results in right heart failure. Despite therapeutic advances, no treatment provides disease progression reversal, resulting in poor long-term survival. Identifying new therapeutic targets for PH therefore requires a deeper understanding of its mechanisms. A defining feature of PH is pulmonary vascular remodeling, a result of excessive proliferation, migration, and apoptosis resistance of pulmonary artery smooth muscle cells (PASMC). SNAI1, a transcription factor regulating cell proliferation and differentiation during development and disease, has recently been implicated in endothelial dysfunction and endothelial-to-mesenchymal transition in PH. However, its role within PASMC remains unknown. Here, we aimed to define the contribution of SNAI1 in PASMC to the pathogenesis of PH.

Methods: Normal human PASMC were exposed to normoxia (21% O2) or hypoxia (3% O2) for 72 hours. PASMC were also isolated from patients with or without PH. Cell proliferation and migration were assessed by EdU incorporation and wound scratch assays, respectively. Mice were exposed to hypoxia (10% O2) or Sugen5416/hypoxia (10% O2 plus Sugen5416 20mg/kg, i.p., weekly) for 6 weeks. PH phenotype was characterized by right heart catheterization, right ventricle (RV) hypertrophy by Fulton index and pulmonary artery (PA) remodeling by H&E staining of lung tissues. Pharmacological blocker GN-25 was used to inhibit SNAI1 in vitro and in vivo.

Results: SNAI1 expression was markedly increased in hypoxic PASMC and in PASMC from patients with PH. Hypoxia also upregulated HIF-1alpha, Vimentin, p-AKT, and PCNA at the protein level in PASMC. EdU incorporation and migration rates were significantly higher in hypoxic cells as well as in PASMC isolated from PH patients compared with controls. In vitro, GN-25 (100 nM) abolished hypoxia-induced PASMC proliferation and migration. Right ventricular systolic pressure (RVSP), a surrogate measurement of PA systolic pressure, estimated mean PAP (mPAP), and RV contractility (RV-±dP/dtmax) were significantly increased in both PH models compared with controls. Additionally, Sugen5416/hypoxia-treated mice exhibited higher RVSP, mPAP, and RV-±dP/dtmax compared to hypoxia-treated animals. The Fulton index (weight of RV vs. left ventricle and septum) was increased in both PH groups, indicating development of RV hypertrophy in PH compared to control. PH mice lost body weight by 5-10%. Additionally, when the Fulton index was normalized by body weight, RV hypertrophy was significantly higher in Sugen5416/hypoxia-treated mice compared with hypoxic animals. Lung tissues from PH mice exhibit PA hypertrophy (media remodeling) with some PA partially or completely occluded. Additionally, we detected protein upregulation of Snai1 and Vimentin in lung tissues of PH mice in both groups. Treatment of PH mice with GN-25 (20 mg/kg, daily, for the last 3 weeks under PH stimuli) in reversal experiments attenuated the PH phenotype in both animal models by decreasing hemodynamic parameters, right ventricular hypertrophy, and pulmonary vascular remodeling.

Conclusions: Our findings identify a key role for SMC SNAI1 in the pathogenesis of lung vascular remodeling during hypoxia-induced PH. Inhibition of SNAI1 in PASMC may constitute a new therapeutic strategy for the treatment of PH.