55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

ROOM 3042

1:45 p.m. **O-18** FCHSD2: A NOVEL MICAL-L1 INTERACTION PARTNER INVOLVED IN ENDOSOMAL FISSION
Presenter: Devin Frisby, UNMC

2:00 p.m. **O-29** POLYMER-BASED MICRORNA DELIVERY SYSTEM IN ALCOHOL-ASSOCIATED LIVER FIBROSIS TREATMENT
Presenter: Marjina Akter Kalpana, UNMC

2:15 p.m. **O-39** CD90 TARGETED MEASLES PSEUDOTYPED LENTIVIRUS FOR HSC THERAPY
Presenter: Ravishankar Madhu, Creighton University

2:30 p.m. **O-41** VISUAL PATHWAY FUNCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE
Presenter: Shaylah McCool, UNMC

2:45 p.m. **O-43** ROLE OF ACTIN REGULATORY PROTEINS CIN85 AND CD2AP IN ORCHESTRATING ENDOSOMAL FISSION
Presenter: Gunjan Misri, UNMC

3:00 p.m. **O-46** THE EFFECTS OF NOVEL GERANYLGERANYL DIPHOSPHATE SYNTHASE INHIBITOR, RAM2061, ON OSTEOCLAST DIFFERENTIATION AND FUNCTION
Presenter: Molly Muehlebach, UNMC

3:15 p.m. **O-47** THE TISSUE LEVEL OUTCOMES OF ALTERING THE NUCLEAR TO CYTOPLASM RATIO OF ENTEROCYTES IN INTESTINAL VILLUS
Presenter: Rosemary Mwithiga, UNMC

3:30 p.m. **O-48** BMX-001 CAN REVERSE RADIATION-INDUCED FIBROSIS
Presenter: Molly Myers, UNMC

3:45 p.m. **O-65** THE TRANSCRIPTIONAL CO-REPRESSOR SAMD1 INHIBITS HEMATOPOIESIS
Presenter: Meg Schaefer, UNMC

4:00 p.m. Break

FCHSD2: A NOVEL MICAL-L1 INTERACTION PARTNER INVOLVED IN ENDOSOMAL FISSION

Devin Frisby, Naava Naslavsky, Steve Caplan

Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE

Background
Internalization is a crucial process in eukaryotic cells and has been extensively studied. Once internalized, receptors are trafficked to the early endosome, where they can be sorted and recycled back to the plasma membrane (PM). Whereas internalization has been extensively studied, recycling is equally important but not as well understood. Receptor recycling requires the fission of budding vesicles from the endosome, a process that necessitates Arp2/3-mediated branched actin polymerization. At the neck of tubular endosomal membranes, the Wiskott Aldrich Syndrome protein and scar homologue complex promotes Arp2/3-mediated branched actin polymerization, which provides the necessary pushing force to generate the membrane tension required during fission, which is mediated by the scaffolding protein MICAL-L1 and key partners such as the ATPase EHD1. However, the precise mechanisms that regulate actin polymerization for endosomal fission remain largely unknown.

Significance of Problem
Disruption of endocytic trafficking pathways inhibits the delivery of receptors to their targeted cellular destinations, which is associated with many diseases, including Alzheimer's and various cancer types. Trafficking and recycling back to the PM are critical for regulating signal transduction pathways as well as maintenance of the PM lipid composition. Receptors destined for the PM may be recycled directly from the EE (fast recycling), or they can first be trafficked through the endocytic recycling compartment before reaching the PM (slow recycling). Fission of budding vesicles from the EE and recycling endosomes is essential for efficient receptor recycling.

Hypothesis, Problem, or Question
At the PM during clathrin-mediated endocytosis, the protein FCH and Double SH3 Domains 2 (FCHSD2) activates Cdc42/N-WASP/Arp2/3-mediated branched actin polymerization to promote vesicle scission. FCHSD2 activity has been implicated predominantly at the PM, but emerging evidence suggests that it might function at the early endosome. We hypothesize that MICAL-L1 recruits FCHSD2 to endosomes to regulate endosomal fission through the enhancement of branched actin polymerization.

Experimental Design
To explore the role of FCHSD2 in cargo recycling from endosomes, we utilized receptor uptake and recycling assays in mock- and FCHSD2 siRNA-treated cells. To determine if FCHSD2 plays a role in fission at endosomes, we used an in-cell endosomal fission assay and an array of quantitative microscopy-based techniques to compare endocytic structures and fission in mock and knockdown cells. Finally, we aimed to elucidate the mechanism of FCHSD2's regulation of fission at endosomes by quantifying the actin network at endosomes in WT-parental and FCHSD2 knock out cells.

