profiles observed in patients before DAA therapy showed a strong macrophage-mediated inflammatory response against HCV infection in the liver that shifted significantly to a tissue remodeling microenvironment after treatment.

260

Breast Cancer Biopsy Triage with Ex-Vivo Microscopy for Downstream Analysis*

Madeline Behr[1], Brett Roberts[2], Layla Alizadeh[3], J. Quincy Brown[4]
[1]Tulane University [2]Ochsner Medical Center, Department of Radiology [3]Ochsner Medical Center, Department of Pathology [4]Tulane University, Department of Biomedical Engineering

OBJECTIVES/GOALS: In this study, the ability of a pathologist to detect malignancy on digital pseudo-H&E slides obtained via structured illumination microscopy (SIM) imaging of fresh diagnostic breast biopsies was assessed. The speed of imaging and processing was also assessed for potential clinical implementation. METHODS/STUDY POPULATION: This study was conducted in accordance with an Ochsner Medical Center of New Orleans IRB. 200 patients undergoing either stereotactic or ultrasound-guided diagnostic breast biopsies were consented and an additional core from the suspicious lesion was collected for research use. Research biopsies were transported to the lab and stained with DRAQ5 and Eosin-Y and imaged with SIM before being submitted for histology processing. Imaging and digital processing times were recorded. The resulting SIM images and histology slides were given to a pathologist for blind review to assess accuracy. RESULTS/ANTICIPATED RESULTS: The ex-vivo structured illumination microscopy images and subsequent histology slides from 79 research cores have been assessed to date. Some samples were excluded from the total data set and not included in the final assessment due to technical failures of the imaging protocol. Of the current set, the pathologist has a specificity of 88% and a sensitivity of 65%, as well as an NPV of 88% and a PPV of 65%. Staining time for each biopsy was completed within 3 and a half minutes and imaging at 20x magnification took between 4 and 12 minutes, depending on size and implementation of autofocus to the imaging system. Image processing took approximately 5 minutes per biopsy and is a direct function of biopsy size. DISCUSSION/SIGNIFICANCE: Decreased time between cancer suspicion and treatment will improve the prognosis of breast cancer patients. SIM imaging of fresh breast biopsies could ultimately allow primary and secondary histology to be performed simultaneously and minimize histopathology time, thus allowing clinicians and patients to implement treatment course more quickly.

261

Characterization of Glycosylation Patterns of Single IgA Molecules Using Single-Molecule Fluorescence Microscopy

Joseph Rubin, Milan Stojanovic, Ali Gharavi, Henry Hess
Columbia University

OBJECTIVES/GOALS: IgA1 nephropathy, which can lead to kidney failure, is caused by complexes formed between aberrant galactose-deficient IgA1 and antibodies directed against it. Our goals are to characterize shifting glycosylation patterns at the level of single IgA1 molecules and to apply this to patient samples for early detection and understanding of the disease. METHODS/STUDY POPULATION: To characterize glycosylation patterns on single IgA1 molecules, labelled IgA1 in low concentration was physisorbed to borosilicate glass in a fluidic cell and labelled Jacalin was flowed in to bind with Glycans on IgA1. The samples were observed with a Nikon TiE epi-fluorescence microscope. FRET images were created by exciting the Jacalin dye with a blue laser and recording the red emission of the IgA1 dye with an EMCCD camera. FRET emission intensities of individual IgA1 molecules over time were analyzed to determine how frequent and how long Jacalin binds to each of them. The rate of binding is roughly inversely proportional to the amount of abnormal glycans on a given IgA1 molecule. After the method is perfected, we intend to compare the glycosylation patterns of healthy and diseased patient samples. RESULTS/ANTICIPATED RESULTS: Addition of the competitive binder Galactose to the solution led to an increase in the off times of Jacalin and IgA1 and a decrease in the on times in a concentration-dependent manner, yielding an increase in the dissociation constant. Dissociation constants of individual molecules within a single experiment vary by 3 orders of magnitude, which cannot be attributed to stochastic fluctuations but rather reflects differences in the adsorption geometry. Nevertheless, the unaffected dissociation constant can be identified. We expect that when this method is applied to samples from healthy and IgA1 Nephropathy patients, specific IgA1 molecules from patients will have higher dissociation constants for Jacalin compared to those from healthy patients. DISCUSSION/SIGNIFICANCE: The binding rates of Jacalin to single IgA1 vary by 3 orders of magnitude. The observed heterogeneity shows the Jacalin probe can differentiate between different IgA1 populations. An understanding of which IgA1 molecules in patient samples are problematic and what their distribution of Glycans is can lead to discovering biomarkers and treatments.

