84084

Team Science to maximize rapid collection and analyses of biosamples from patients with Covid-19

Sharon M Moe, Brooke Patz, Yunlong Liu, Christie Orschell, Andy Yu, Scott Denne, Peter Embi, and Tatiana Foroud
Indiana University, Embi also from Regenstrief Institute

ABSTRACT IMPACT: Indiana CTSI Team Science to maximize rapid collection, analyses and dissemination of biosamples collected from patients with Covid-19 to provide preliminary data for grant applications on the pathogenesis and outcomes of patients with Covid-19. OBJECTIVES/GOALS: When Covid-19 hit Indiana in April, there was an immediate need to respond rapidly to coordinate research across our healthcare systems. The CTSI became a point of contact for coordinating research endeavors including activation of clinical trials and use of precious samples from patients with Covid-19 to maximize preliminary data for grants. METHODS/STUDY POPULATION: The Indiana CTSI coordinated collection of biospecimens at multiple hospitals using in person and remote consenting via telephone or on a smartphone utilizing a QR code. We also retrieved existing samples from the Indiana Biobank previously collected for future research and from subject positive for Covid-19 by search of the linked electronic health record (EHR). A total of 224 subject samples (7 children, 36 previously collected, and 6 with both acute and recovered specimens) were obtained over a four month period. Our CTSI cores ran varied analyses collated to a single database, linked to the EMR for use as preliminary data for grant applications to avoid redundancy of measures on limited samples. RESULTS/ANTICIPATED RESULTS: The 224 subject samples were used for whole exome DNA sequencing, RNA seq, analyses of 48 plasma cytokine/chemokines by multiplex analyses, and PBMC isolated for culture and assessment of secreted cytokines. The clinical data were linked and included demographics, hospitalization length of stay and need for mechanical ventilation, max and min oxygen levels, liver function tests, IL-6, D-dimer, CRP, LDH, and ferritin, need for dialysis, and echocardiography. Additional clinical data were available upon request. A survey was sent to our CTSI email to query for potential interest in the data with 87 inquiries, and to date 46 investigators have requested data and/or additional samples. DISCUSSION/SIGNIFICANCE OF FINDINGS: During the first surge of Covid-19, the CTSI coordinated analyses for the dissemination of results for use by CTSI investigators to minimize duplication of assays and increase availability. The collaboration of research coordinators, biobank, research cores, and informatics demonstrates the power and agility of team science in the Indiana CTSI.

86583

The role of creatine in developmental myelination and remyelination*

Lauren Rosko[1], Tyler Gentile[1], Victoria Smith[2] and Jeffrey K. Huang[1]
[1]Georgetown University, [2]George Washington University

ABSTRACT IMPACT: This study highlights the importance of creatine in developmental myelination and remyelination to investigate whether creatine provides a therapeutic value during a central nervous system (CNS) demyelinating insult with a potential value in patients with Multiple Sclerosis. OBJECTIVES/GOALS: Creatine is vital for ATP buffering in the brain. Interestingly, the cells that generate myelin express the main enzyme for creatine synthesis, Gamt. Patients with Gamt mutations display intellectual delays and impaired myelination. Therefore, we hypothesize that creatine is essential for developmental myelination and improves remyelination. METHODS/STUDY POPULATION: To investigate these hypotheses, we developed a new transgenic mouse model with LoxP sites flanking exons 2-6 of the guanidinoacetate methyltransferase (Gamt) gene where excision leads to expression of a green fluorescent tag allowing us to track the cells normally expressing Gamt. We used immunohistochemistry techniques to look at the corpus callosum, the main white matter tract in the brain, and evaluate the number of oligodendrocytes (OL), glial cells responsible for generating myelin. We also used the cuprizone model of toxic demyelination to investigate whether dietary creatine and cyclocreatine, a planar analog of creatine that more efficiently crosses the blood-brain barrier, can enhance remyelination. RESULTS/ANTICIPATED RESULTS: In this mouse model, we show a 95% (+/-0.47%, n=3) co-localization of Gamt within mature OL during postnatal (P) day P14 with no co-localization in neurons or other glial cells. This suggests that mature OL are the main cells making creatine in the CNS. Next, we show that knocking out Gamt leads to a significant reduction in OL in the developing corpus callosum, at P14 and P21 (P14: 0.007, n=3; P21: 0.04, n=3). We also show that creatine supplementation causes a trending increase in mature OL density in the corpus callosum following cuprizone demyelination (2% creatine; p=0.052; n=4). Interestingly, cyclocreatine supplementation significantly increased mature OL density in the corpus callosum following cuprizone demyelination (0.1% cyclocreatine; p=0.034; n=4). DISCUSSION/SIGNIFICANCE OF FINDINGS: These studies highlight the important role creatine plays in developmental myelination and remyelination to investigate whether creatine and cyclocreatine provide a therapeutic value during a CNS demyelinating insult. This work investigates a potential therapeutic value of creatine to patients with Multiple Sclerosis.

