A Comparative Analysis of the Molecular Characteristics of Canine and Human Gliomas

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• Glioma is a common type of tumor originating in the brain. About 33% of all brain tumors are gliomas
• Most common type of primary malignant brain tumor
• Originate in the glial cells that surround and support neurons in the brain
  • astrocytes (astrocytomas), oligodendrocytes (oligodendrogliomas), (also ependymal cells (ependymomas), but these are a separate class of tumors)
• Gliomas grow within the substance of the brain and often mix with normal brain tissue
• The need for post-operative treatment for a glioma depends on its grade
  • There are four grades of brain tumors
    • grades I or II are "low grade"
    • grades III or IV are “high grade”
    • based on the tumor’s growth potential and aggressiveness
Gliomas

- Separated into types by histology including:
  - astrocytomas - include glioblastoma (GBM),
  - oligodendrogliomas
  - oligoastrocytoma (aka “mixed” or “undefined”)
- High grade tumors have a dismal prognosis; GBM is nearly universally fatal

<table>
<thead>
<tr>
<th>Type of Tumor</th>
<th>5-Year Relative Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-grade (diffuse) astrocytoma</td>
<td>73% 46% 26%</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>58% 29% 15%</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>22% 9% 6%</td>
</tr>
<tr>
<td>Oligodendrogliaoma</td>
<td>90% 82% 69%</td>
</tr>
</tbody>
</table>

ACS survival statistics:
<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Easy control of variables</td>
<td>• Environment and housing not applicable to humans</td>
<td>• Develop spontaneous tumors</td>
<td>• Difficult to control environment and standardize treatment</td>
</tr>
<tr>
<td>• Controlled environment</td>
<td>• Limited options for spontaneous tumors</td>
<td>• Shared environment with humans</td>
<td>• Breed variability</td>
</tr>
<tr>
<td>• Availability of GEM &amp; humanized mice</td>
<td>• PDX mice are immunocompromised</td>
<td>• Outbred and heterogeneous genome comparable to humans</td>
<td>• Limited standardized reagents</td>
</tr>
<tr>
<td></td>
<td>• GEM &amp; humanized mice are expensive</td>
<td>• Immunocompetent</td>
<td>• Heterogeneous tumor biology and immune responses</td>
</tr>
<tr>
<td></td>
<td>• Too small for surgery, blood draw ...</td>
<td>• Heterogeneous tumor biology and immune responses</td>
<td></td>
</tr>
</tbody>
</table>
Canine Gliomas

- Over 50% of all gliomas in dogs occur in brachycephalic breeds
  - eg Boxers, Bulldogs, Boston terriers
- A 2018 publication by the NIH Comparative Brain Tumor Consortium is used to type and grade canine glioma
- Incidence of brain tumors in adult dogs is 2.8% to 4.5% and, although individual studies vary
- Gliomas represent 36% to 70% of primary brain tumors in dogs
- As with people, high grade more common than low grade
- Oligodendrogliomas more common in dogs, but astrocytoma also occurs
- Gaps in knowledge regarding biology and molecular characteristics of glioma in dogs
• How similar are gene expression and mutation profiles between canine and human glioma?

• Identify molecular differences between astrocytomas and oligodendrogliomas

• What genes differ in expression between low and high grade gliomas? How does the tumor microenvironment differ by grade?

• Are any changes observed in immune cell composition between low and high grade gliomas? Between astrocytomas and oligodendrogliomas?
Datasets Used in this Study

1. Amin et al., Cancer Cell. 2020 37, 243–257.

12 true normal samples
3 NAT
83 canine gliomas
   • 46 oligodendroglioma
   • 31 astrocytoma
   • 6 undefined glioma

464 true normal samples - GTEx
1,115 adult gliomas
   • 190 oligodendroglioma
   • 793 astrocytoma
   • 131 mixed glioma

15 NAT samples
530 pediatric gliomas
   • 1,260 oligodendroglioma
   • 18,009 astrocytoma
   • 463 brainstem glioma (DIPG)

Key pathways are mutated in GBM

- PI3K, TP53, and Rb pathways are commonly dysregulated in high grade gliomas

- p53 plays critical roles in tumor prevention

- TP53 is one of the most commonly dysregulated genes in cancer

- Rb dysregulation has been shown to be critical in the transformation of low grade to high grade tumors
Genes in key pathways are altered
Genes in key pathways are altered

- 8% of samples have a TP53 pathway mutation
- 24% of samples have a PI3K pathway mutation
- 48% of samples have a RTK-RAS pathway mutation
- high grade gliomas harbor more mutations in known oncogenic pathways
Genes in key pathways are altered

In adult glioblastoma data, the most frequent alterations:
- **CDKN2A** gene deletion/mutation
- **CDK4** amplification
- **RB1** mutation/deletion

