Canine Cancer Genomics

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Dogs in Genetics

• 78.2 million dogs owned in U.S.
  – ~35% of 131 million U.S. households have dogs
• Dogs live with and are exposed to the same environment as their human owners
• Dogs receive preventative and diagnostic healthcare
• Dogs live to old age
• Population structure well suited to genetic studies
Cancer is the #1 Cause of Disease Associated Death in Dogs

More than 1 in 4 dogs will develop cancer in their lifetime

Many cancers show strong breed predisposition:

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Breed</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric Carcinoma</td>
<td>Chow Chow</td>
<td>10-20</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>Golden Retriever</td>
<td>12.4</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Boxer</td>
<td>4.5</td>
</tr>
<tr>
<td>Mammary Carcinoma</td>
<td>Doberman Pinscher</td>
<td>3.2</td>
</tr>
<tr>
<td>Mast Cell Tumor</td>
<td>Boxer</td>
<td>16.7</td>
</tr>
<tr>
<td>Pancreatic Carcinoma</td>
<td>Airedale</td>
<td>16.9</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>Giant breeds</td>
<td>60.1</td>
</tr>
<tr>
<td>Bladder Cancer</td>
<td>Scottish Terrier</td>
<td>19</td>
</tr>
</tbody>
</table>
Canine TCC of the Bladder

• Transitional cell carcinoma of the bladder accounts for 2% of all canine tumors
• Affects up to 20,000 dogs each year
• Scottish terriers are 19 times more likely to get TCC than the average dog (Shetland sheepdogs and West Highland white terriers have a 5x increased risk)
RNaseq of Canine TCC

- Survey sequence of 4 tumors (2 ST, 1 WHWT, 1 SSD), and 2 normal bladder urothelial tissues.

- Sequenced complete transcriptome using Illumina Hiseq with paired end libraries

Increase information base:
- Identify transcripts that are not annotated
- Find genes that are expressed in only tumor or only normal bladders
- Identify somatic mutations
Mutation in Canine BRAF is Identical to Human BRAFV600E

Human BRAF Somatic Mutation Frequency

Canonical Human BRAF Mutation: V600E
The BRAF Mutation Activates the MAPK Pathway By Phosphorylating MEK

<table>
<thead>
<tr>
<th></th>
<th>K9TCC-Sh</th>
<th>K9TCC-An</th>
<th>K9TCC-AxA</th>
<th>K9TCC-Nk</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAFV595E</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vemurafenib (1µM)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Time (hrs)</td>
<td>2</td>
<td>24</td>
<td>2</td>
<td>24</td>
</tr>
</tbody>
</table>

- pMEK
- MEK
- Actin
BRAF Inhibitor Vemurafenib Reduces Cell Growth in Cells with BRAFV595E

Concentration of Vemurafenib (µM)

Percent Growth of Control

K9TCC-Sh
K9TCC-AxA
K9TCC-Nk
K9TCC-An

Percent Growth of Control vs Concentration of Vemurafenib (µM)
Next Generation Sequencing Can Identify the Mutation in <1 Allele per 1000

Normalized Adenine Bases per 10,000 Reads

- 1:1 Allelic Ratio
- 1:10 Allelic Ratio
- 1:100 Allelic Ratio
- 1:1000 Allelic Ratio
- Urine Sample Only Positive by AmpliSeq
- Tumor Sample Only Positive by Ampli-Seq
- Sample Negative by Ampli-Seq
Additional RNAseq in TCC

- Completed 22 tumors and 8 normal bladder urothelial tissues.
- Sequenced transcriptome using Illumina Hiseq with paired end libraries

**Analyses underway:**

- Identify somatic mutations: Filter out mutations found in normal tissues and the multi-breed database
- Analyze expression patterns
- Compare BRAF+ and – tumors: identify alternate drivers, additional players, compare gene set with human tumors
Canine Transmissible Venereal Tumor (CTVT)

Survival Strategies of an Ancient Clonally Transmissible Canine Tumor
Canine Transmissible Venereal Tumor (CTVT)

- Clonally transmissible parasite - single founder tumor spreads from dog to dog via **sexual transfer of malignant cells**

- World’s oldest known continuously propagating somatic cell lineage – thousands of years, endemic across the globe

- Transmitted during months-long period of evasion of host immune defenses, later eliminated

