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

Kulani T. Simafranca-Narte, Ryan G. Toedebusch, Christine M. Toedebusch
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Features of Canine Malignant Melanoma

• Malignant melanoma (MM) accounts for 7% of all canine cancers

• Oral cavity is the most common primary tumor site – also the most deadly

• 38% of MM will have central nervous system (CNS) metastasis

Simpson et al., *Pigment Cell Melanoma Research*, 2014

Razmara et al., *Frontiers in Oncology*, 2022
Presence of clinical brain disease accelerates euthanasia

Median survival time (MST) = 9.5 days

Razmara et al., Frontiers in Oncology, 2022
Microglia have been implicated as being permissive to brain metastasis

Conales et al., unpublished observations, STAR 2021
**Hypothesis:** The brain microenvironment and primary tumor transcriptomic signatures will be distinct between groups, correlating with the presence or absence of brain metastasis in canine metastatic melanoma.

- **Aim #1:** Identify the transcriptomic differences of cells within the brain microenvironment in the following canine groups: Malignant melanoma brain 1) with and 2) without brain metastasis, and 3) Normal brain.
- **Aim #2:** Identify transcriptomic differences of the primary tumor microenvironment in canine malignant melanoma between dogs with and without brain metastasis.
Study Design: Case Selection

Inclusion criteria:
- Histopathological diagnosis from a board-certified pathologist of oral melanoma that has metastasized to at least one organ
- Brain histopathological evaluation
- Tissue availability of brain and primary oral melanoma tumor

Exclusion criteria:
- Coexisting metastatic cancer
- Additional brain disease
Study Design: Spatial Transcriptomics

GeoMx Canine Cancer Atlas Panel (1,963 genes)

Microglia = IBA1
Endothelial cells = CD31
Melanocytes = PNL2
Data: Quality Control, Filter, Normalization

Segment QC
- Raw reads < 1000
  - Sequencing failed (ex. pipetting error during library prep)
- Percent aligned reads < 80%
  - Contaminated or low quality
- Sequencing saturation < 50%
  - Depth of sequencing not sufficient to capture low expressing unique targets
- *Negative probe count < 10
  - Background noise could not be estimated
- No template control count > 1000
  - Contamination during PCR
- Minimum nuclei count < 50
- *Minimum surface area < 16,000 um^2

Biological Probe QC
- Probes in all segments/Probes within target < 0.1
  - Excludes probes performing poorly relative to other probes for same target
- Fails Grubbs outlier test in > 20% of segments
  - Excludes probes that are consistent outliers
- Calculate limit of quantitation (LOQ) = 2 SD above mean of negative probes
  - Determines confidence threshold of probe expression
- Results: 2010 total probes = 1997 passed, 13 local outliers

Filter
- By ROI - exclude if expression < LOQ or frequency < 5%
  - Results: 93/100 ROIs passed = 93% of ROIs expressed at least 5% of panel genes
  - Removed all PNL2 ROIs in one case
- By target gene – exclude if expression < LOQ or frequency < 5%
  - Results: 1118/1963 genes passed = 57% of target genes from panel were detected in at least 5% of ROIs

Q3 Normalization
- Normalized to top 25% expressors to reduce variance of gene expression
CNSmet samples segregate from NormalBrain and NonCNSmet
Microglia in dogs with brain metastasis express pro-tumorigenic genes

- ALDOC
- KIF5C
- IL13RA2
- LGALS3

Increases angiogenesis and tumor growth in melanoma mouse model

Stimulates angiogenesis and tumor growth in human melanoma cell lines

Okamoto et al., Scientific Reports, 2019; Braeuer et al., Cancer Research, 2012
22 unique differentially expressed genes (DEGs) identified in microglia across groups.

CNSmet vs Normal: Total DEGs = 84

CNSmet vs NonCNSmet: Total DEGs = 75

NonCNSmet vs Normal: Total DEGs = 2

52 shared

1 shared

Unique DEGs = 22
Are there region-specific gene signatures of microglia within the brain tumor?
Microglia in the core and border of the brain metastasis segregate from peri-tumoral microglia

**Patient_Group**
- **CNSmet**
- **NormalBrain**
- **NonCNSmet**

**Location**
- **No specific location**
- **Adjacent**
- **Border**
- **Core**
Border and core microglia may have distinct functions

Increased expression in border

- log10(P-value)

-3 -2 -1 0 1 2 3 4 5

log2(Fold change)

Increased expression in core

- log10(P-value)

-3 -2 -1 0 1 2 3 4 5

log2(Fold change)

CCL2

Immune cell recruitment, BBB microvessel leakage

ERO1A

Implicated in metastasis and immune cell escape (e.g., PDL1)

- - - Fold change = 2
- - P-value = 0.05

Errede et al., Fluids and Barriers of the CNS, 2022; Johnson et al., Journal of Cancer Immunology, 2020
Summary and Conclusion

- Gene expression is distinct between patient groups

- Microglia function in CNSmets have pro-tumorigenic signature
  - Role of microglia in NonCNSmets is unclear

- Microglia signature in brain metastases are distinct between location
  - Microglia along the border play a role in recruitment
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