Enrichment tools to better understand the types of circulating nucleosomes and their genome patterns in the plasma of dogs with lymphoma

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Introduction

❖ Nucleosomes = most basic structural components of DNA
❖ Methods by which circulating nucleosomes are produced
  ❖ Dying/activated WBCs
  ❖ Apoptotic cells
  ❖ Tumor cell secretion
❖ Elevated nucleosome levels in sepsis, severe burns, immune-mediated diseases, cancer
❖ H3.1 ELISA validated for measurement of nucleosome levels in canine lymphoma (LSA) patients
❖ H1 Nu.Q® Capture

AIM: To isolate and sequence cancer-derived nucleosomes from dogs diagnosed with LSA
Sample Collection

- Fasted >6hrs
- K2-EDTA tubes
- 3000xg for 10 min
- -80°C

Nu.Q® Capture

- Long DNA + Beads → Incubation → Linker Protein
- Supernatant
- Short DNA
Whole genome sequencing
- Illumina 2x150bp
- Average Depth 40x

Align to CanFam4
- Bwa-mem2

Variant calling
- GATK 4.1.10
- HaplotypeCaller
- Mutect2

Variant annotation
- SNPeff
Results

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Nucleosome Content (ng/mL)</th>
</tr>
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<tbody>
<tr>
<td>Chester (c)</td>
<td>24.4</td>
</tr>
<tr>
<td>LSA (10016)</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>LSA (60010)</td>
<td>&gt;2500</td>
</tr>
</tbody>
</table>

Figure 1: Plasma-derived nucleosome sequencing histograms. A: Sequencing histogram of lymphoma patient 10016 immunoprecipitated. B: Sequencing histogram of lymphoma-patient 10016 supernatant. C: Sequencing histogram of supernatant fraction highlighting enrichment of 147bp insert size. D: Sequencing histogram of control (healthy) patient supernatant fraction lacking the peak at 147bp.
Conclusions

- Canine lymphoma patients have circulating nucleosomes lacking linker DNA (i.e., shorter nucleosomes) that are not detected in plasma from healthy canines.
- The Nu.Q® Capture is capable of enriching canine cancer-associated nucleosomes in plasma of naïve multicentric lymphoma patients.
- These shorter, canine lymphoma-associated, nucleosomes demonstrate rare genetic variants, most notably gain of chromosome 13, loss of chromosome 14, and potential aberrations on chromosome 31.

References


Future Directions

- Optimize Nu.Q® Capture assay for enhanced cancer-associated nucleosome enrichment.
- Deeper analysis of candidate genes and known drivers of canine lymphoma with the goal of identifying diagnostic and prognostic markers in a wider variety of lymphoma cases.
- Expansion of the Nu.Q® methodology and sequencing pipelines utilized in this study to additional canine cancers, including hemangiosarcoma and osteosarcoma.
- Combine H1 Nu.Q® Capture with additional assays to better understand cancer derived circulating nucleosomes.

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