Precision Medicine

Basic Science

48352

Mechanisms Underlying Lipidomic Changes in Major Depressive Disorder

Ana Paula Costa, Milenna T. van Dijr, Ardesheer Talati, Myrna M. Weissmann and Laura Beth McIntire
Columbia University

ABSTRACT IMPACT: Lipidomics is emerging as a powerful strategy to identify biomarkers for Major Depressive Disorder, as well as therapeutic targets in lipid metabolic pathways. OBJECTIVES/GOALS: Lipidomics is increasingly recognized in precision psychiatry for global lipid perturbations in patients suffering from Major Depressive Disorder (MDD). We will test the hypothesis that lipid metabolism dysregulation is associated with familial risk of depression. METHODS/STUDY POPULATION: Patients with MDD (G1), children (G2), and grandchildren (G3) have been part of a longitudinal study since 1982. If a parent G2 and grandparent G1 have MDD, G3 is considered a high risk of depression. Biospecimens (saliva and serum) were collected for full exome sequencing and RNA analysis. Samples will also be extracted for lipid content and lipids will be identified by mass spectrometry. A panel of nearly 600 lipid species can reliably be identified and quantified using liquid chromatography paired with tandem mass spectrometry

https://doi.org/10.1017/cts.2021.656 Published online by Cambridge University Press

(LC-MS/MS). Dysregulated lipids will be correlated with familial risk of depression in samples of G3. RESULTS/ANTICIPATED RESULTS: We hypothesize that dysregulation of lipids and lipid metabolism will be apparent in biospecimens from the high risk compared to the low risk of depression. Also, alterations in RNA transcriptomics of genes involved in lipid metabolic networks are associated with familial risk of depression. Several differential lipid species were previously identified to be associated with MDD. Reduced phosphatidylcholine(PC), phosphatidylethanolamine(PE), phosphatidylinositol(PI), and increased LysoPC, LysoPE, ceramide, triacylglycerol, and diacylglycerol levels have been correlated to MDD. However, these results need to be replicated in independent studies using lipidomics analysis. DISCUSSION/SIGNIFICANCE OF FINDINGS: It is highly likely that completely novel cellular targets will emerge from these studies by uncovering the convergence of lipidomics and genetic variance of lipid metabolic enzymes as biomarkers for predisposition to MDD as well as potential targets for therapeutic development for MDD.

78511

Synthesis of Novel Core/Shell Polymeric Nanoparticles for Controlled Drug Release