Results/Data
FCHSD2 siRNA knockdown and FCHSD2 depletion in CRISPR knockout cells led to a significant enlargement of EEA1-decorated endosomes and MICAL-L1-decorated tubular recycling endosomes, suggesting impaired endosome fission at steady-state. Using receptor uptake and recycling assays, we showed that siRNA knockdown of FCHSD2 significantly reduced the recycling of transferrin and Major Histocompatibility Complex class I, which are clathrin-dependent and -independent cargoes, respectively. To directly test if FCHSD2 plays a role in fission at endosomes, we used a newly designed in-cell endosomal fission assay coupled with quantitative microscopy. In this assay we inhibited and synchronized endosome maturation and fission during transferrin uptake. Upon reversal of this inhibition we directly monitored the decrease in endosome size as a measure of fission occurring in mock- and FCHSD2 siRNA-treated cells. Our data show that FCHSD2 siRNA knockdown significantly reduced endosome fission. Finally, to determine if FCHSD2 regulates actin polymerization at endosomes, we transfected an active Rab5 mutant into WT-parental and FCHSD2 knockout cells. This mutant causes highly enlarged endosomes, allowing clear visualization of actin on these organelles. In these assays, we found a significant decrease in the overlap of Rab5 and cortactin, a protein that binds and stabilizes branched actin, in our FCHSD2 knockout cells, suggesting a key role for FCHSD2 in potentitating actin branching at endosomes.

Conclusions
In summary, we identify FCHSD2 as a novel MICAL-L1-interaction partner at endosomes that promotes actin branching and endosome fission, thereby supporting receptor recycling.

POLYMER-BASED MICRORNA DELIVERY SYSTEM IN ALCOHOL-ASSOCIATED LIVER FIBROSIS TREATMENT

Marjina Akter Kalpana, Benita McVicker, David Oupicky

Department of Genetics, Cell Biology & Anatomy, Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center, Omaha, NE, 68198

Alcohol-associated liver disease (AALD) turned into the ninth leading cause of death in the United States in 2021. In 2017, 50% of the deaths in the US were related to alcohol consumption. AALD is a liver pathogenesis which ranges from fatty liver, liver fibrosis, to liver cirrhosis. Currently, there is no FDA-approved treatment for AALD treatment, which makes a new drug development essential. Binding of the stromal cell-derived factor-1 (SDF-1) to the CXC chemokine receptor 4 (CXCR4) on activated hepatic stellate cells (HSC) and the high expression of microRNA 155 (miR155) in Kupffer cells (KC) causes the pathogenesis of alcoholic liver fibrosis (ALF), which develops into cirrhosis if untreated. We synthesized a CXCR4 antagonizing polymer PAMD from AMD3100 and modified it with cholesterol (PAMD-Ch) to develop a microRNA-based therapeutic polyplex nanoparticles to treat ALF. Many of the polymer-based drugs successfully passed the preclinical trials in the early twentieth century, but they have been challenged by the lack of specificity and inadequate drug bioavailability. We developed a strategically engineered polymer-based PAMD-Ch/anti-miR155 polyplexes, which provides extended availability in blood combined with more target-specificity. We hypothesize that PAMD-Ch with the CXCR4 antagonizing property will inhibit the CXCR4 signaling in activated HSCs and will deliver the therapeutic anti-miR155 to the activated KCs, which will reduce the miR155 expression in KCs and attenuate the ALF. PAMD-Ch binds antimiR155 efficiently at 1.5 w/w excess ratio (Fig. 1 A) and forms self-assembled nanoparticles with hydrodynamic size ~80 nm and zeta potential 12 mV, which makes them suitable for entry through the liver fenestrae and reach to the targeted liver cells (Fig. 1B). Our formulated PAMD-Ch/miR polyplexes have satisfactory colloidal stability in physiological body fluid conditions for 3 days (Fig. 1C). The integrity of the PAMD-Ch/miR polyplex is maintained in the blood serum and indicates the controlled payload release over 24 h (Fig. 2). The PAMD-Ch/miR polyplexes are resistant to heparan sulfate mediated disruption as tested against high concentration of heparin (above 100 µg/ml) (Fig. 3). Overall, we developed the CXCR4 antagonizing PAMD-Ch which can efficiently bind the therapeutic anti-miR155 and form the PAMD-Ch/miR complex of an effective hydrodynamic size, provides improved stability of the microRNA in different physiological conditions, and serves as a potential drug delivery system in the treatment of ALF. Discovery of novel therapy to treat ALF is essential because many people develop fatty liver, causing liver fibrosis or liver cirrhosis even after abstinence from alcohol consumption. Our research will benefit people who quit alcohol consumption but still suffer from liver problems because of their past drinking habits. Overall, this research directly impacts the area of new drug development in alcoholic liver fibrosis treatment.

