Nu.Q[®] Capture-MS allows the epigenetic profile analysis of circulating nucleosomes in non-Hodgkin lymphoma patients

P. Van den Ackerveken¹, A. Lobbens¹, J-V. Turatsinze¹, V. Solis-Mezarino², M. Völker-Albert², A. Imhof^{2,3}, M. Herzog¹

¹ Belgian Volition SRL, 22 Rue Phocas Lejeune, Parc Scientifique Crealys, 5032, Isnes, Belgium

² EpiQMAx GmbH, Am Klopferspitz 19, 82152, Planegg-Martinsried, Germany

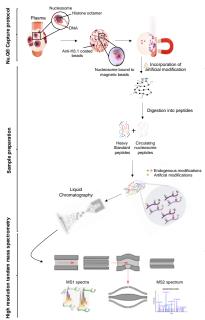
³ BioMedical Center, Division of Molecular Biology, Faculty of Medicine, Ludwig-Maximilians Universität München, Großhaderner Strasse 9, 82152 Planegg-Martinsried, German



Summary

There are over 544,000 new cases of Non-Hodgkin lymphoma (NHLym) diagnosed worldwide each year and approximately 260,000 deaths. The non-specific symptoms of lymphoma often delay diagnosis. Alterations of epigenetic modifications have been demonstrated as an important player in human cancer including lymphoma. However, the epigenetic profile of histone post-translational modifications (PTMs) on circulating nucleosomes is still not well described. We have developed a fast and robust enrichment method to isolate circulating nucleosomes from plasma for further downstream proteomic analysis (Nu.Q®-MS). We implemented this innovative method in a pilot study composed by plasma samples from NHLym cancer patients (n=9) and healthy controls (n=6). By comparing NHLym vs healthy plasma samples after Nu.Q® Capture-MS protocol, we identified 56 histone proteoforms. Core histone proteins such as histone H3 and H4 were identified in all immunoprecipitated samples confirming the immunoprecipitation of circulating nucleosomes. Among the histone peptides identified, we found 5 histone PTMs, located at 5 different sites, differentially represented in plasma from NHLym patients or healthy donors (p < 0.05). Altogether these data suggest that the Nu.Q® Capture protocol is effective to isolate circulating nucleosomes from plasma samples of NHLym patients to allow their subsequent analysis by mass spectrometry and the discovery of potential biomarkers for NHLym cancer diagnosis

1) Nu.Q[®] Capture—Mass spectrometry protocol



- 900 µl of plasma samples containing circulating nucleosomes were incubated with anti-H3.1 coated magnetic beads (Nu.Q® Capture protocol) to nucleosomes captured isolate from the rest of plasma.

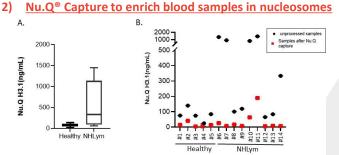
Then, chemical derivatization of histones by acylation was used to block the lysine residues and generate compatible peptides for LC-MS analysis.

After trypsin digestion, heavy amino acid labeled histone H3 were added during peptides preparation to sample each sample. These synthetic histone used to peptides are for normalization eliminate potential bias caused by sample preparation or instrumentation.

 Next, desalted peptides were injected in а liauid chromatography system (Ultimate 3000 RSLCnano). The eluent from the HPLC was directly electro-spraved into a Q Exactive HF mass spectrometer (Thermo Fisher Scientic, San Jose, CA).

- The mass spectrometer was operated in MS/MS acquisition mode

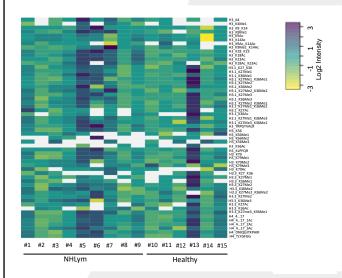
Van den Ackerveken, P., Lobbens, A., Turatsinze, JV. et al. A novel proteomics approach to epigenetic profiling of circulating nucleosomes. Sci Rep 11, 7256 (2021). <u>https://doi.org/10.1038/s41598-021-86630-3</u>



Higher level of circulating nucleosome in NHLym patients. Box plots showing the (A) concentration of circulating H3.1-nucleosomes (ng/mL) quantified by Nu.Q®H3.1 immunoassay from plasma samples of healthy donors (n = 5) or NHLym patients (n = 9). Box plot shows mean value and 25^{th} and 75^{th} percentiles, the whiskers represent the standard deviation (Mann-Whitney test ; p-Value = 0,0599)

Nu Q®H3.1 results showing the depletion of nucleosomes after Nu Q® Capture (B) immunoprecipitation (red dots) in comparison to the level present in the initial plasma (black dots). Thanks to Nu.Q® capture protocol, we were able to isolate 92.7% ± 5.3% of the nucleosomes present in the plasma sample of NHLym patients.

3) Quantitative analysis of histone PTMs from circulating nucleosomes by LC-MS/MS



Heat map showing the histone PTMs peptides identified in plasma samples from NHLym patients (n=9; from #1 to #9) and Healthy donors (n=6; from #10 to #15). Using our Nu.Q® Capture - Mass spectrometry protocol, we identified 56 histone proteoforms. Core histone proteins such as histone H3 and H4 were identified in all immunoprecipitated samples confirming that the immunoprecipitation protocol isolate circulating nucleosomes. Data are shown as Log2 Intensity of histone PTM levels.

H3 1 K9Ac K14Ac H3.1 K9Me1 Α. P=0,015 P=0.02 24 22.5 22 ntensity 20 20 log2 | 17 : 15 NHLy NHLvm D. Ε. H3.1_K23A H3.1_K18Ac Box plots showing the abundance (Log2 Intensity) of 5 histone peptides, 27 24 P=0.005 P=0.04 23 All 26 22 Log2 Int H3.1_K9Ac_K14Ac (A): 25 H3_K9Me1(C) 21 H3.1_K18Ac (E). 20 Healthy NHLym Healthy NHLym

Histone PTMs on circulating nucleosomes 4) differentially represented in NHLym patients

representing 7 histone PTMs, differentially abundant in plasma from NHLym patients vs Healthy donors : H3_K27Me2_K36Me2 (B): ; H3_K23Ac (D) and The box plot shows the median and the

25th and 75th percentiles; the whiskers indicate the 5th and 95th percentiles.

Contact

www.volition.com

+32 (0) 81 40 79 14 E-mail: M.Herzog@volition.com