Identification of an epigenetic profile of circulating nucleosomes in Non-Hodgkin Lymphoma as potential biomarkers of the disease

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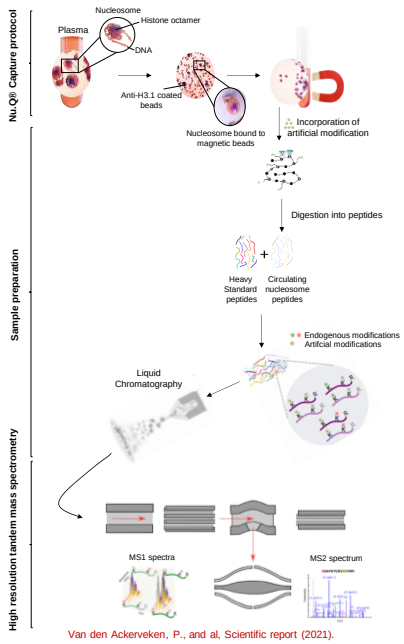
Summary

During cell death, nucleosomes are released into the blood stream and elevated levels have been found in the plasma of patients with solid cancers.
In this study, we demonstrate an increase in cell free circulating H3.1-nucleosomes levels in plasma samples from patients with non-Hodgkin lymphoma (NHL), relative to healthy donors. As histone post-translational modifications (PTMs) of circulating nucleosomes are described as potential biomarkers of various solid cancers, we investigated the epigenetic profile of nucleosomes from NHL patients following nucleosome enrichment (Nu.Q® Capture) combined with mass spectrometry. Eight histones PTMs, including the acetylation of histone H3K9Ac, H3K14Ac, H3K18Ac as well as the H3K9Me1, H3K27Me3 and H3K36Me3, were identified at a higher level in the plasma of NHL patients compared to healthy donors. These results were confirmed in a larger clinical cohort by Nu.Q® immunoassay. Subsequently, the temporal profile of these histone PTMs in NHL patients undergoing treatment course highlighted the potential use of these new biomarkers to monitor treatment response and/or disease progression. Our results substantiate that levels of H3.1nucleosomes are particularly elevated in NHL patients and may be a useful diagnostic tool. Moreover, our work emphasizes the crucial roles of the epigenetic marks present on circulating nucleosomes to detect and monitor tumor progression and/or treatment response of non-Hodgkin Lymphoma.

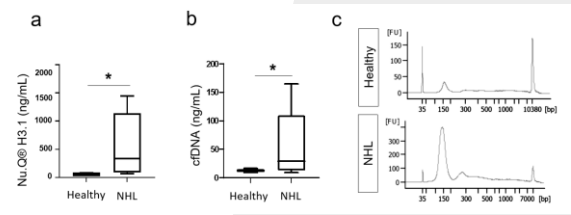
1) Nu.Q® Capture—Mass spectrometry protocol



Van den Ackerveken, P., and al, Scientific report (2021).

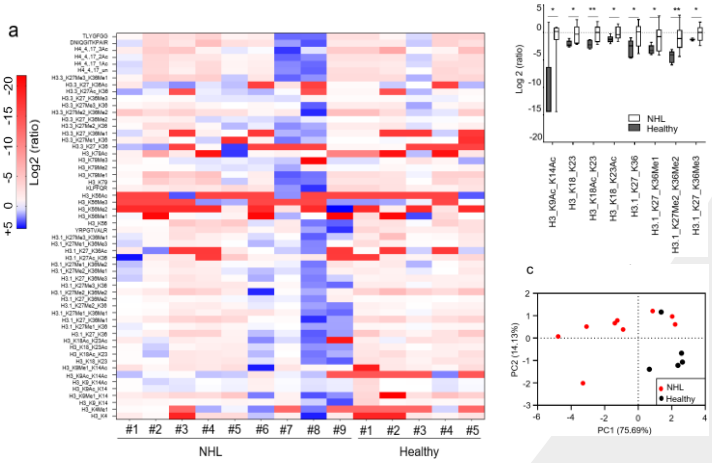
- 900 µl of plasma samples containing circulating nucleosomes were incubated with anti-H3.1 coated magnetic beads (Nu.Q® Capture protocol) to isolate nucleosomes captured from the rest of plasma.
- Then, chemical derivatization of histones by acylation was used to block the lysine residues and generate compatible peptides for LC-MS analysis.
- After trypsin digestion, heavy amino acid labeled histone H3 peptides were added during sample preparation to each sample. These synthetic histone peptides are used for normalization to eliminate potential bias caused by sample preparation or instrumentation.
- Next, desalted peptides were injected in a liquid chromatography system (Ultimate 3000 RSLCnano). The eluent from the HPLC was directly electro-sprayed into a Q Exactive HF mass spectrometer (Thermo Fisher Scientic, San Jose, CA).
- The mass spectrometer was operated in MS/MS acquisition mode.

