Characterizing the hAβ^{SAA} Alzheimer's Model Using Spatial Whole Transcriptome and Proteomic Analysis

Adrian L. Oblak[1], Christoph Preuss[2], Dylan Garceau[3], Alison VanSchoiack[3], Marty Ross[3], Kathy Ton[3], Alyssa Rosenbloom[3], Bridget R. Kulasekara[3], Daniel R. Zollinger[3], Steve Lianoglou[4], Dan Xia [4], Gil Di Paolo [4], Pascal Sanchez [4],Joseph M. Beechem[3], Michael Sasner[2], Gregory W. Carter[2]

1. Indiana University School of Medicine, Indianapolis, IN. USA. 2. The Jackson Lab Bar Harbor, ME, USA. 3. NanoString Technologies, Inc., Seattle, WA, USA. 4. Denali Therapeutics, Inc., South San Francisco, CA, USA.

NanoString Technologies
530 Fairview Avenue North, Seattle, WA 98109

Background and Study Design

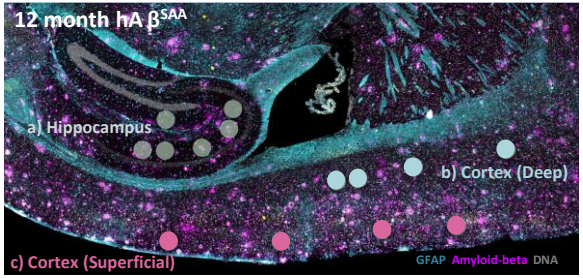
- Alzheimer's Disease (AD) is a debilitating neurodegenerative disorder affecting nearly 50 million patients worldwide, with no effective treatment currently available.
- The Model Organism Development and Evaluation for Late-onset AD consortium (MODEL-AD) is charged with creating, defining, and distributing novel mouse models of late-onset AD carrying human-relevant risk factors for broad use.
- NanoString's GeoMx® Mouse Whole Transcriptome Atlas (muWTA) is used here to map the relationship between AD pathology and gene expression in a novel knockin AD model (hAβ^{SAA}).
- Spatial profiling provides a means for measuring all gene activity in tissue sections, across brain regions and ages, and to map where disease-related changes in expression occur.

GeoMx Workflow

Region of Interest Selection

hAβ^{SAA} Mouse Alzheimer's disease model

The hAβ^{SAA} model is a newly created knock-in mouse model of AD. It carries three humanizing mutations in the Aβ region of the mouse App locus, as well as the Swedish, Arctic and Austrian AD-related mutations (Xia et al, biorxiv doi.org/10.1101/2021.01.19.426731). Amyloid pathology is detected at 4 months of age with cortex and hippocampus having the most pronounced pathology.



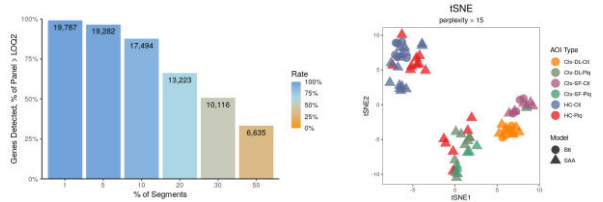
muWTA

- In 12 month hAβ^{SAA} mice (n=3), 200um diameter geometric ROIs were placed on amyloid-beta plaques or control areas with low/no plaques.
- ROIs were placed in a) Hippocampus, b) Cortex (Superficial), or c) Cortex (Deep).
- Control ROIs were collected in age matched C57BL/6 mice (n=2) in all three brain regions.

Protein

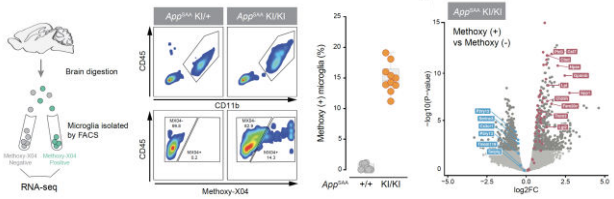
- Amyloid-beta plaques and non-plaque control regions in hippocampus and cortex (layers not distinguished) were profiled at both early (4 month) and late (10/12 month) timepoints in hAβ^{SAA} mice.
- Control regions were profiled in age matched C57BL/6 mice (n=3 for each genotype/timepoint).

muWTA distinguishes both regional and plaque associated gene expression signatures



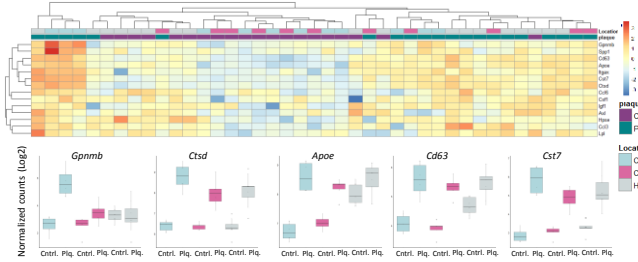
Left: Total genes detected by muWTA panel across all ROIs profiled across all mice included in this study. Right: tSNE plot of all ROIs reveals distinct clustering by anatomical region and ROI pathology.

Transcriptional profiling of FACS-sorted plaque-associated microglia



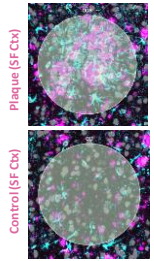
Transcriptional profiling of isolated plaque-associated microglia identified increased expression of disease associated microglia (DAM) genes and genes involved in innate immunity, and lipid clearance and metabolism (relative to non-plaque associated microglia) (Xia et al, biorxiv doi.org/10.1101/2021.01.19.426731).

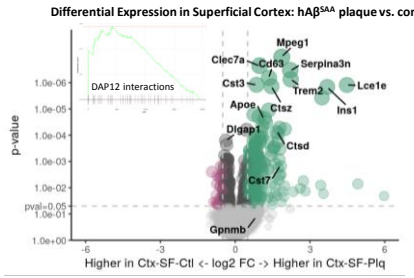
GeoMx *in situ* spatial transcriptional profiling



GeoMx muWTA profiling confirms upregulation of DAM genes around plaques in hAβ^{SAA} and provides spatial context. Top: Heatmap of DAM genes upregulated as detected by FACS-sorted RNA-seq confirms plaque associated expression but highlights heterogeneity of expression across plaques and anatomical location. Bottom: Box plots demonstrate heterogeneous expression of DAM genes by anatomical location and ROI pathology. Expression of DAM genes is generally higher in deeper cortical layers compared to hippocampus or superficial cortex.

Differential expression and GSEA confirms region specific AD pathology in hAβ^{SAA} mice





Differential expression and GSEA between plaque and control regions highlight genes/pathways known to be enriched in AD patients and other AD model systems (i.e. Trem2 & Apoe) and provides spatial context. Upregulation of top GSEA enriched pathway in plaque ROIs (DAP12 interactions) in humans is an inflammatory hallmark of AD.

Spatial protein analysis provides confirmatory and complimentary model characterization

Spatial profiling of 65+ targets with GeoMx mouse Neuroscience protein panel identifies timepoint and region specific changes in DAM and AD associated proteins/phospho-proteins in amyloid-beta plaques in hAβ^{SAA} mice.

Conclusions

- Spatial profiling with GeoMx muWTA generates a highly sensitive transcriptional readout with over 17,000 genes detected in at least 10% of all ROIs profiled.
- GeoMx confirms upregulation of DAM genes localized to plaques in hAβ^{SAA} mice as identified by RNA-seq
- Both transcriptomic and proteomic spatial analysis identifies regional and plaque associated genes and proteins.