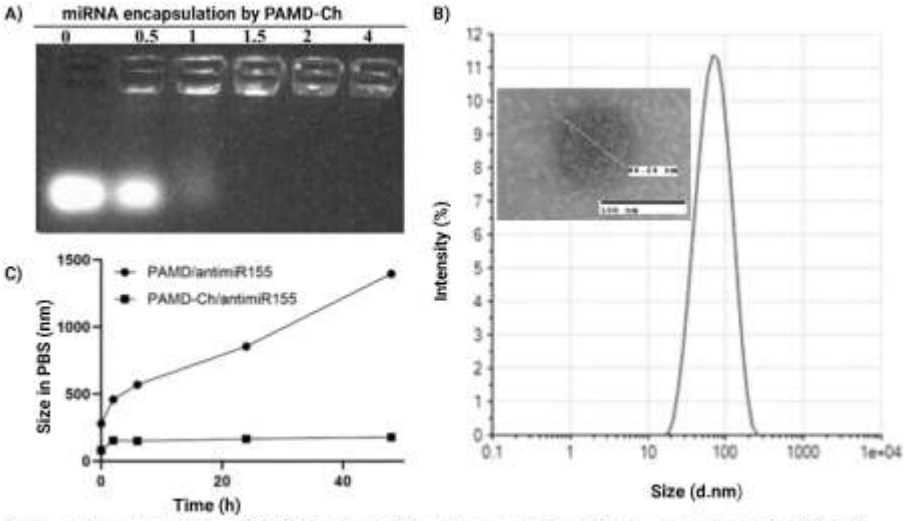

Figure 1. Characterization of PAMD-Ch/antimiR155 polyplexes. A) microRNA encapsulation by PAMD-Ch B) Hydrodynamic size and TEM image of PAMD-Ch/anti-miR155 polyplexes C) Colloidal stability of PAMD-Ch/anti-miR155 polyplexes in PBS.

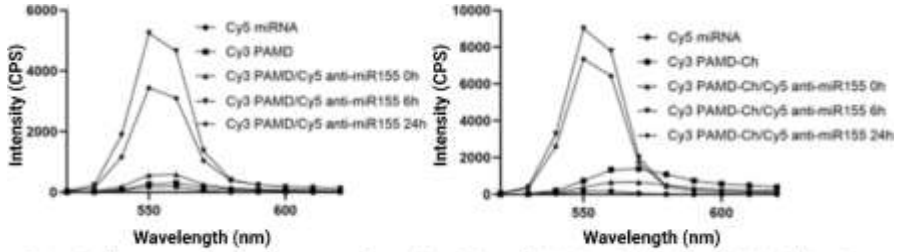

Figure 2. Fluorescence emission spectra after coincubation of PAMD/anti-miR155 and PAMD-Ch/anti-miR155 polyplexes with FBS for 0, 6, and 24 h.

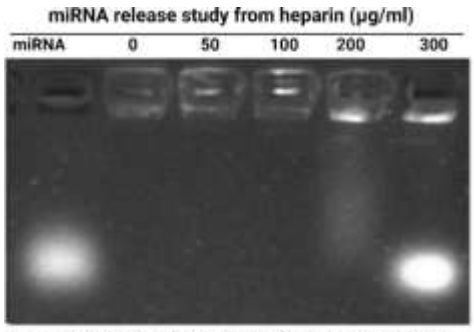

Figure 3. Release of microRNA from PAMD-Ch/anti-miR155 polyplexes against increased concentration of heparin exchange.

CD90 TARGETED MEASLES PSEUDOTYPED LENTIVIRUS FOR HSC THERAPY

Kurt Berckmueller[2,4], Justin Thomas[2,3], Eman A. Taha[2], Seunga Choo[2], **Ravishankar Madhu[1,2],** Greta Kanestrom[2], Peter B. Rupert[2], Roland Strong2, Hans-Peter Kiem[2,4,5], and Stefan Radtke[2]

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Introduction: Current gene therapy approaches rely on the modification of hematopoietic stem cells (HSCs) outside the patient's body (ex vivo) and require highly specialized facilities similar to bone marrow transplantation, severely limiting the accessibility of this treatment option. Injection of gene therapy agents and modification of HSCs directly in the patient (in vivo) would overcome all these limitations. However, a major obstacle to perform gene therapy in vivo is the lack of HSC-targeted gene therapy agents able to specifically deliver the therapeutic cargo and avoid off-target effects. Targeted delivery depends on the uptake of the vector after attaching to the cell/antigen, which has not been achieved with CD34-targeted HSC gene therapy vectors. Previous work has shown that the CD90+ subset of CD34+ HSCs is exclusively responsible for rapid recovery onset, robust long-term multi-lineage engraftment, and an entire reconstitution of the bone marrow stem cell compartment.
Results: Measles-pseudotyped lentiviral vectors efficiently transduce a wide variety of cell types that recognize the broadly expressed cell surface antigens CD46 and CD150. Here, we stepwise developed the measles envelope to remove the native targeting and instead exclusively recognize CD90 (Figure 1).
The Transduction ability was tested by incubating our CD90 targeted viral vectors with both CD90 expressing Jurkat cell lines. Determined by flow cytometry, we observed transduction of Jurkat cells with variant H1. Relative to variant H1, knock-out of CD46 and CD150 recognition reduced the ability of variant H2 to transduce Jurkat cells by 81.6% ± 13.4%. Addition of the CD90 scFv (variant H3) had no impact on the transduction ability in comparison to the WT variant H1. Finally, variant H4 (KO/CD90) regained transduction capability over variant H2 (KO), reaching on average 44.8% ± 17.9% transduction efficiency. (Figure 2)
On- and off-target activity of our CD90 targeted viral vectors was performed by co-culture experiments mixing CD90-lacking (off-target) and CD90-expressing (on-target) suspension cell lines. GFP(+) Jurkat cells and GFP(-) Raji cells were mixed and exposed to measles variants H1 and H4 in a serum-free, transduction enhancer-free, suspension culture system, and mScarlet expression flow cytometrically determined on day 3–5 post-transduction. The WT H1 virus showed mScarlet expression for both Raji as well as Jurkat cells. However, when the mix was exposed to variant H4, a strong preference of mScarlet signal was seen in the CD90+ Jurkat cells (on-target). (Figure 3)
Conclusion: We demonstrate the successful design of measles pseudotyped lentiviral vectors with a novel anti-CD90 scFv to target HSCs. Surface engineering impacted neither the binding nor fusion of viral vectors to CD90 expressing cell lines and primary human HSCs. Most important, targeted vectors demonstrated enhanced on-target specificity in mixed cultures of cell lines, as well as for human HSCs within bulk CD34+ HSPCs ex vivo.