- All CTVT tumors have **shared origins** in the single founder tumor - strong genetic identity with one another, markedly distinct from their transient host
CTVT has Propagated and Evolved for Thousands of Years

*CTVT Founder*

*Most Recent Common Ancestor Tumor*

*Modern Tumor*

*CTVT 79T* Brazil

*CTVT 24T* Australia

*Murchison, et al. Science, 2014*
The CTVT Genome =
An Ancient Canid Genome + Somatic Mutations

Host Contamination

Inherited Alleles from Founder

Somatic Drivers of Clonal Transmissibility

Somatic Mutations

Lineage-Specific Mutations

Germline Genome of CTVT Founder Canid

Systematic Errors

CTVT 79T

CTVT 24T
Nearly All Canine Variation is Present in Panel of 186 Diverse Canids

Our catalog encompasses vast majority of genetic variation in modern canids. Therefore:

1) Shared CTVT variants **found** in catalog are likely **inherited alleles** from the founder canid

2) Shared CTVT variants **not found** in catalog are likely enriched for **somatic mutations**

95.63% of SVs
Canine Variation Catalog Segregates Founder Alleles from CTVT Somatic Mutations

CTVT Founder → CTVT 79T → CTVT 24T

High-Quality Shared

Inherited Alleles from CTVT Founder

YES → Found in Ostrander Lab Canid WGS Catalog?

NO

Somatic Mutations

Raw Variants
SNV = 8,623,150
Indel = 3,683,400
SV = 1,531,180

HQ + Shared
SNV = 3,728,916
Indel = 1,435,051
SV = 8,903

Somatic Candidates
SNV = 910,376
Indel = 113,026
SV = 7,338
All steps of antigen presentation are disrupted in CTVT cells, preventing non-self recognition by host T-cells.
Somatic Mutations Disrupt All Aspects of Somatic Cell Participation in Immunosurveillance

CTVT is perfectly adapted to its niche as a transmissible allograft

Unique evolutionary pressures + Thousands of years

Functionally overlapping somatic mutations enable evasion of host immunosurveillance
Ancient CTVT Mutations Have a Plausible Role in Establishment of Clonal Transmissibility

<table>
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<th>Immunosurveillance and Cell Cycle Genes with Ancient Somatic Mutations</th>
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<tr>
<td><strong>CASP3</strong></td>
</tr>
<tr>
<td><strong>CDKN2A/B</strong></td>
</tr>
<tr>
<td><strong>TAP2</strong></td>
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<tr>
<td><strong>TP53</strong></td>
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- Ancient LOH events: founder inherited allele homozygosity fraction $>0.85$, somatic mutation homozygosity fraction $<0.5$
- Ancient somatic mutations: homozygous mutations within those regions
The CTVT Genome is Highly Rearranged

Disarrayed Genomic Architecture at Base-Pair Resolution

- 7,338 high-quality SVs shared by both tumors
- 2,329 disrupt at least one exon
- 247 potential gene fusions
- Somatic mutations may have contributed to genome instability – *ATM, BRCA1, BRCA2, MRE11A, MLH1, PMS1, RAD21, TP53*
CTVT-Specific Genome Assembly

- Requisite for annotation and future population and expression studies.
- Obtained fresh frozen samples
  - 150x coverage (Illumina 250bp paired end)
  - PacBio anchoring backbone (10kb-40kb reads)
    - Optical mapping using BioNano IRY

  - Expression / annotation using total RNA
Conclusions from CTVT

• Our canid variation catalog enables segregation of CTVT founder inherited alleles from somatic drivers of clonal transmissibility

• CTVT is exquisitely adapted to its transmissible allograft niche, with overlapping mutations at every step of somatic cell participation in immunosurveillance

• Identification of some early somatic mutations points to genes that may have contributed to establishing clonal transmissibility

• Understanding CTVT biology may shed light on host-tumor interactions in human cancers

• Sequencing underway for a new CTVT tumor with plans for novel assembly instead of alignment.
Dogs acquire multiple forms of cancer through natural means as a course of aging, interaction with the environment, and/or inheritance.

Analysis of TCC somatic mutations reveal a driver in canine tumors that is identical to a human driver but in an unexpected place.

The tumor parasite, CTVT, can provide data regarding how cancers to escape immune detection.
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