Spatial transcriptomic analysis of canine metastatic melanoma: Defining RNA signatures of primary tumors and the brain microenvironment

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Introduction

- **Features of canine malignant melanoma (MM)**
  - Accounts for 7% of all canine cancers [1]
  - 38% of dogs will develop brain metastasis [2]
  - Brain metastasis associated with morbidity and mortality
    - Median survival time post neurological signs: 9.5 days [2]
- **Microglia may be permissive to brain metastasis**
  - Heterogeneously infiltrate brain metastases [3]

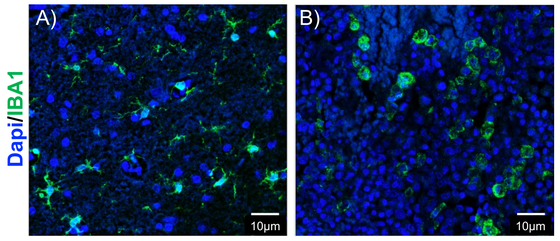


Figure 1: Microglia, identified by positive immunoreactivity to IBA1, within **(A)** normal brain and **(B)** melanoma brain metastasis. Representative image of high-density IBA1+ cells with an ameboid morphology infiltrating the tumor. [3]

What is the role of microglia in MM brain metastasis?

Hypothesis

The brain microenvironment and primary tumor transcriptomic signatures will be distinct between groups, correlating with the presence or absence of central nervous system (CNS) metastasis in canine metastatic melanoma.

Aim #1

Identify the transcriptomic differences of **cell types within the brain microenvironment** of dogs with MM in following groups: 1) CNS metastasis, 2) Non-CNS metastasis, and 3) Normal brain.

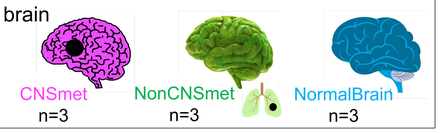
Aim #2

Identify transcriptomic differences of the **primary tumor cells and the microenvironment** in MM between dogs with and without CNS metastasis.

Methods: Aim #1 Case Selection

Inclusion criteria:
- Histopathological diagnosis of metastatic oral melanoma
- Histopathological evaluation of brain
- Tissue availability

Exclusion criteria:
- Coexisting metastatic cancer
- Additional brain disease



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- Toedebusch Lab: C. Toedebusch, R. Toedebusch, N. Wei, F. Catacci, D. Jimenez, J. Furth-Jacobus, M. Gragg, T. Pechnikova

References

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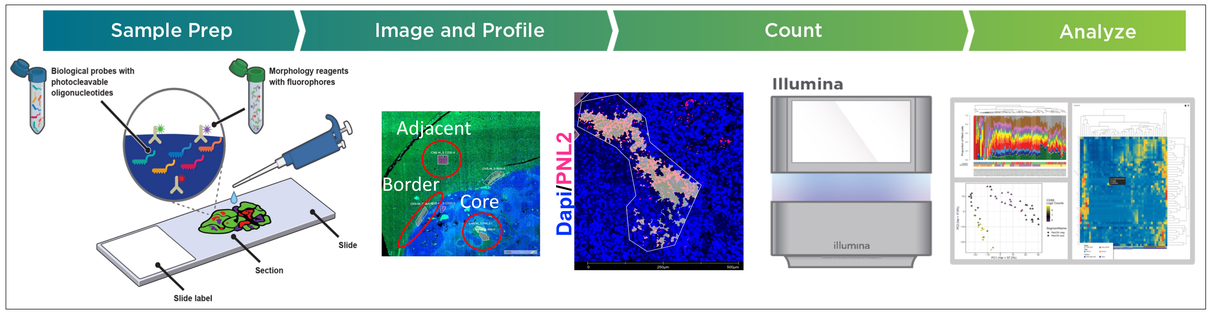
Methods: Spatial Transcriptomics



Conclusions

- Gene expression is **distinct between patient groups**
- Microglia in brain metastases have a **pro-tumorigenic signature**
  - Role of microglia in dogs without brain metastasis is unclear
- Microglia along the border of brain metastases may play a **role in recruitment**
- Ongoing work: investigate DEGs, evaluate endothelial cells and primary tumor characteristics

Cell types from CNS metastasis samples segregate from Normal Brain and Non-CNS metastasis samples

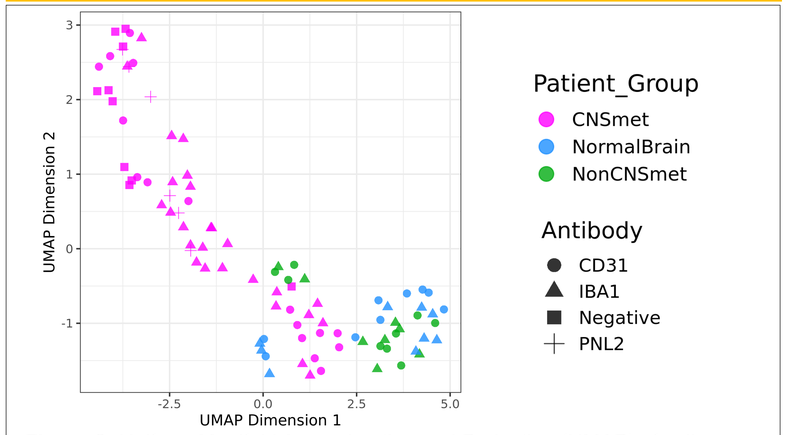


Figure 2: Uniform Manifold Approximation and Projection (UMAP) of cell types in the brain microenvironment (microglia, IBA1; endothelial cells, CD31; melanocytes, PNL2) across groups. Gene signatures of cells within CNS metastases are widely variable, while gene signatures of cells within normal brain and non-CNS metastases cluster together.