265

Connecting Electronic Medical Record Sub-phenotypes of Obstructive Sleep Apnea to Adverse Outcome Risks

Victor Borza[1], Raghu Upender[2], Wei-Qi Wei[2], Bradley Malin[2]
[1]Vanderbilt University and Vanderbilt University Medical Center [2]Vanderbilt University Medical Center

OBJECTIVES/GOALS: Prior work has established subtypes of OSA and linked them to risks of future adverse events but rarely with the longitudinality and richness of data available in the EMR. Our goal is to leverage EMR data identify clinically meaningful sub-phenotypes of OSA and better study how they affect risks of adverse outcomes. METHODS/STUDY POPULATION: Vanderbilt's EMR database has over 61,000 adult patients with a literature-validated EMR definition of OSA with a median EMR follow-up period of 4 years after OSA diagnosis. Of these patients, 12,516 have fully recorded sleep study data in addition to EMR variables such as age at study and most recent BMI. We applied several clustering methods including to identify natural sub-phenotypes of OSA and assessed cluster quality. We also applied techniques which allow a single patient to belong to multiple clusters in various degrees. After selecting final clusters, we plan to analyze the associations between OSA sub-phenotypes and risks using statistical tools like logistic regression and Cox proportional hazards regression, with and without adjusting for factors such as age, gender, and certain medications. RESULTS/ANTICIPATED RESULTS: Preliminary clustering with primarily sleep study data has shown overlap with literature-described patient clusters, including a severe, high non-REM stage 1 sleep, high BMI cluster and a high

https://doi.org/10.1017/cts.2023.324 Published online by Cambridge University Press

nocturnal limb movement cluster. As we incorporate more EMR variables, we will select a final set of OSA sub-types. We anticipate patients in different clusters to have different risks of various adverse OSA-associated outcomes that are tracked in our EMR data. Notable outcomes with sufficient incidence rates (>3%) after OSA diagnosis include essential hypertension (43.4%), hyperlipidemia (28.8%), type 2 diabetes (21.9%), anxiety disorder (19.2%), coronary atherosclerosis (14.9%), cerebrovascular disease (7.7%), and pulmonary heart disease (5.9%). DISCUSSION/SIGNIFICANCE: If our results match anticipations, we will show how EMR data can be used to define OSA sub-phenotypes and predict patient risks of various OSA-associated outcomes. This analysis enables work in personalized risk and treatment predictions for OSA patients. By better understanding these risks, providers can better tailor treatments to patients.

266

Decoding the role of polyamine metabolism on anti-tumor immunity in head and neck cancer

Richard Alexander Harbison[1], William Andrews[2], Michael Mikula[1], Aleksandra Ogurtsova[1], Liz Engle[1], Ogechi Nwankwoala[1], Reagan Willis[1], Pritam Sadhukhan[1], Mark Burns[3], Drew Pardoll[1], Tanguy Seiwert[1], Carole Fakhry[1], Robert A. Casero[1], Elana Fertig[1], Erika Pearce[1]
[1]Johns Hopkins University [2]University of Maryland [3]Aminex Therapeutics