Red - CNV in > 10% of samples
Purple - CNV in > 5% of samples
Black - CNV in < 5% of samples
Genes in key pathways are altered

- RTK/RAS/PI3K signaling pathway is altered in 65% of samples
- TP53 signaling pathway is altered in 16% of samples
- Rb signaling pathway is altered in 47% of samples
TME in High vs Low Grade

% Expression

- B cells memory
- B cells naive
- Dendritic cells activated
- Dendritic cells resting
- Eosinophils
- Macrophages M0
- Macrophages M1
- Macrophages M2
- Mast cells activated
- Mast cells resting
- Monocytes
- Neutrophils
- NK cells activated
- NK cells resting
- Plasma cells
- T cells CD4 memory activated
- T cells CD4 memory resting
- T cells CD4 naive
- T cells CD8
- T cells follicular helper

Tumor Grade
- Normal
- Low-Grade Glioma
- High-Grade Glioma
• MAPK and PI3K are active in all samples including normal, but are much more enhanced in gliomas

• JNK is active in gliomas, but not active in normal samples

• Significant heterogeneity is seen in immune signatures
  • exemplifies the strength of using the canine model

• Sample-wise gliomas have enhanced immune suppressive signatures
  • especially clear in high grade gliomas
  • only 3 high grade glioma samples have stronger immune enhancing signatures than immune suppressive signatures
Immune modulators are critical for cancer immunotherapy.
Astro vs Oligo DEG

Dysregulated Pathways in Canine Oligodendrogliomas Vs Astrocytomas

- Log_{10} adjusted FDR

Log_2 fold change

total = 16336 variables

Pathway Z-score

- Up-regulated
- Down-regulated
- Not Available
Immune and stromal differences

- Little difference seen between pathologies in terms of immune score, but stromal score showed differences.
- Both immune and stromal scores were significantly different between gliomas of each pathology and normal samples.
Pathology classifier

• An SVM was initially used to subset features that classify canine glioma pathologies
• 109 genes were selected that optimize the classification accuracy rate
• Of these genes, 65 were statistically significantly differentially expressed (at FDR < 0.25)
• These 65 genes were used in a Naïve Bayes classifier using leave-one-out-cross-validation
• 88.6% prediction accuracy

<table>
<thead>
<tr>
<th></th>
<th>Astrocytoma</th>
<th>Oligodendroglia</th>
<th>Accuracy</th>
<th>F1 Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>9</td>
<td>2</td>
<td>81.82</td>
<td>0.8182</td>
</tr>
<tr>
<td>Oligodendroglia</td>
<td>2</td>
<td>22</td>
<td>91.67</td>
<td>0.9167</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td>88.57</td>
<td>0.8675</td>
</tr>
</tbody>
</table>
Consensus Clustering

- Stability was verified by calculating sample co-clustering frequency using iterative hierarchical clustering by applying consensus clustering using the 65 genes included in the NB classifier
  - $k=2$, 1000 bootstraps, 80% subsamples
- Consensus score is denoted ranges from 0 (white, never clustered together) to 1 (dark blue, always clustered together)
- Two distinct clusters containing 90% of the samples with high (>0.8) consensus scores
- Only $\sim 10\%$ of the samples were unstable with low (<0.8) consensus score.
  - Unstable samples = same as those misclassified by the NB classifier
Classifying human glioma samples

- Used the 65 gene canine Naïve Bayes model to classify the human data
- Only 45 genes were ultimately used (not all genes from canine had one-to-one homologs in human)
- Achieves 81.8% prediction accuracy
- F1 score of 0.803

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<tr>
<th>Actual</th>
<th>Predicted</th>
<th>Astrocytoma</th>
<th>Oligodendrogioma</th>
<th>Accuracy</th>
<th>F1 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytoma</td>
<td>295</td>
<td>53</td>
<td></td>
<td>84.77</td>
<td>0.8576</td>
</tr>
<tr>
<td>Oligodendrogioma</td>
<td>45</td>
<td>145</td>
<td></td>
<td>76.32</td>
<td>0.7474</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td>81.78</td>
<td>0.803</td>
</tr>
</tbody>
</table>
Conclusions

- Key canonical pathways that are known to be altered in human gliomas are also altered in canine gliomas.

- The canine tumor microenvironment (TME), like that in humans, appears to be immunosuppressive.

- Gene expression profiles of astrocytomas and oligodendrogliomas show alterations in a number of signaling pathways, including several immune-related and TME-specific pathways.

- A Naïve Bayes classifier was developed that accurately classifies canine glioma pathologies based on gene expression profiles alone.

- The classifier developed based on canine data also classifies human glioma pathologies based on gene expression profiles.
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The families who have donated their beloved pets’ tissue for these studies