Evaluation of Plasma Nucleosome Concentrations in Healthy Dogs and Dogs with Various Common Cancers.

Heather Wilson-Robles, DVM, DACVIM (Oncology)

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FINAL DISCLOSURE:
Grant/Research Support- Volition Veterinary Diagnostic Development
Grant/Research Support- Fred and Vola Palmer Chair in Comparative Oncology (Texas A&M University)
Consulting Engagement- Volition Veterinary Diagnostic Development
The Basic Concept

- Cancer & cell death results in chromatin fragmentation and release of nucleosomes into the blood.

- Nucleosomes contain >200 possible modifications that regulate every fundamental cellular process.

- Measuring nucleosome levels and modifications in circulation have the potential to be both prognostic and diagnostic markers for disease.
Circulating Nucleosomes in Cancer

Prediction of response to neoadjuvant chemotherapy in breast cancer patients by circulating apoptotic biomarkers nucleosomes, DNase, cytokeratin-18 fragments and survivin

Oliver J Stoetter, Debora M Fersching, Christoph Salat, Oliver Steinkohli, Christian J Gabka, Ulrich Hamann, Michael Braun, Axel-Mario Feller, Volker Heinemann, Barbara Siegele, Dorothea Nagel, Stefan Holdenrieder

Circulating nucleosomes predict the response to chemotherapy in patients with advanced non-small cell lung cancer

Stefan Holdenrieder, Petra Stieber, Joachim von Pawel, Hannelore Raith, Dorothea Nagel, Knut Feldmann, Dietrich Seidel

Predictive and prognostic value of circulating nucleosomes and serum biomarkers in patients with metastasized colorectal cancer undergoing Selective Internal Radiation Therapy

Yvonne Nadine Fahmuller, Dorothea Nagel, Ralf-Thorsten Hoffmann, Klaus Talsch, Tobias Jakobs, Petra Stieber, and Stefan Holdenrieder
H3.1 ELISA Assay

- Proprietary epigenetic immunoassay platform
- Determine levels of circulating nucleosomes
- Profiles nucleosome epitopes
  - Histone post translation modifications
  - Histone variants
  - DNA modifications
- Flexibility of platform and diversity of modifications enables the development of disease specific panels
Materials and Methods
Samples were either collected at the Texas A&M Small Animal Teaching Hospital (AUPs CA 2019-0211 and 2017-0350) or from the NCI Division of Cancer Treatment and Diagnosis Biorepository.

A total of 134 healthy animals were recruited for this study.

A total of 528 canine cancer samples were either prospectively collected at TAMU or purchased from the NCI DCTD.

Plasma was isolated within 1 hour of collection and stored at -80°C until samples could be run in batches.

Animals were all fasted a minimum of 4 hours before collection.

All samples were run on the Nu.Q® H3.1 ELISA assay (Belgian Volition, SRL, Isnes, Belgium) according to the manufacturers protocol.

Statistical analysis was performed using Receiver Operator Characteristic (ROC) curves, Wilcoxon rank sum tests and Kruskal-Wallis test for repeat measures with a Dunn’s multiple comparison test using Graph pad prism v.9 and R v. 3.4.3.
Results

H3.1 Nucleosome Levels Across Multiple Cancer Types

Diagnosis
- Healthy
- Cancer

ng/mL

Healthy, HSA, LSA, Histiocytic sarcoma, Melanoma, OSA, STS, MCT
AUC 68.74%
Additional Cases
Circulating Nucleosomes in Dogs with Lymphoma

Elevated Nucleosome Levels in Lymphoma (LSA)

Variability in Nucleosome Levels Across Lymphoma Samples
Lymphoma

Characterizing Circulating Nucleosomes in the plasma of dogs with lymphoma BMC Vet Res 17, 276 (2021)
Diagnosis by Lymphoma Stage

Stage I: 7 of 11 cases detected (63.6%)
Stage II: 1 of 7 cases detected (14.3%)
Stage III: 28 of 37 cases detected (75.7%)
Stage IV: 31 of 39 cases detected (81.6%)
Stage V: 27 of 33 cases detected (81.8%)

