

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

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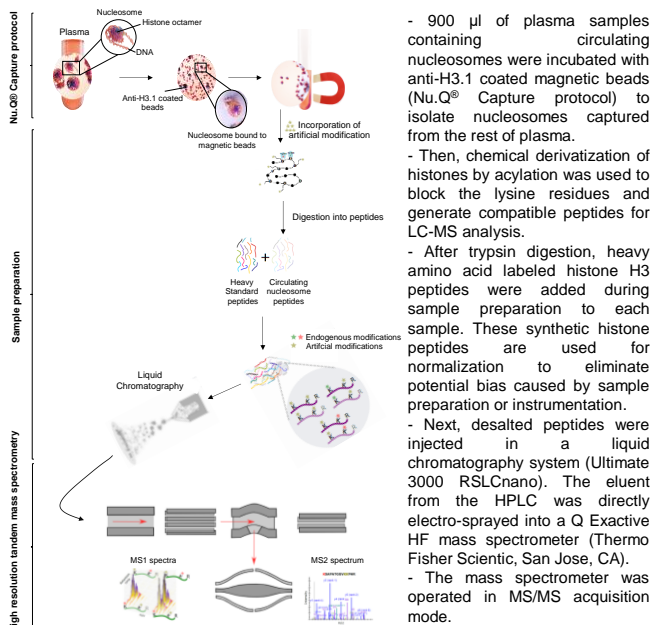
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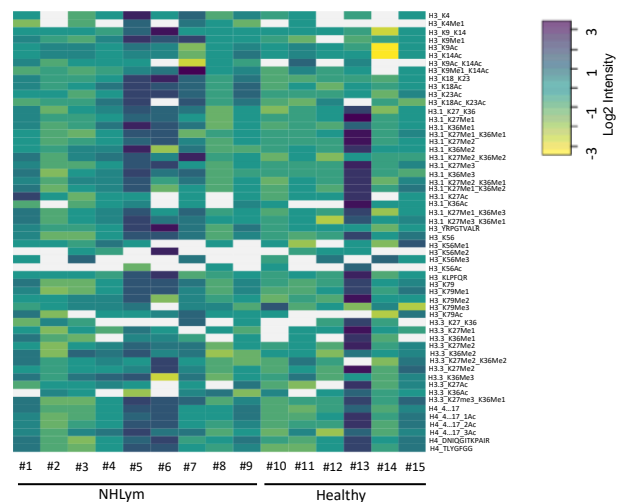
Summary

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



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.

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

2) Nu.Q® Capture to enrich blood samples in nucleosomes

