Evaluation of plasma nucleosome concentrations as a tool for treatment and disease monitoring in cancer bearing dogs.

Wilson-Robles, H[1], Miller, T[1], Miller, P[1], Jarvis, J[1], Butera, T[2], Matsushita, M[1], Terrell, J[2], Kelly, TK[2]

Texas A&M University College of Veterinary Medicine & Biomedical Sciences[1]; Volition America and Volition Veterinary Diagnostics[2]

Introduction

- Nucleosomes are small fragments of DNA wrapped around a histone octamer comprised of 4 sets of duplicate histones. These structures make up the very basic units of the chromosome. During cell death, fragments of the chromosome are released into the blood as nucleosomes.
- Certain diseases, such as cancer, can lead to increased concentrations of circulating nucleosomes in the blood in the form of cell free DNA.
- Histone modifications and nucleosome remodeling have been implicated in both the genesis of cancer as well as metastasis in humans. Nucleosomes also play a vital role in immune modulation through the formation of NETs (Neutrophil extracellular traps). NETosis has been implicated as a cause of disease progression, metastasis and thromboembolism in a variety of human cancers.
- In humans, circulating nucleosomes concentrations have been used as a surrogate for measuring treatment response to chemotherapy or radiation therapy in breast cancer, non-small cell lung cancer and colorectal cancer, among others.
- Elevated plasma nucleosome concentrations have been detected in dogs with lymphoma, hemangiosarcoma, histiocytic sarcoma, malignant melanoma and other cancers at the time of diagnosis; however, very little is known about the effects of treatment and disease response on circulating nucleosome concentrations in these patients.
- The goal of this study was to assess circulating concentrations of nucleosomes containing Histone H3.1 in dogs undergoing treatment and remission monitoring for a variety of malignancies.

Results

Breed	Age at Diagnosis	Body weight (kg)	Gender	Disease/Stage	H3.1 Concentration at diagnosis
Australian Cattle Dog	11	36	Neutered Male	Lymphoma, B cell, IVa	225.85
Mixed Breed	11	20.4	Neutered Male	Lymphoma, B cell, IVa	525.61
Mixed Breed	9	37.2	Neutered Male	Lymphoma, T cell, Visceral, IVb	397.26
Rottweiler	5	38.4	Spayed Female	Lymphoma, B cell, IVa	224.01
Labrador	7	34.2	Spayed Female	Lymphoma, B cell, IVa	655.4
Labrador	5	35.6	Neutered Male	Lymphoma, B cell, IVa	145.612
Pit Bull Terrier	9	21.2	Spayed Female	Lymphoma, B cell, IIIa	183.52
Min. Schnauzer	6	4.9	Spayed Female	Lymphoma, B cell, IIIa	650.84
Labrador	6	32.2	Neutered Male	Lymphoma, T cell, Cutaneous, Va	224.13
Maltese	8	5.3	Spayed Female	Lymphoma, B cell, IVa	154.67
Rhodesian/Leonberger	5	52.2	Neutered Male	Lymphoma, B cell, IIIa	80.98
Chow Chow	9	34.8	Neutered Male	Multiple Myeloma	71.76
Shetland Sheepdog	10	17.8	Neutered Male	Malignant Melanoma, IV	128.79
German Shepherd	8	35.4	Neutered Male	Nasal Chondrosarcoma, III	109.25
St. Bernard	9	43	Spayed Female	Osteosarcoma IIb	40.8
Catahoula	12	26	Neutered Male	Hemangiosarcoma (SC), III	646.65
Labrador	10	19.4	Neutered Male	Hemangiosarcoma (Rt. Auricle), II	249.47
German Shepherd	10	33	Spayed Female	Hemangiosarcoma (Spleen), III	93.66
Poodle	12	4.6	Neutered Male	High grade pulmonary carcinoma, IV	76.27
Siberian Husky	7	29.8	Spayed Female	AGASACA, III	101.22
Goldendoodle	10	33.8	Neutered Male	AGASACA, I	816.2

