

Building the Neuro Myeloid Cell Atlas: From the periphery to the brain

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Abstract

Background:

Myeloid populations within the brain have been implicated in various neurodegenerative diseases (NDDs). Neurodegeneration/inflammation leads to an increase in the diversity of macrophage and microglia subpopulations. The functional role of the various myeloid sub-populations in disease have not been fully clarified. Furthermore, it remains unresolved how these populations correlate with populations in the periphery.

Methods:

To address the question of how myeloid populations in neurodegeneration correlate with those found in peripheral inflammatory conditions, we integrated 19 high-quality, single-cell RNA-Seq datasets from different NDDs to create a Neuro myeloid cell atlas (NeuroMCA) composed of 3.8M cells from 315 human donors. We then cross correlated this with our peripheral macrophage cell atlas (MCA) encompassing conditions of fibrosis and inflammation from across peripheral tissue compartments. To annotate functional contributions we leveraged monocyte derived macrophages and performed genetic perturbation of defining gene features.

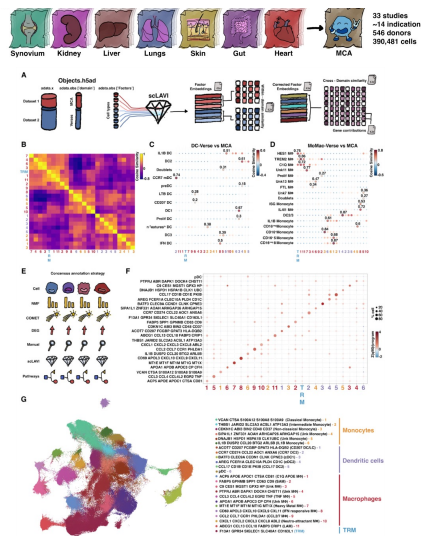
Results:

Pathogenic populations in neurodegeneration were found to strongly correlate with pathogenic peripheral populations/subsets. Staining for Scar Associated Macrophages (SAM)(FABP5, GPNMB, CD63, SPP1 and CD9) and areas of brain pathology were found to overlap, with an additional association of collagen deposition. Genetic knockout of receptors associated with pathogenic macrophage markers demonstrated reduced amyloid uptake with knockout of GPNMB or Trem2, but no change in uptake with CD9 or CD63 knockout.

Conclusions:

We demonstrate changes in the myeloid compartment in the brain potentially emerge from peripheral populations over time. These populations were found to overlap between macrophage populations associated with populations associated with inflammation and fibrosis, highlighting the need for a new approach to addressing macrophage biology in neurodegeneration.

Introduction



We have established a comprehensive multi-modal discovery platform named Mapping Associated Cell Subsets (M.A.C.S.). This platform integrates advanced computational workflows with a newly developed Myeloid Cell Atlas (MCA), designed to preserve critical phenotypic, tissue-specific, and functional gene signatures (tiers 1, 2, and 3) by deploying a frequency-weighted union approach for variable gene selection to retain tissue-specific programs. The MCA contains 498,642 myeloid cells from 33 studies and 546 donors that cover 14 indications and 7 tissue compartments. Altogether, we identified 24 unique myeloid subsets using the consensus of differential expression (DEG), Combinatorial Marker detection from single-cell Transcriptomics (COMET), consensus Non-negative Matrix Factorization (cNMF) and pathway enrichment analysis. We then built an AI/ML tool called scLAVI (single cell Latent Approximate Variational Inference) to enable rapid and robust cell type alignment between datasets.

Methods



We have integrated 19 high-quality, single-cell RNA-Seq datasets from different NDDs to then create a Neuro myeloid cell atlas (NeuroMCA) composed of 3.8M cells from 315 human donors. We subsequently cross correlate the NeuroMCA to the MCA to identify overlapping myeloid populations. We subsequently validate identified populations through tissue staining of cell markers using 7 healthy donors and 8 patients diagnosed with Alzheimer's disease.



All procedures performed on subjects were in accordance with regulations and established guidelines, and were reviewed and approved by an Institutional Animal Care and Use Committee.

Statements

Results

1. The neuro myeloid cell atlas defines the myeloid landscape in neurodegeneration and identifies correlations with MCA

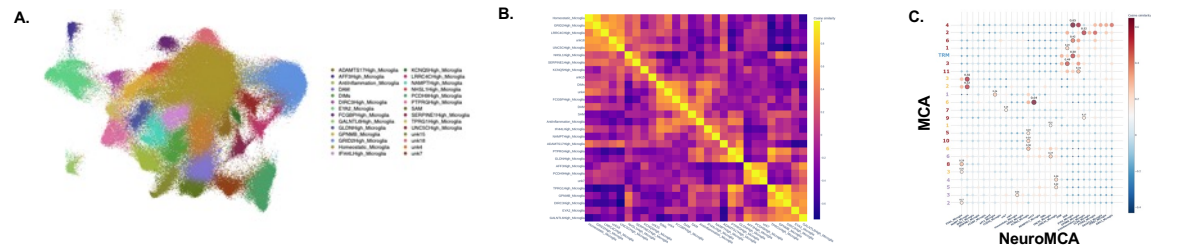


Figure 1: A. UMAP visualization of neuroMCA comprised of 348,548 microglia cells from 172 donors (80 Control, 67 AD, 26 PD) across 13 studies, with 28 microglia subtypes. B. Heatmap of the internal cosine similarity between the 24 subsets identified in the neuroMCA. C. Dot plot of the cosine similarity between the neuroMCA and MCA. Outline circles represent significant p-value, bold number shows cosine similarity value

