Characterizing immune cell populations in the normal canine brain

Daniela A. Jimenez[1,2], Ryan G. Toedebusch[3,4], and Christine M. Toedebusch[3,4]

[1]Veterinary Scientist Training Program, [2]Students Training in Advanced Research Program
[3]Department of Surgical & Radiological Sciences, [4]UC Davis Comprehensive Cancer Center
School of Veterinary Medicine, University of California Davis, CA

Introduction

- **Canine glioma is a fatal primary brain tumor**
  - Second most common primary brain tumor
  - Median age at diagnosis: 7-8 years
  - Median survival time: 9-14 months
- **Glioma associated microglia/macrophages (GAMs)** are actively recruited to the tumor microenvironment
  - Largest population of tumor infiltrating cells
  - 30-50% of tumor mass

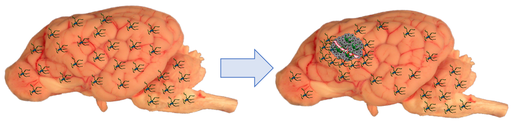

Figure 1: Schematic depicting resident microglia, the immune cells of the brain, in the normal canine brain and their recruitment into the tumor microenvironment.

Degree of GAM invasion ∝ tumor grade

- **Multiple immune cells infiltrate canine glioma:**
  - Microglia, macrophages
  - Lymphocytes: T cells, T regulatory cells, B cells

The unmet needs:

- Immune cell function in glioma is unclear
- Immune cell 1) composition and 2) function in normal canine brain is unclear

The immune cell signature in normal canine brain must be defined to begin to understand changes occurring with brain pathology

Hypothesis

Single cell RNA sequencing (scRNA-seq) will define distinct mononuclear cell populations and corresponding transcriptomic profiles <u>across different brain regions</u> in the <u>normal</u> canine brain.

Specific Aims

- **Aim 1A**: Define the immune composition across four distinct canine brain regions.
- **Aim 1B**: Characterize the transcriptome of mononuclear cell subpopulations in canine brain.

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Pilot Study Design

- Archived mononuclear cells from two normal dog brains:
  - Canine 1 - “Cooper” MC Lab Mix ~8yrs
  - Canine 2 - “Gemma” FS Chihuahua ~7yrs
- Cells sorted using Fluorescence Activated Cell Sorting (FACS) – to enrich live cell proportion
- Four brain regions per dog:
  - Left Frontal Cortex
  - Left Temporal Cortex
  - Left Mesencephalon
  - Left Occipital Cortex

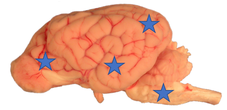

Figure 2: Brain regions submitted for scRNA seq.

Methods

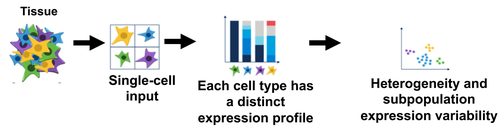

Figure 3: Single cell RNA sequencing analysis can reveal rare subpopulations. Figure adapted from 10xGenomics website.

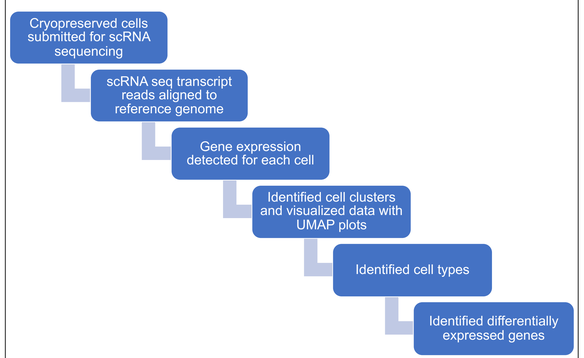

Figure 4: Sequencing and Bioinformatics Workflow

Single cell RNA sequencing is feasible on cryopreserved brain mononuclear cells

Patient ID	Signalment	Brain Region	Estimated number of cells	Mean reads per cell	Median genes per cell	%Reads mapped to genome	%Reads mapped to transcriptome
719245	7yr FS Chihuahua	Left Frontal Cortex	1370	153,631	1188	90.1	54.8
719245	7yr FS Chihuahua	Left Temporal Cortex	2110	123123	1251	91.7	58.4
719245	7yr FS Chihuahua	Left Mesencephalon	1797	174850	1153	88.6	50.6
719245	7yr FS Chihuahua	Left Occipital Cortex	2523	203946	1250	91.3	53.6
702942	8yr MC Labrador Mix	Left Frontal Cortex	6155	106945	1579	93.9	57.4
702942	8yr MC Labrador Mix	Left Temporal Cortex	6912	68422	1294	95.4	61.1
702942	8yr MC Labrador Mix	Left Mesencephalon	2598	128755	1248	94.4	60.7
702942	8yr MC Labrador Mix	Left Occipital Cortex	2753	103058	1102	93.5	60.1
Average +/- SEM			3277.25 +/- 731.79	132841.25 +/- 15279.06	1258.125 +/- 50.85	92.3625 +/- 0.82	57.0875+/- 1.33

Table 1: Results from single cell sequencing experiment.