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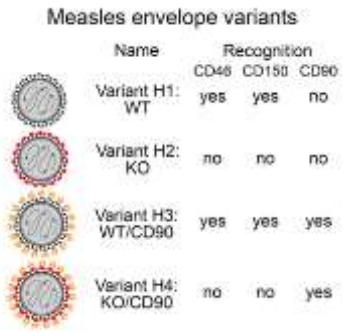

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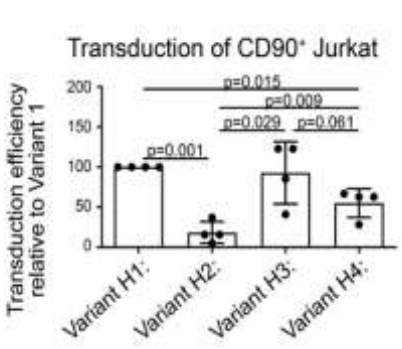

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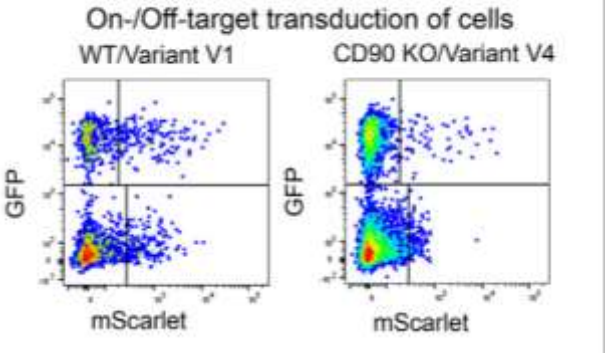

VISUAL PATHWAY FUNCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Background

Vision is a critical source of sensory information and is one of the first reported symptoms of Alzheimer's disease (AD). There are several visual manifestations of AD including deficits in contrast sensitivity and visual acuity, loss of visual field and color vision, and ocular motor dysfunction due to inability to suppress saccades. The two primary neuropathological changes that contribute to a decline in memory and cognitive impairment in AD are neurofibrillary tangles and amyloid beta (Aβ) plaques. Due to the widespread pathological nature of AD, many brain regions are affected including regions important for vision such as the dorsal lateral geniculate nucleus (dLGN) where plaques have been found. The dLGN is an important structure in the pathway for conscious vision as it directly receives visual input from the retina and relays that information to higher cortical areas. As vision declines, quality of life for those suffering from AD also declines indicating a need for determining the mechanisms behind vision loss and visual processing issues in AD.

Significance of Problem

More than 6 million Americans are currently living with AD and that number is projected to grow to over 12 million in the coming years. With this in mind, the goal of this project is to provide evidence for the development of early diagnostics and therapeutics for individuals affected by AD to improve their visual health and overall quality of life. Utilizing a wide range of techniques including electrophysiological approaches, in vivo and ex vivo imaging methods, and immunohistochemistry in an animal model with progressing amyloidosis, we aim to increase knowledge in both the fields of vision research and AD research by providing an in-depth analysis of potential structural and functional deficits related to formation of Aβ plaques.

Hypothesis, Problem, or Question

Does Aβ lead to deficits in structural components or function of the retina and dLGN?

Experimental Design

For this research, we utilized 6-, 9-, and 12-mo male and female 5xFAD mice. These mice develop Aβ plaques around 2mo of age. These AD mice are on a C57 background so C57 mice were aptly used as controls. Thioflavin s was used to stain 5xFAD tissue containing sections of dLGN. Aβ plaque density was quantified, and a high burden of plaques were identified in the dLGN in all age groups of our 5xFAD mice compared to controls which presented with zero plaques. We are using a range of functional assays, histological approaches, and imaging techniques to collect the data which has been analyzed via two-way ANOVA or nested ANOVA where appropriate.