OBJECTIVES/GOALS: The effect of immunosuppressive metabolites on anti-tumor immunity in human papillomavirus (HPV)-associated vs carcinogen-driven head and neck cancer is unknown. The objective of this study is to define the extent to which metabolites impair this response and identify novel metabolic targets for enhancing anti-tumor immunity. METHODS/STUDY POPULATION: HPV-associated and carcinogen-driven head and neck squamous cell carcinoma specimens were frozen following surgical excision, and tumor sections were cut onto glass slides. Slides were coated in alpha-cyano-4-hydroxy-cinnamic acid (CHCA) matrix and subjected to mass spectrometry imaging using matrix-assisted laser desorption ionization (MALDI) on a Bruker SolariX XR 12T Hybrid QqFT-ICR mass spectrometer run in positive mode. Slides were then stained for immunohistochemistry (IHC) using markers of CD8 T cells, macrophages (CD163), B cells (CD20), and tumor cells (panCK). Mass spectrometry imaging and IHC spatially resolved data will be co-registered and metabolite intensity in regions of interest (cell types) quantified. RESULTS/ANTICIPATED RESULTS: A total of seven HPV-associated (three metastatic lymph nodes and four primary tumors) and six carcinogen-driven (primary tumors) HNSC specimens were subjected to MALDI and IHC. Metabolites significantly enriched in HPV-associated HNSC relative to carcinogen-driven HNSC include 2,3-diphosphoglyceric acid, xanthine, 2,3,5-Trichloromaleylacetate, and indole-3-carboxyaldehyde. Metabolites significantly enriched in carcinogen-driven HNSC relative to HPV-associated HNSC include hesperetin 3'-O-sulfate, hypoxanthine, phosphorylcholine, and L-homocysteine sulfonic acid. In ongoing analyses, we anticipate identifying a relationship between CD8+ T cell enriched vs depleted regions and immunosuppressive metabolites (e.g., kynurenine, adenosine monophosphate). DISCUSSION/SIGNIFICANCE: Defining the extent to which CD8+ T cells interact with the metabolic milieu of the microenvironment will provide a foundation for metabolic Precision Medicine. Strategically targeting metabolic pathways to enhance the anti-tumor immune response will be leveraged for the design and implementation of immune modulatory metabolic therapy.

267

Defining the single-cell transcriptomes of splenic adaptive Natural Killer cells in donors with latent human cytomegalovirus infection

Mohamed Khalil, Scott Terhune, Subramaniam Malarkannan
Medical College of Wisconsin

OBJECTIVES/GOALS: The primary objective of this study was to define the transcriptomes and transcriptional regulatory network required for the development and function of adaptive Natural Killer (NK) cells in donors with latent human cytomegalovirus (HCMV) infection. METHODS/STUDY POPULATION: Eight healthy adult human spleens were obtained from four HCMV seropositive and four HCMV seronegative donors. Spleens were provided by the Versiti Organ Donor Center of Wisconsin and were processed to a single cell suspension. CD7+ CD3E- CD14- CD19- CD20- NK cells were isolated, using the BD FACSAria sorter. Following cell sorting, single-cell RNA sequencing (scRNA-seq) was performed, and cDNA libraries were constructed and sequenced via NextSeq 550. Cell Ranger was then used to algin the cDNA reads and the Seurat R package was used to analyze the transcriptional data. Cells were filtered and clustered based on the number of uniquely expressed genes. The monocle software was used for single cell trajectory analysis and the SCENIC software was used to decipher gene regulatory networks. RESULTS/ANTICIPATED RESULTS: Eight healthy spleens from four HCMV seropositive and four HCMV seronegative donors were obtained and their NK cells were sorted and captured for scRNA-seq. Donor median age was 59 [IQR 48.5-56.5], 50% (n=4) were female and all donors were not experiencing any acute or chronic symptoms. Using scRNA-seq, we observed elevated numbers of NKG2C+ adaptive NK cells in HCMV seropositive individuals when compared to HCMV seronegative individuals. In addition, we identify a set of transcription markers and regulators that are responsible for the development and function of adaptive NKG2C+ NK cells. Finally, our trajectory analysis of adaptive NKG2C+ NK cells revealed a unique developmental pathway. DISCUSSION/SIGNIFICANCE: Here, we demonstrate that HCMV infection can induce the formation of adaptive NKG2C+ NK cells that display a unique transcriptional and developmental profile. These findings have the potential to influence the future application of adaptive NK cells in cellular immunotherapies.

268

DEGAS: Deep transfer learning reveals cancer-like transcriptional signatures in histologically normal prostate tissue and adjacent-normal tissues in pancreatic cancer

Justin Couetil[1], Ziyu Liu[2], Jie Zhang[1], Kun Huang[3], Travis Johnson[3]
[1]IUSM, Medical and Molecular Genetics [2]Purdue University, Statistics [3]IUSM, Biostatistics and Health Data Sciences

OBJECTIVES/GOALS: Single-cell and spatial transcriptomics have revealed high heterogeneity in the tumor and microenvironment. Identifying populations of cells that impact a patient's prognosis is an important research goal, so researchers can generate hypotheses and clinicians can provide targeted treatment. METHODS/STUDY POPULATION: DEGAS uses deep-transfer-learning to identify

https://doi.org/10.1017/cts.2023.324 Published online by Cambridge University Press