Canine brain has distinct immune cell populations

- There are similar cell types between different brain regions within the same dog.

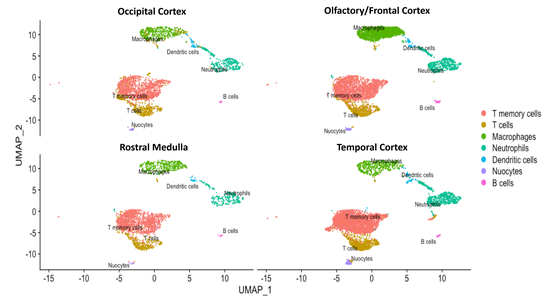

Figure 5: Uniform Manifold Approximation and Projection (UMAP) displaying cell types present within the four brain regions of Canine 1. Similar cell types are present across brain regions.

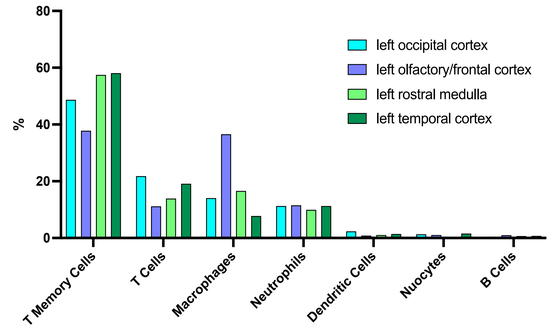

Figure 6: Proportion of cell types across brain regions in Canine 1.

Cell populations within the same brain region are similar between dogs

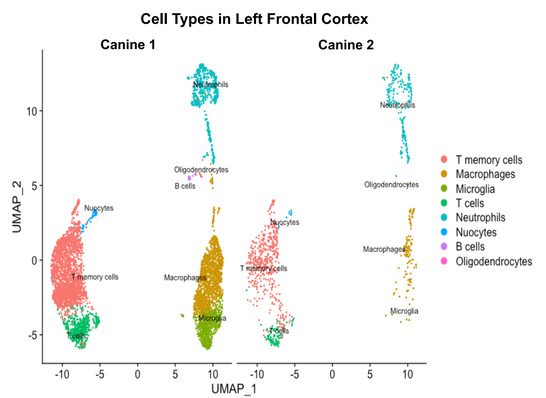

Figure 7: UMAP displaying cell types present within the left frontal cortex of Canine 1 and 2. Similar cell types are present between the two dogs.

Proportion of cell types differ between dogs within the same brain region.

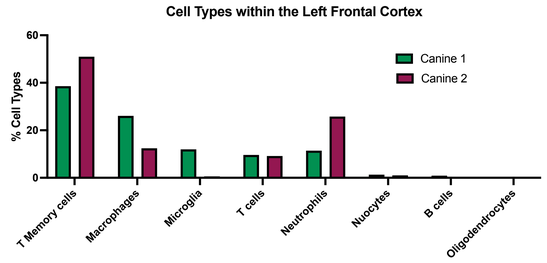

Figure 8: Proportion of cell types within the same brain region of different dogs.

Canonical microglia markers are expressed within the macrophage/microglia populations identified.

- Macrophages/Microglia have similar expression profiles.

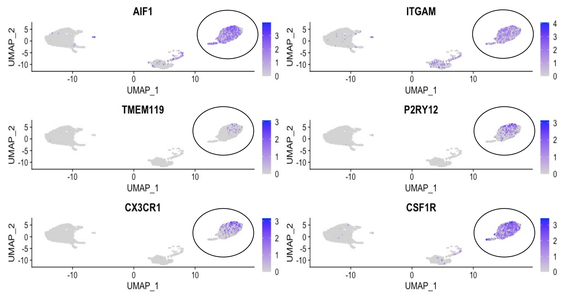

Figure 9: Uniform Manifold Approximation and Projection (UMAP) displaying expression levels of canonical microglia markers (top 6 markers from PanglaoDB – human markers).

Conclusions

- Unexpected lack of myeloid cells suggests our cryopreservation method likely not microglia/macrophage friendly.
  - However, clinical cases randomly arrive – cannot avoid archiving samples.
- There are distinct cell populations within the normal canine brain.
- There are functional differences between cell types (to be further explored).

Future Directions

- Future efforts will work to:
  - Refine secondary analysis – continue to explore changes across brain regions and between dogs.
  - Identify differentially expressed genes within the clusters identified.
  - Submit additional samples for scRNA sequencing – including brain tumor patients to see how the immune cell transcriptome changes with pathology