Results/Data

Using electroretinogram (ERG) recordings, we measured the amplitude of the A and B waves corresponding to function of the photoreceptors and bipolar cells respectively. We found a decrease in A and B wave amplitude in our 9- and 12-mo 5xFAD mice compared to controls. To determine if this decrease in amplitude is attributed to the photoreceptors or the bipolar cells, we took the ratio of the B wave to the A wave and found no difference between groups indicating normal synaptic function, and therefore, the dysfunction is coming from the photoreceptors. Optical coherence tomography (OCT) was used to take retinal images allowing us to measure retinal layers. No difference in thickness of inner retinal layers was seen. However, when we measured the outer nuclear layer (ONL) and external limiting membrane (ELM), which contains the nuclei of the photoreceptors, a thickening at 1000 μm from the optic nerve head was seen in this layer indicating structural changes to the retina in our 9-mo 5xFAD mice. Immunohistochemical staining of the dLGN for vGlut2, a marker for the retinal ganglion cell (RGC) axon terminals that synapse there, showed decreased puncta density between the 6-, 9-, and 12-mo 5xFAD mice compared to 6-mo controls. This indicates a potential decrease in RGC axon terminals in the dLGN. Miniature excitatory postsynaptic current (mEPSCs) recordings of thalamocortical (TC) neurons show normal function of the retinogeniculate synapse. Similarly, single-cell dye fills of TC neurons show no structural alterations to dendrites analyzed via Sholl analysis. Finally, optomotor response (OMR) assay was used to measure reflexive visual behavior, however no significant changes were observed.

Conclusion

Even with pronounced histopathological evidence of disease in the brain, we see only mild changes in the dLGN of the 5xFAD mice. There are fewer RGC axon terminals but normal TC neuron dendritic structure indicating presynaptic structural changes in the dLGN of the 5xFAD mice. Despite presynaptic structural change, we don't see changes in mEPSC frequency which might indicate changes in synaptic strength in the dLGN. There is dysfunction in the photoreceptors of the 6, 9, and 12-mo 5xFAD mice. Finally, there is normal reflexive visual behavior in the 5xFAD mice. These data suggest that Aβ might have fairly modest influences on the visual system or may point to adaptive mechanisms that preserve function of visual pathways in this model. Investigating the mechanisms by which Aβ pathology acts on the early visual system may provide critical insights into how the visual system functions during an AD-like state, and results of this work could further the development of early diagnostic tools or drug therapies for AD.

ROLE OF ACTIN REGULATORY PROTEINS CIN85 AND CD2AP IN ORCHESTRATING ENDOSOMAL FISSION

Gunjan Misri, Kanika Dhawan, Ajay Murakonda, Devin Frisby, Naava Naslavsky and Steve Caplan
Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, United States

Background: There is an enormous diversity in the repertoire of cell surface receptors, which are essential for signaling and cellular communication. These receptors undergo continuous and dynamic internalization, sorting, and trafficking in a highly regulated manner. Once internalized, receptors found on early/sorting endosomes are sorted and either sent to the lysosome via late endosomes for degradation or recycled to the plasma membrane through recycling endosomes. A key event after receptor sorting is endosome fission, mediated by the ATP-dependent scission protein EHD1. EHD1 is essential for recycling internalized receptors, through the fission of transport vesicles or tubules. At specialized tubular-shaped endosomes known as tubular recycling endosomes, a scaffolding protein known as MICAL-L1 functions in endosome fission and receptor recycling through its interactions with EHD1, various other proteins, and the actin cytoskeleton. The process of membrane fission requires the actin cytoskeleton for membrane remodeling needed to form membrane buds or tubules. However, our comprehensive knowledge of the proteins that regulate actin at the endosomes mediating fission is still unclear. We have recently carried out a yeast two-hybrid screen with MICAL-L1 as bait and identified the actin-regulatory proteins CIN85 and CD2AP as novel MICAL-L1 interaction partners. CIN85 and CD2AP are homologous proteins that regulate branched actin through their ability to bind and sequester the actin-capping protein and remove it from actin branching points. This study elucidates the critical role of CIN85 and CD2AP in regulating endosome fission and receptor recycling by adopting branched actin at the endosomes.

Significance: Receptor recycling and trafficking are critical for human health, impairment of which can lead to cardiovascular diseases and different types of cancer. Mutations in the EHD1 fission protein have been documented in patients with polycystic kidney disease and hearing impairment. Accordingly, there is high significance in better understanding how the complex process of endosome fission is orchestrated and, in identifying the different players involved. We propose novel roles for two proteins that promote actin-branching, CIN85 and CD2AP, at the endosomes mediating fission and receptor recycling.

Hypothesis: Src homology 3 (or SH3) domains are found in many proteins that facilitate interactions with proline-rich sequences found in other proteins. Based on the knowledge that CIN85 and CD2AP have three SH3 domains, and MICAL-L1, a vital scaffolding protein on tubular recycling endosomes, has fourteen proline-rich motifs, we hypothesize that one or more SH3 domains found in CIN85 and CD2AP might interact with MICAL-L1 to localize to tubular recycling endosomes. Both CIN85 and CD2AP proteins have a conserved CPI or Capping Protein Interaction motif, which removes the actin capping protein from branched actin, thereby facilitating actin branching. We hypothesize that the branched actin-promoting ability of the two homologous proteins aids in the synergistic formation of constricted endosome tubules, leading to endosome fission by EHD1 and subsequently, to receptor recycling.