Disease monitoring: H3.1	% of dogs <67.4 ng/mL		H3.1 (ng/mL) at CR	Time to lowest H3.1	Time from CR to lowest H3.1	Time to CR
Round Cell Tumors	12/12	Mean	294.97 (71.6-655.4)	49.5 (6-210)	35.8 (13-125)	28.1 (6-85)
	100%	Median	224.07	19.5	16	23
Sarcomas	3/5	Mean	227.97 (40.8-646.6)	129 (24-282)	181.5 (81-282)	123.6 (7-282)
	60%	Median	109.25	81	181.5	82
Carcinomas	3/3	Mean	331.23 (76.3-816.2)	20 (14-28)	20 (14-28)	23 (18-28)
	100%	Median	101.22	18	18	23
All Cancers	19/21	Mean	95.3 (40.8-816.2)	55.1 (6-282)	51.1 (13-282)	44.35 (6-282)
	90.4%	Median	42.7	23	19.5	24

Conclusions

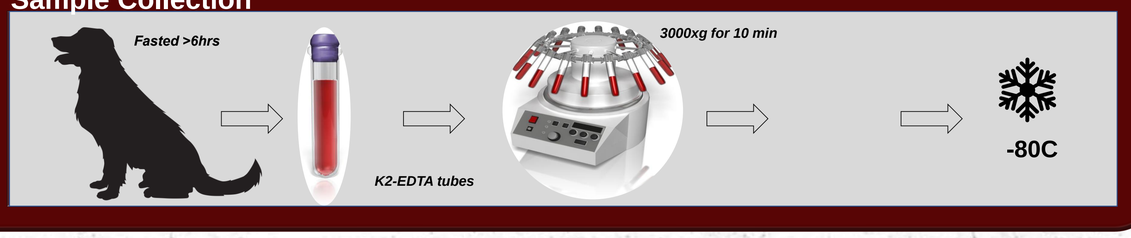
- Plasma nucleosomes can be used to monitor for disease response and progression.
- Plasma nucleosome concentrations reach healthy dog levels (<67.4 ng/mL) in most patients achieving clinical remission.
- Plasma nucleosome concentrations lag behind physical exam findings for clinical remission and may serve as a more sensitive measurement of residual disease.
- Plasma nucleosome elevations often precede obvious clinical progression, however, due to the other possible causes of elevated plasma nucleosomes it is recommended to have 2 consecutive elevations before altering the treatment or staging protocol in canine cancer patients.

References

1.Snoderly, HT, Boone, BA, et al. Neutrophil extracellular traps in breast cancer and beyond: current perspectives on NET stimuli, thrombosis and metastasis, and clinical utility for diagnosis and treatment. Breast Cancer Res 2019; 21, 145.
2.Stoetzer OJ, Fersching DM, et al. Prediction of response to neoadjuvant chemotherapy in breast cancer patients by circulating apoptotic biomarkers, nucleosomes, DNAse, cytokeratine-18 fragments and surviving. Cancer Lett. 2013; 336 (1):140-8.
3.Holdenrieder S, Stieber P, et al, Circulating nucleosomes predict the response to chemotherapy in patients with advanced small cell lung cancer. Clin Cancer Res. 2004; 10:5981-7.
4. Fahmueller YN, Nagel D, et al. Predictive and prognostic value of circulating nucleosomes and serum biomarkers in patients with metastasized colorectal cancer undergoing selective internal radiation therapy. BMC Cancer 2012; 12:5.
5.Wilson-Robles H, Miller T, Jarvis J, et al. Evaluation of nucleosome concentrations in healthy dogs and dogs with cancer. *PLOS ONE* 2020;15:e0236228.
6.Dolan C, Miller T, Jill J, et al. Characterizing circulating nucleosomes in the plasma of dogs with lymphoma. *BMC Veterinary Research* 2021;17.

Methods

Sample Collection



Sample Processing

- Twenty-one dogs with a variety of cancers were recruited for this study. Owner consent was achieved before participation. All dogs were fasted for a minimum of 6 hours before sample collection.
- Up to 5 mL of whole blood was collected into EDTA tubes from a jugular or peripheral vein. Samples were centrifuged at room temperature for 10 min at 3000 g within 1 hour of collection. The plasma was collected and stored at -80C and run in batches.
- The Nu.Q® H3.1 ELISA was performed on all samples according to the manufacturer's protocol. Thymidine kinase-1 was also performed using the Abcam canine TK1ELISA kit. CRP was run on all patient samples through the TAMU GI Laboratory. Plates were read using a standard plate reader at OD 450 nm.
- A standard curve was generated using Graphpad Prism 9 software and kit controls were confirmed to be in range for all plates. Unknown data sets were interpolated using a sigmoidal 4PL, X is concentration model. Descriptive statistics were performed using Microsoft Excel.