2) Elevated levels of circulating H3.1-nucleosomes are associated with NHL and correlated to cfDNA levels



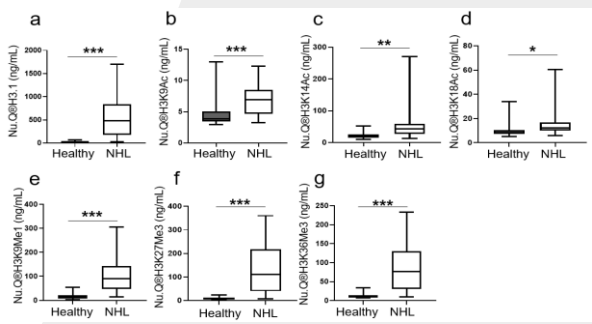
(a) Significant increase of the H3.1-nucleosome concentration (Nu.Q® H3.1 : ng/mL) and (b) cfDNA (qubit dsDNA : ng/mL) in NHL samples compared to healthy samples. p-values were determined by Mann–Whitney (*p<0.05). (c) cfDNA Fragment size distribution of a representative healthy sample compared to NHL sample using Agilent 2100 Bioanalyzer.

3) Nu.Q® Capture—MS allows the epigenetic profilling of circulating nucleosomes of NHL patients



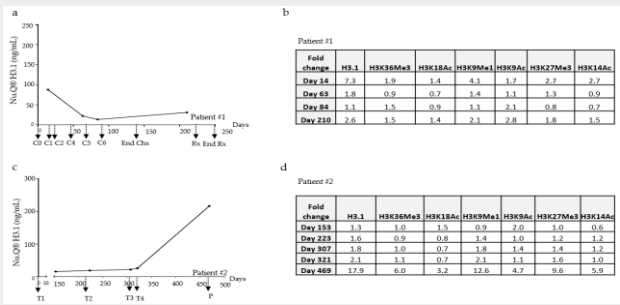
(a) Heat map showing 56 histone PTMs peptides identified on captured circulating nucleosomes from NHL samples (n=9 labelled from #1 to #9) and healthy samples (n=5 labelled from #1 to #5). Data are shown as log2 ratio of histone PTMs levels. (b) Box plot showing the abundance of histone peptides detected by Nu.Q® Capture-MS in plasma sample from healthy donors compared to NHL patients. p-values were determined by ANOVA Test (*p<0.05; **p<0.01) and results are expressed as log2 (ratio) of histone PTMs levels. (c) Principal component analysis. Each point represents a sample. The variance explanation of the principal component (PC) is expressed in % under the related axis.

4) Confirmation of elevated histone PTMs levels in NHL patients by Nu.Q® immunoassay



Box plot showing quantifications by immunoassays of modified circulating H3.1- (a), H3K9Ac- (b), H3K14Ac- (c), H3K18Ac- (d), H3K9Me1- (e), H3K27Me3- (f), H3K36Me3- nucleosomes (g) from NHL samples (n=24) compared to healthy samples (n=34). p-values were determined by Mann–Whitney. (*p<0.05; **p<0.01; ***p<0.001)

5) Nucleosome concentrations are altered in response to chemotherapy



Patient #1

Fold change	H3.1	H3K36Me3	H3K18Ac	H3K9Me1	H3K9Ac	H3K27Me3	H3K14Ac
Day 14	7.3	1.9	1.4	4.1	1.7	2.7	2.7
Day 63	1.8	0.9	0.7	1.4	1.1	1.3	0.9
Day 84	1.1	1.5	0.9	1.1	2.1	0.8	0.7
Day 210	2.6	1.5	1.4	2.1	2.8	1.8	1.5

Patient #2

Fold change	H3.1	H3K36Me3	H3K18Ac	H3K9Me1	H3K9Ac	H3K27Me3	H3K14Ac
Day 153	1.3	1.0	1.5	0.9	2.0	1.0	0.6
Day 223	1.6	0.9	0.8	1.4	1.0	1.2	1.2
Day 307	1.8	1.0	0.7	1.8	1.4	1.4	1.2
Day 321	2.1	1.1	0.7	2.1	1.1	1.6	1.0
Day 469	17.9	6.0	3.2	12.6	4.7	9.6	5.9

(a, c) Temporal distribution of H3.1-nucleosomes level expressed in ng/mL at different timepoints. (b, d) Fold change levels of specific PTMs-nucleosomes(H3.1-, H3K36Me3-, H3K18Ac-, H3K9Me1-, H3K9Ac-, H3K27Me3- and H3K14Ac-nucleosomes) compared to the samples. C1 to C6: chemotherapy cycle from 1 to 6; End of Chx: end of chemotherapy, T1 to T4: treatment from 1 to 4, P: progression, (End)Rx: (End of the) radiations.

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This program benefited from a financial support of Walloon Region

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