Experimental Design: To validate MICAL-L1 interactions with CIN85 and CD2AP in mammalian cells, we used endogenous HeLa cell lysates and determined that MICAL-L1 co-immunoprecipitated with both CIN85 and CD2AP. Since CIN85 and CD2AP each have three SH3 domains, we identified which SH3 domains are needed for interaction with MICAL-L1. To map the binding of the SH3 domains, we used a) Selective yeast-two-hybrid or b) GST-pulldown assays. We then asked whether MICAL-L1 is needed to recruit CIN85 and CD2AP onto endosomes, to test this we used siRNA knock-down of MICAL-L1, coupled with immunofluorescence and quantification of CIN85 and CD2AP found on tubular recycling endosomes in HeLa cells. To establish the roles of CIN85 and CD2AP in endosome fission and recycling, we carried out a) Receptor recycling studies using CD98 receptor, and b) Endosome fission assays. This is a novel assay that we developed where enlarged endosomes are generated using a Phosphoinositide 3 Phosphate Kinase (PI3PK) inhibitor, and the rate of fission is quantified after removing the inhibitor and measuring the decrease in endosome size over time. Since CIN85 and CD2AP promote actin branching through their CPI motif, we used cortactin (a marker of branched actin) to test changes in branched actin at early/sorting endosomes upon depletion of CIN85 or CD2AP in Non-Small Cell Lung Cancer Cells.

Results/Data: 1) MICAL-L1 interacts with CIN85 and CD2AP in mammalian cells. 2) The second and third of three SH3 domains of CIN85 interact with MICAL-L1. 3) MICAL-L1 independently recruits both CIN85 and CD2AP to tubular recycling endosomes. 4) Both CIN85 and CD2AP are required for optimal endosome fission and receptor recycling. 5) When either CD2AP or CIN85 is depleted, branched actin at endosomes is significantly reduced.

Conclusion: Endosome fission is indispensable to the intricate process of receptor recycling and trafficking. Elucidating the various players involved and the regulatory aspects can provide valuable insights into physiological and pathological contexts. Our findings have highlighted CIN85 and CD2AP as novel proteins at the early/sorting endosomes, recruited by MICAL-L1. Through our study we have shown that CIN85 and CD2AP play a vital role in regulation of endosome fission and receptor recycling through facilitating branched actin dynamics. Overall, these studies shed new light on endosomal trafficking, which is essential for all receptor-mediated events in the cell.

THE EFFECTS OF NOVEL GERANYLGERANYL DIPHOSPHATE SYNTHASE INHIBITOR, RAM2061, ON OSTEOCLAST DIFFERENTIATION AND FUNCTION

Molly E Muehlebach, Sarah A Holstein – University of Nebraska Medical Center; Omaha, NE

Myeloma bone disease (MBD) affects nearly 90% of patients with multiple myeloma (MM), significantly impacting patient morbidity. MBD is characterized by the presence of osteolytic lesions and diffuse osteopenia leading to complications such as pathological fractures and spinal cord compression. Such effects are attributed to the overactivation of osteoclast cells initiated by MM cell localization in the bone marrow, leading to excessive bone resorption and suppression of new bone formation. Current treatments include nitrogen bisphosphonates (NBPs) such as zoledronic acid which target osteoclasts, inhibiting their excessive resorptive activity. NBP's inhibit the enzyme farnesyl diphosphate synthase (FDPS) which is responsible for production of farnesyl pyrophosphate (FPP). Isoprenoids such as FPP and downstream product geranylgeranyl pyrophosphate (GGPP) are utilized for the post-translational modification (prenylation) of proteins in the small GTPase family. Agents that inhibit FPP and/or GGPP synthesis thereby disrupt localization and function of these proteins. Importantly, NBP-induced anti-osteoclastic effects are attributed to depletion of GGPP alone, rather than depletion of FPP. Thus, inhibition of GGPP synthesis may represent a more selective approach for targeting MBD. Our lab has developed a novel α-methyl homoneryl triazole bisphosphonate (RAM2061) which inhibits the enzyme geranylgeranyl diphosphate synthase (GGDPS). Our studies have shown that RAM2061 has direct anti-MM effects through disruption of geranylgeranylation of Rab proteins necessary for secretory cell processes essential for MM cell function. Understanding that our inhibitor demonstrates direct anti-MM effects, we next investigated whether it exhibits anti-osteoclastic effects. Using the RAW264.7 pre-osteoclast cell line, we found that RAM2061 results in induction of ER stress pathways in pre-osteoclast cells, inducing the unfolded protein response (UPR) pathway and ultimately pre-osteoclast apoptosis. Further investigation into the effects of RAM2061 on the differentiation of RAW264.7 cells revealed that GGDPS inhibition disrupts osteoclast differentiation, downregulating expression of osteoclast-specific markers and decreasing generation of tartrate resistant acid phosphatase (TRAP)-positive osteoclast numbers. Furthermore, treatment of fully differentiated osteoclasts resulted in a decrease in TRAP enzyme activity and inhibition of osteoclast resorption in a concentration-dependent manner. Confocal microscopy studies revealed that RAM2061 disrupts actin cytoskeletal dynamics essential for osteoclast differentiation and function. With the geranylgeranylated proteins of the Rho GTPase family being responsible for processes such as cytoskeletal reorganization, we suggest that GGDPS inhibition leads to depletion of intracellular GGPP levels, inhibiting Rho protein geranylgeranylation, and ultimately preventing cytoskeletal reorganization necessary for proper osteoclast differentiation and function. Future directions will focus on understanding the impact of GGDPS inhibition on osteoblast cells as well as determining the effects on bone remodeling processes in vivo.

