Utility of Serial Plasma Nucleosomes Concentrations for Monitoring Treatment Response and Disease Progression in Canines with Hematopoietic Malignancies

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• Volition Veterinary Diagnostic Development, LLC
• Fred and Vola Palmer Chair in Comparative Oncology (Texas A&M)
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• 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 Stoetzer, Debora M I Fersching, Christoph Salat, Oliver Steinkohl, 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 Fahmueller, Dorothea Nagel, Ralf-Thorsten Hoffmann, Klaus Tatsch, Tobias Jakobs, Petra Stieber, Stefan Holdenrieder
Nu.Q® Technology

- 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
Circulating Nucleosomes in Dogs with Lymphoma (previously published)

Elevated Nucleosome Levels in Lymphoma (LSA)

Variability in Nucleosome Levels Across Lymphoma Samples

Materials and Methods

• 25 dogs with hematopoietic malignancies were recruited for this study.
• Blood samples were drawn at diagnosis and at chemotherapy appointments and rechecks thereafter.
• Samples were centrifuged at 3000g for 10 min and the plasma was immediately removed.
• Samples were frozen at -80C to be run later in batches.
• Plasma was provided to the Texas A&M GI laboratory to determine C-Reactive Protein levels.
• Thymidine kinase-1 Canine ELISA kit
Materials and Methods

• Medical records were available for review in all 25 cases.

• All dogs with multicentric lymphoma were staged with the following tests:
  – CBC, Chemistry, UA
  – Thoracic radiographs
  – Abdominal Ultrasound
  – Bone marrow aspirate
  – Flow cytometry
Materials and Methods

- One dog with cutaneous lymphoma was staged with a skin biopsy, CBC, Chemistry, UA, thoracic radiographs and abdominal ultrasound.
- One dog with AML staged with CBC, Chemistry, AUS, CXR and Flow cytometry.
- One dog with Multiple Myeloma staged with CBC, Chemistry, Thoracic and Abdominal CT.
Materials and Methods

- Signalment, stage, phenotype and disease response information was recorded for each patient and matched to corresponding H3.1, CRP and TK1 plasma concentrations.
- CRP and TK1 analysis was not complete at the time of presentation for all cases.
Results

- Signalment Summary:
  - 25 cases meeting criteria for enrollment
  - 15 MN, 9 FS
  - Median age 7 years (range 5-14 years)
  - Median weight 26.8 kg (range 4.9-78 kg)
  - 5 mixed breed dogs, 4 Labrador retrievers, 2 miniature schnauzers, 2 Australian cattle dogs
Results

• Disease Summary:
  – 15 dogs with B cell LSA
    • 4 stage IIIa and 1 IIIb multicentric LSA
    • 9 stage IVa and 1 IVb multicentric LSA
  – 6 dogs with T cell lymphoma
    • 2 indolent T zone LSA (IIIa)
    • 2 stage IVa, 1 IVb visceral lymphoma
    • 1 stage Va cutaneous lymphoma
  – 2 unknown phenotype
    • Both Vb
Results

- 20/25 dogs had elevated nucleosome concentrations at diagnosis.
  - 2 T zone lymphomas within healthy range
  - 3 B cell lymphomas within healthy range
- Plasma H3.1 concentration at diagnosis:
  - 224.99 ng/mL median
  - 331.18 ng/mL (mean)
  - Range 78.2- >842.2 ng/mL
Results

• Plasma H3.1 concentration at first CR
  – 32.49 ng/mL (median)
  – 80.37 ng/mL (mean)
  – Range: 0-561.6 ng/mL

• Lowest plasma H3.1 concentration during treatment.
  – 11.1 ng/mL (median)
  – 16.8 ng/mL (mean)
  – Range: 0-53.8 ng/mL
20/20 dogs had nucleosome concentrations drop into the healthy range during treatment.
  - Median time: 14.5 days
  - Mean 33.6 days
  - Range: 4-210 days

Time to clinical remission or best response:
  - Median 24.5 days
  - Mean 29.95
  - Range: 6-85 days
Results

• Median percent change from highest to lowest plasma H3.1 concentration:
  – Median percent change: 95.6%
  – Range: 75.2-100%

• Progression Free Survival
  – All dogs- (range 23-844) days
  – Elevated H3.1 (n=20)- (range 23-844) days
  – Normal H3.1 (n=3)- (range 213-522) days
Results

Survival based on H3.1

- All dogs
- Elevated H3.1
- Normal H3.1
- H3.1 > 200
- H3.1 < 200

Days elapsed

Probability of Survival
Results

• Correlation Statistics
  – PFS vs % H3.1 Decrease
    • $p = 0.0976$
    • $r = -0.38$, 95% CI (-0.7-0.07), $R^2 = 0.15$
  – PFS vs Days to Clinical Remission
    • $p = 0.78$
    • $r = -0.06$, 95% CI (-0.4942 – 0.38), $R^2 = 0.004$
  – PFS vs Days to Normal Plasma H3.1 Concentration
    • $p = 0.54$
    • $r = 0.15$, 95% CI (-0.32-0.55), $R^2 = 0.02$
Cases: B cell lymphoma
Results

Trends in Nucleosome Concentrations During Treatment
Patient 1: Stage IVa B cell LSA

- CRP
- TK
- Nucleosomes

Represented 67.4 ng/mL
Results

Trends in Nucleosome Concentrations
During Treatment for LSA
Patient 2: Stage IVa B cell LSA

- CRP
- TK
- Nucleosomes
- Represents 67.4 ng/mL
Results

Trends in Nucleosome Concentrations During Treatment for LSA
Patient 4: Stage IVa B cell LSA

H3.1 Nucleosomes (ng/mL) vs. CRP (mg/L)

- Nucleosomes
- CRP
- TK

1:1 V out of remission
1:1 V out of remission

Represents 67.4 ng/mL
Cases: T cell lymphoma
Results

Trends in Nucleosome Concentrations During Treatment
Patient 2: Stage Va T cell LSA (Cutaneous)

- **H3.1 Nucleosomes (ng/mL)**
- **CRP (mg/L)**
- **TK**
- **Nucleosomes**

Represents 67.4 ng/mL
• Circulating nucleosome concentrations are elevated in many lymphoma cases (20/25 (80%) in this cohort).
• Circulating nucleosome concentrations change from week to week and appear to mirror disease response.
• There is no correlation between CRP and TK1 concentrations and circulating nucleosome concentrations.
Conclusions

• No significant survival benefit for LSA cases based on H3.1 concentrations in this cohort.
  – Dogs with normal H3.1 had low numbers (n=3)
  – Larger cohort needed

• No correlation between PFS and:
  – % decrease in H3.1 concentration
  – Days to clinical remission
  – Days to normal H3.1 concentration
Future Directions

- Currently enrolling dogs with various chemotherapy or radiation responsive diseases to evaluate the utility of circulating nucleosomes as a surrogate for treatment response.
- Investigating the role circulating nucleosomes can play in determining MRD.
- Investigating prognostic significance of changes in nucleosome concentrations during treatment.
Questions???

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