Individual Case Examples

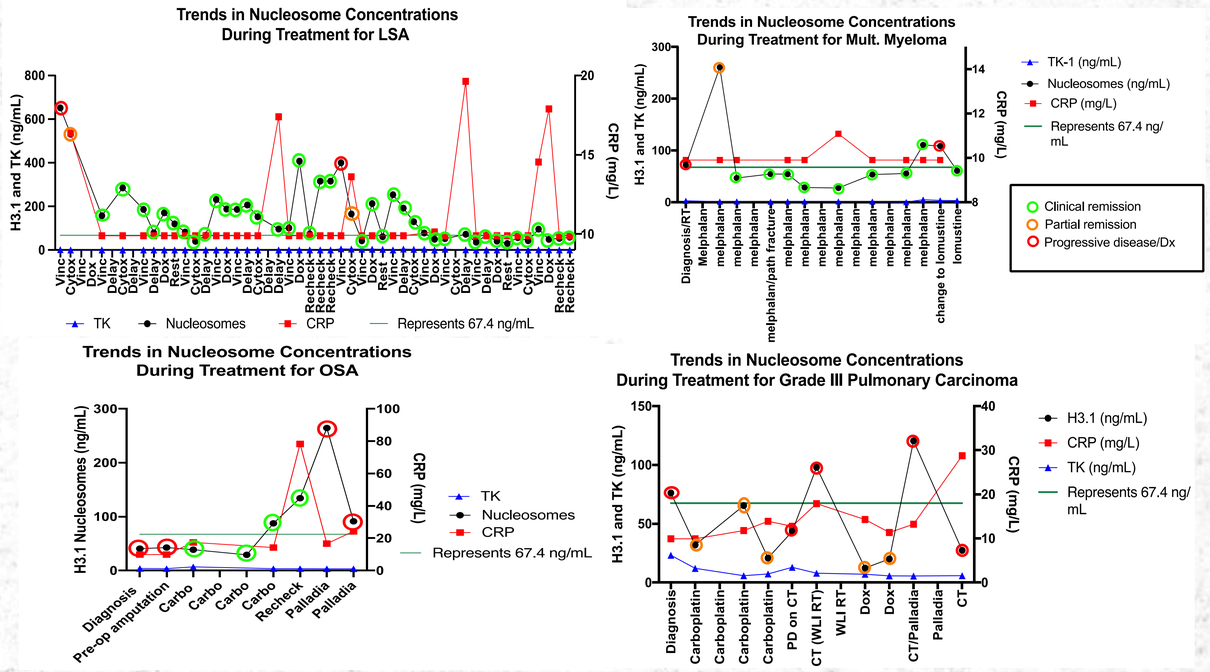


Figure 1:Individual Case examples: A. Dog with stage IVa B cell lymphoma. This dog's H3.1 concentrations rarely dip into the healthy range and she has a short remission duration before disease progression is noted. She has consistently lower H3.1 concentrations through her 2nd round of CHOP. H3.1 levels increase 2 months before clinical progression is noted. **B**. Dog with Multiple Myeloma with an acute increase in H3.1 immediately after radiation and starting chemotherapy that may indicate acute cell death. H3.1 levels increased 3 weeks before clinically detectable progression was noted. **C**. Dog with osteosarcoma that had H3.1 concentrations in the healthy range at diagnosis but the levels increase starting 2 months before obvious metastatic disease is detected. **D**. Dog with a high grade metastatic pulmonary carcinoma treated with carboplatin, whole lung irradiation, doxorubicin and Palladia.

Future Directions

- Currently we are enrolling additional dogs with a variety of cancers undergoing definitive therapy at 5 additional institutions to increase the number of dogs with complete data sets.
- Additionally, we plan to open a study in early 2022 to prospectively monitor treatment and remission status for dogs enrolled at 2 institutions with real time H3.1 data to validate the predictive value of nucleosome concentrations in cancer therapy.

Acknowledgments

- Texas A&M University College of Veterinary Medicine Veterinary Small Animal Clinical Sciences Department
- Texas A&M University Department of Veterinary Integrative Biosciences
- Fred and Vola Palmer Chair in Comparative Oncology
- Research in this study has been funded by Volition Veterinary Diagnostic Development.