THE TISSUE LEVEL OUTCOMES OF ALTERING THE NUCLEAR TO CYTOPLASM RATIO OF ENTEROCYTES IN INTESTINAL VILLUS

Rosemary Mwithiga and Subu Ramanathan
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Background and significance of the problem: The enterocytes on the intestinal villi are morphologically uniform and have stereotypical cell and nuclear size. However, tumor cells, including those from the intestine, can exhibit abnormal variations in the size of cells and nuclei. Our previous studies indicate that the aberrant cell size influences how cells pack and organize tissues. Nevertheless, how a shift in the physical properties of enterocytes, including the variation in the nuclei and cell size, on intestinal disease progression is not well understood.

Hypothesis: This study will examine the mechanical outcomes of altering cytosol to nuclei-dominated packing on aberrant tissue organization in epithelial tumors of the intestine.

Experimental Design: To test this hypothesis, genetic mosaic villi were generated using conditional alleles to alter the mTOR and Wnt signaling pathways which play a role in the regulation of cell size and have been associated with the development of epithelial cancers. The alteration in cellular packing to allow changes in cell and nuclear morphology will be analyzed using confocal microscopy, imaging, and 3D enteroid cultures to model the impact of altered cell/nuclear size in maintaining epithelial order.

Results: Previous studies have shown in various diseases and cancer, the nuclei are stiffer than the cytosol by an order of magnitude and our preliminary data indicates there is a strong correlation between nuclear diameter and cellular width in intestinal organoids.

Conclusions: This study will enhance our understanding of the outcome of tissues diverging from uniform cell size and shape which could shed light on the initiation of epithelial tumors and how the structure of the tissue is altered in disease.

BMX-001 CAN REVERSE RADIATION-INDUCED FIBROSIS

Molly S. Myers[1], Elizabeth A. Kosmacek[1], Chia Sin Liew[2], Arpita Chatterjee[1], Jean-Jack Riethoven[2], and Rebecca Oberley-Deegan[1]

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2. Center for Biotechnology, University of Nebraska-Lincoln, Lincoln, Nebraska

Background

Prognoses of pelvic cancers, such as prostate, gynecologic, and gastrointestinal cancer, have improved due to advancements in diagnostic and treatment strategies, including the use of radiation as a main treatment modality. Patients who receive pelvic irradiation are at risk of long-term, therapy-induced side effects caused by radiation-induced fibrosis, such as fecal and urinary incontinence. Radiation generates reactive oxygen species (ROS), including superoxide, which persists in tissues long after radiation and can initiate and propagate fibrosis. ROS adducts DNA, which can disrupt methylation and consequently transcription. Radiation-induced DNA damage can also induce fibroblast senescence via p21 induction, which cycles with superoxide to maintain senescence and produces a senescence-associated secretory phenotype (SASP), which can cause nearby fibroblasts to adopt a fibrotic phenotype. Traditionally, fibrosis is thought to be an irreversible process, and no agents exist to treat post-radiation side effects. BMX-001, a superoxide scavenger, is currently in clinical trials to prevent radiation-induced side effects in brain, head and neck, rectal, and anal cancers, but our data suggests that BMX-001 can reverse radiation-induced fibrosis and senescence when given three weeks after irradiation.

Significance of Problem

Radiation-induced fibrosis diminishes the quality of life of pelvic cancer survivors, and an agent that reverses fibrosis has the potential to improve radiation-induced pelvic pain and incontinence.

Hypothesis, Problem, or Question

We hypothesize that BMX-001 can reverse radiation-induced fibrosis by epigenetically modifying genes responsible for fibrosis and by scavenging p21-induced superoxide to disrupt senescence.

Experimental Design

We evaluated BMX-001 both *in vitro* and *in vivo* to establish its ability to reverse fibrosis. *In vitro,* we isolated and cultured primary murine fibroblasts. To evaluate fibroblast activation, fibroblasts were given physiologically relevant doses of 2 Gy of radiation and BMX-001 two- or four-days post-irradiation and either stained for α-SMA or seeded on collagen discs to evaluate contractile capacity. To evaluate fibroblast senescence, fibroblasts were treated and stained for senescence-associated β-galactosidase, p16, and p21. *In vivo,* mice received 7.5 Gy of pelvic irradiation every day for 5 days and given BMX-001 three weeks later. Two months after radiation, fibroblasts were isolated, cultured, and methylation and RNA sequenced. Gene targets that were both differentially methylated and expressed were identified. Fibroblasts were also stained for β-galactosidase to evaluate senescence.