B cell lymphoma: 41/43 cases detected (95.3%)
T cell lymphoma: 10/18 cases detected (55.6%)

Circulating Nucleosomes in Dogs with Hemangiosarcoma

Variability in Nucleosome Levels Across Hemangiosarcoma Samples

Diagnosis by Stage in HSA

- **Stage I:** 6 of 9 cases detected (66.7%)
- **Stage II:** 19 of 25 cases detected (76%)
- **Stage III:** 27 of 30 cases detected (90%)

Histiocytic Sarcoma

- 26 cases total
- H3.1 Concentration
  - Median 69.6 ng/mL
  - Mean 261.22 ng/mL
  - Range 21.8-1800
- Location
  - Visceral (n=21)
  - Bone (n=5)
- Versus healthy
  - p< 0.0001

Plasma Nucleosome Concentrations
Histiocytic Sarcoma

![Graph showing H3.1 nucleosome concentrations in different locations: Bone, Other Site, Lung, Lymph node, Spleen. The x-axis represents different sites, and the y-axis represents H3.1 nucleosomes (ng/mL). There are data points indicating variability in nucleosome concentrations across different sites.]
Oral Melanoma

- 49 cases in total
  - 7 haired skin
  - 42 oral tumors
- H3.1 Concentration
  - Median for cutaneous tumors – 24.8 ng/mL
  - Median for oral tumors – 60.0 ng/mL
- Tumor Size
  - Median for cutaneous tumors- 3 cm
  - Median for oral tumors- 4 cm
- Versus Healthy
  - p= 0.000025

Plasma Nucleosome Concentrations

Melanoma

<table>
<thead>
<tr>
<th></th>
<th>Cutaneous</th>
<th>Oral/Lip</th>
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<tbody>
<tr>
<td>H3.1 Nucleosomes (ng/mL)</td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Mast Cell Tumors

- 126 cases in total
- Tumor grade
  - 9 grade 1
  - 87 grade 2
  - 26 grade 3
- H3.1 concentrations
  - Low grade median 21.68 ng/mL; mean 41.9 ng/mL
  - High grade median 38.2 ng/mL; mean 149.7 ng/mL
  - p = 0.005
- When compared to healthy dogs
  - p > 0.99 (low grade)
  - p = 0.005 (high grade)
Osteosarcoma

- 49 total cases
  - 17 elevated
  - Sensitivity 60.7% at 97% specificity
- H3.1 Concentration
  - Median 43.2 ng/mL; mean 72.7 ng/mL
  - Range 0.1-446 ng/mL
- Versus healthy
  - $p=0.035$
Soft Tissue Sarcoma

- 51 total cases
  - 15 elevated
  - Sensitivity 48.19% with a specificity of 97%
- H3.1 Concentration
  - Median 25.09 ng/mL; mean 200.07 ng/mL
  - Range 0.1-2500 ng/mL
- Versus Healthy
  - $p = 0.704$
In this case series, plasma nucleosome concentrations were able to identify 174 of 229 (76%) systemic cancers (lymphoma, hemangiosarcoma and Histiocytic sarcoma).

Overall, in 7 of the most common cancers, plasma nucleosome concentrations were able to identify 49.8% of all cancers tested.
Discussion

- Similar findings to the PATHFINDER study (Galleri test by GRAIL)
- Positive Predictive Value 49% in their study of over 50 different cancers in humans.
Discussion

• Also similar to the OncoK9 test
  • Able to identify 54.7% of cancers they tested.
  • 85.4% of lymphoma, hemangiosarcoma and osteosarcoma cases.
Elevated plasma nucleosome concentrations may be a useful tool for the early detection of cancer in geriatric pet dogs.

This test should not be used in lieu of traditional diagnostics but as a companion test.

The ELISA platform lends itself to being a low-cost test that requires a small sample with a quick turnaround time.
Questions?

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