2. Pathogenic macrophage populations are increased in neurodegeneration

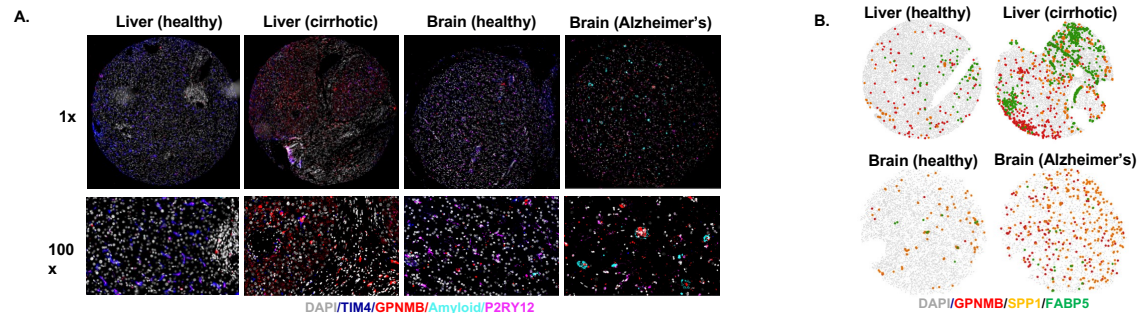


Figure 2: A. Tissue microarray staining of TIM4 (blue), P2RY12 (pink), GPNMB (red), Amyloid Aβ42 (teal) of healthy and diseased tissue. Serial staining was performed followed image registration through scale invariant feature transform (SIFT) and then subsequent image transformation via MUMPS. The aligned images were then fully merged into a single multi-round one.tif file for analyses. Representative images are shown at 1x and 100x magnification. B. ProteoMAP visualization extrapolated from IF images showing total cells (grey), SPP1 (green), FABP5 (yellow) and GPNMB (red) macrophages in healthy and diseased tissues

3. Infiltrating pathogenic macrophage populations are associated with amyloid

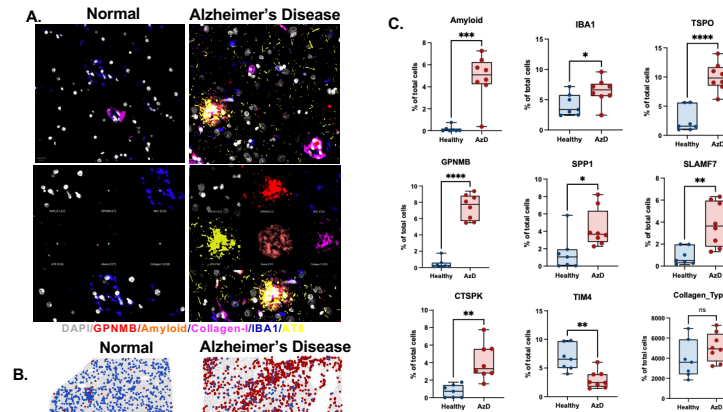


Figure 3: A. Representative IF images following ProteoMAP workflow for image alignment. Staining showing GPNMB (red), IBA1 (blue), Amyloid Aβ42 (teal), Collagen1 (pink), Amyloid Aβ42 (orange), and p-Tau (yellow) in healthy and diseased tissues. Scale bar: 50 μm. B. ProteoMAP visualization of IF images showing total cells (grey), TIM4 (blue) and GPNMB (red) macrophages in healthy and diseased brain sections. C. Quantification of cell associated staining as quantified following proteoMap image alignment followed by image thresholding and quantification in Omics. Welch's t-test with, *p<0.05, **p<0.01 and ***p<0.001.

4. MDMs upregulate GPNMB in response to amyloid

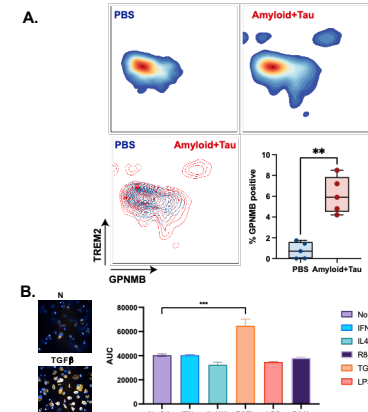


Figure 4: A. Contour density plot of monocyte derived macrophages stimulated by PBS or the combination of amyloid and tau. B. Uptake of lyso-labeled amyloid beta by GM-CSF matured macrophages. Uptake was quantified using imageJ software. One-way ANOVA was used for statistical testing *p<0.05, **p<0.01 and ***p<0.001.

Conclusions

1. The Neuro Myeloid Cell Atlas identified significant myeloid heterogeneity arising during Alzheimer's disease

2. Correlations between the neuro MCA with the peripheral Myeloid Cell Atlas (MCA) demonstrate significant correlation between macrophage populations in neurodegeneration and peripheral inflammation and fibrosis

3. Staining demonstrates populations of SAM (FABP5, GPNMB, SPP1, CD63, TREM2) macrophages associating with regions of amyloid burden

4. Functional assays demonstrate upregulation of GPNMB in GM-CSF matured macrophage with amyloid treatment, and increased amyloid uptake in conditions previously demonstrated to result in increased GPNMB expression

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