Braden Hahn
Auburn University Samuel Ginn College of Engineering

ABSTRACT IMPACT: This work will develop a novel drug delivery system that has improved biocompatibility and controlled release than current systems and allow for customizable loading and drug delivery to unique patient and treatment requirements. OBJECTIVES/GOALS: The goal of my project is a novel hybrid core/shell nanoparticle system for controlling in vivo chemotherapeutic concentration. The current goal is to confirm core and shell polymeric nanoparticle formation via emulsion technique and validate predictive model developed to optimize shell formation efficiency and control shell thickness. METHODS/STUDY POPULATION: Though early results are promising, they are not proof that the desired core/shell structure is being formed via my novel process. I constructed a theoretical model to use to optimize and control the process for precise shell thicknesses. Therefore, the current experimental plan focus is to not only visually confirm the predicted formation of my core/shell design but use these experiments to validate the model.
1. Gel-Suspended SEM: nanoparticles suspended in gel matrix, bisected to reveal inner structure
2. Fluorescent Conjugation Microscopy: visually-distinct dyes used to show polymer distribution and validated against the theoretical model predictions.
3. Modified Hydrophobic Dye Release: different mixtures of polymers with release showing if previous promising results due to core/shell structure RESULTS/ANTICIPATED RESULTS: As stated, the experiments will confirm the core/shell nanoparticle structure, validate the developed theoretical model, or provide direct evidence against any formation. This core/shell structure is key to the current design for controlling payload release rate and thus in vivo drug concentration. For the gel-suspension experiment, the interior core will be labeled with ultrasmall SPIONs and thus any layers within the particles will be distinct. While this result is qualitative, high magnification fluorescent microscope images will be analyzed using image processing software to determine core/shell formation efficiency and compared to estimated efficiencies from the model. Finally, the mixed release will clarify previous experiments' release mechanism and either support or disprove shell influence. DISCUSSION/SIGNIFICANCE OF FINDINGS: The significance of this work is twofold: core/shell particles have been proven to provide variable control of release on the micron scale but not yet at the nanoscale, allowing for a circulating, targeted system that can finely control release. The process is also novel for producing this type of structure, at highly consistent quality and size.

81030

Utilizing a synergistic drug combination to target relapsed/refractory FLT3 mutant AML

LaQuita Jones[1], Katelyn Melgar[1], Scott Hoyt[2], Mark Wunderlich[1], Eric O'Brien[1], John Perentesis[1], Craig Thomas[2] and Daniel Starczynowski[1]
[1]Cincinnati Children's Hospital Medical Center; Katelyn Melgar; [2]National Center for Advancing Translational Sciences

ABSTRACT IMPACT: This work will help to understand a novel therapeutic approach to a common type of acute myeloid leukemia. OBJECTIVES/GOALS: FMS-like tyrosine kinase 3 (or FLT3) mutations occur in ~30% of acute myeloid leukemia (AML) cases. FLT3 tyrosine kinase domain (TKD) mutations are particularly important in relapsed/refractory FLT3 mutant AML, which portends poor prognosis. This study describes a therapeutic approach to overcoming resistance conferred by FLT3-TKD mutations. METHODS/STUDY POPULATION: To understand the efficacy of a novel type 1 FLT3 inhibitor (NCGC1481), as a monotherapy and combination therapy, several assays were utilized to interrogate functionality of these therapies. Cell lines and patient samples containing aspartate 835 to tyrosine mutations (D835Y, the most common TKD alteration) and phenylalanine 691 to leucine (F691L) were utilized to examine the effects of NCGC1481 with and without other targeted therapies like MEK inhibitors. Specifically, assays measuring viability, cell death using flow cytometry, in vitro clonogenicity, cellular signaling, and xenograft survival were examined in these FLT3-TKD AML models. Synergy was also measured using well described methods, which also allowed for appropriate dose finding for drug combination experiments. RESULTS/ANTICIPATED RESULTS: Our novel type 1 FLT3 inhibitor (NCGC1481) was particularly effective in the most common FLT3 TKD mutant, D835Y. NCGC1481 reduced viability and cell signaling, while also inducing cell death and prolonging xenograft survival in the FLT3-D835Y model system. In contrast, clinically approved FLT3 inhibitors were less effective at suppressing AML cells expressing FLT3-D835Y. In the case of FLT3-F691L, most of the FLT3 inhibitors tested, including NCGC1481, suppressed canonical FLT3 signaling, but did not significantly reduce viability or leukemic clonogenicity. However, when NCGC1481 was combined with other targeted agents like MEK inhibitors, at synergistic doses, eradication of the FLT3-F691L AML clone was substantially increased. DISCUSSION/SIGNIFICANCE OF FINDINGS: In AML, response to FLT3 inhibitor therapy is often short-lived, with resistance sometimes occurring via FLT3-TKD mutations. Given the dismal prognosis of relapsed FLT3 mutant AML, novel therapies are necessary. This study describes efficacy of a novel FLT3 inhibitor, along with its synergistic activity when combined with other targeted agents.

https://doi.org/10.1017/cts.2021.656 Published online by Cambridge University Press