Microglia in dogs with CNS metastasis express pro-tumorigenic genes

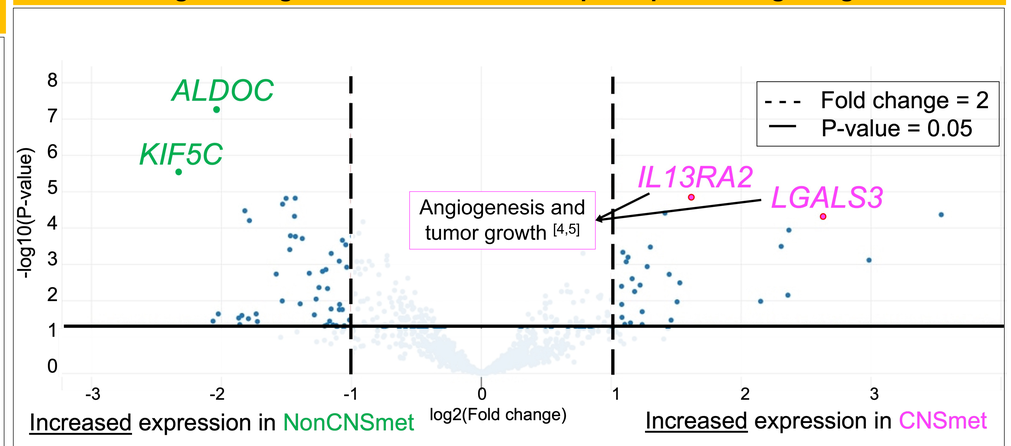


Figure 3: Volcano plot of differentially expressed genes (DEGs) of microglia in CNS metastases and non-CNS metastases. *ALDOC* and *KIF5C* expression is increased in microglia in non-CNS metastases, while *IL13RA2* and *LGALS3* expression is increased in microglia in CNS metastases.

Microglia in the core and border of brain metastases segregate from peri-tumoral microglia

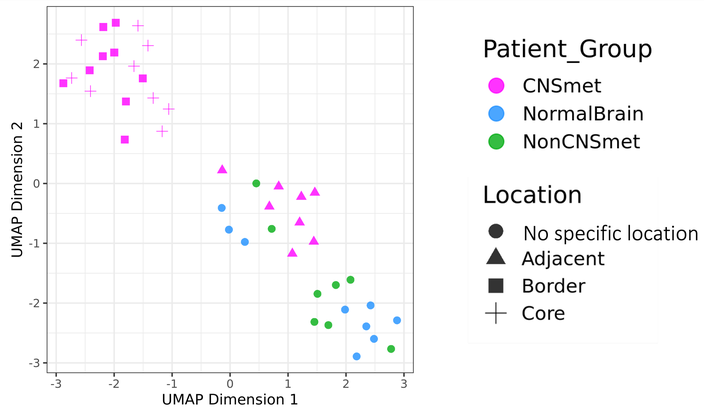


Figure 4: UMAP of microglia across groups. Microglia in dogs with CNS metastasis were evaluated in the tumor core, tumor border, and tissue adjacent to tumor separately. Microglia signature within the core and border of the tumor cluster together and separately from microglia in normal brain and non-CNS metastases.

Microglia in the core and border of brain metastases *may* have distinct functions

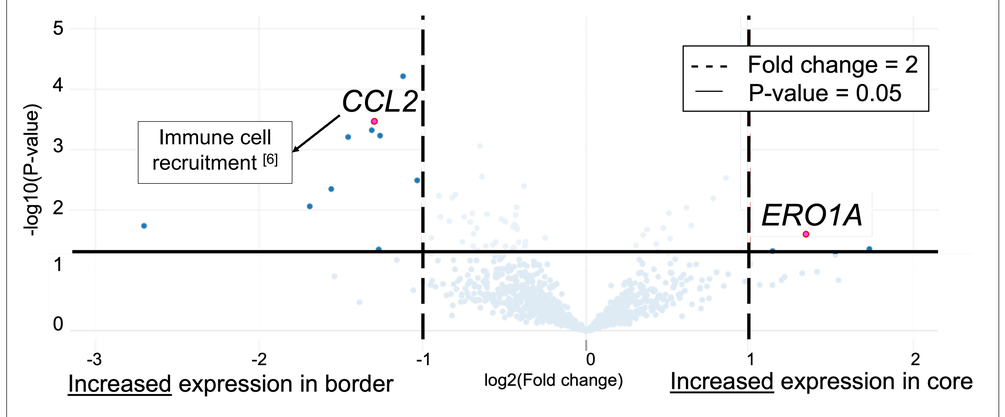


Figure 5: Volcano plot of DEGs of microglia in the border and core of brain metastatic melanoma foci. *CCL2* expression is increased in microglia along the border of the brain metastases, while *ERO1A* expression is increased in microglia in the tumor core.