Results/Data

In vitro, irradiated fibroblasts stained positive for α-SMA, which BMX-001 treatment administration 2 and 4 days later reversed (55.6% positive vs. 24.0% positive). Irradiated fibroblasts contracted collagen discs to a smaller size, indicating activation, while fibroblasts that received BMX-001 2 days after did not contract the disc (1.3 mm^2 vs 1.9 mm^2). The percentage of senescent fibroblasts increased with radiation, which BMX-001 administration 2 and 4 days later reversed (55.8% vs. 5.4%). *In vivo,* BMX-001 treated mice had fewer senescent fibroblasts compared to their PBS-treated counterparts (4.3% vs. 46.5%) and decreased cell size (6 mm^2 vs. 16 mm^2). Pathways involving genes that were both differentially methylated and expressed in fibroblasts treated with BMX-001 post-radiation include cardiovascular development and connective tissue.

Conclusions

These data demonstrate BMX-001's ability to reverse previously established fibrosis and senescence, which provides a strong foundation for BMX-001 as an agent that can reverse fibrosis.

THE TRANSCRIPTIONAL CO-REPRESSOR SAMD1 INHIBITS HEMATOPOIESIS

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Background
Hematopoietic stem and progenitor cell (HSPC) differentiation is marked by states of progressively restricted lineage-committed cells (e.g., the megakaryocyte-erythrocyte progenitor (MEP)). Transcriptomics studies have highlighted significant cell state heterogeneity during the process of differentiation. Within these datasets, a family of related sterile alpha motif (SAM)-containing proteins are elevated in MEPs. The commonly ascribed roles for SAMs in regulating cell signaling and transcription via homo- or heterologous interactions with other SAMs suggest shared control mechanisms. However, in many cases, their function remains uncharacterized. We identified that the sterile alpha motif protein-1 (SAMD1) protein is expressed highly in hematopoietic stem cells (HSCs) and MEPs and is predicted to control key hematopoietic transcription factors such as GATA2. In other contexts, SAMD1 acts as a transcriptional co-repressor with LSD1 to control transcription. SAMD1 knockout is embryonic lethal, and no conditional knockout mouse currently exists.

Significance of Problem
SAM-containing proteins are involved and/or implicated in several human pathologies. For example, a mutation in the SAM of the Ephrin A4 cell surface receptor increases kinase activity and promotes melanoma progression. Two additional SAM-containing proteins, Samd9 and Samd9l, are involved in hematologic disorders, myelodysplastic syndrome, and leukemia, promoting cell signaling via the Kit receptor. Both SAMD1 and LSD1 are commonly upregulated in acute myeloid leukemia (AML), and high expression is correlated with poor prognosis. Linking the contribution of SAMD1 hematopoietic signaling and transcription opens the door to translational avenues for studying SAMD1 in hematologic pathologies.

Hypothesis
SAMD1 is required for transcription and/or signaling during hematopoiesis.

Experimental Design
I tested my hypothesis using an shRNA knockdown system in mice and a CRISPR-Cas9 KO system in human hematopoietic progenitors. Following knockdown/knockout of Samd1, I used a standardized protocol to differentiate mouse bone marrow-derived cells or human CD34+ hematopoietic progenitors along the erythroid lineage. These experiments allowed me to analyze the ability of hematopoietic progenitors to differentiate into red blood cells using flow cytometry and Wright-Giemsa staining. To test Kit signaling activation, I observed an erythroid progenitor's response to Kit's ligand stem cell factor (SCF). To examine SAMD1's involvement in transcription, RNA-seq was performed on erythroid-differentiated CD34+ primary cells. To clarify the role of Samd1 in hematopoiesis, we conducted in vivo competitive transplant experiments in mice using shRNA knockdown HSCs.

Results/Data
SAMD1 knockout increased late erythroid cell ($CD71^{-}CD235a^{+}$) frequency by 8-fold. In mouse and human erythroid progenitors, Kit signaling was decreased by 2.7-fold in the absence of SAMD1. Consistent with the observation that SAMD1 knockout increased erythropoiesis, an RNA-seq analysis identified a cohort of SAMD1 repressed genes were involved in heme metabolism (TRIM10 and TRAK2) and ROS pathways (GLRX2 and GLCM). Conversely, SAMD1-activated genes include those involved in hemostasis and platelet activity pathways (F2RL3 and FLNA) and signal transduction (MAPK3 and PIK3CG), suggesting a role for SAMD1 in MEP cell fate. Finally, Samd1 knockdown versus control HSCs revealed an increase in HSC repopulation with 2.7-fold more $CD45.2^{+}$ after 16 weeks.

Conclusions
SAMD1 inhibits erythropoiesis, which may also promote megakaryopoiesis. We are currently testing SAMD1 transcriptional mechanisms using ATAC-seq and CUT&RUN and a role for SAMD1 in erythropoietin signaling. Additionally, my competitive transplant suggests a role for Samd1 in inhibiting HSC activity. While increased HSC repopulating efficiency is a relatively rare phenotype for gene knockdown, it is consistent with our data demonstrating that SAMD1 knockdown dampens MAPK signaling responses. These mechanisms may be exploited to improve HSC expansion ex vivo, and studies examining Samd1's role in HSC activity are ongoing.