# Nu.Q<sup>®</sup> Capture- Mass spectrometry is a novel proteomics approach to epigenetic profiling of circulating nucleosomes

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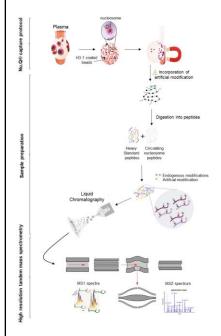
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## Summary

Alteration of epigenetic modifications plays an important role in human cancer. Notably, the dysregulation of histone post-translational modifications (PTMs) has been associated with several cancers including colorectal cancer (CRC). However, the signature of histone 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) . This method enabled us to quantify the global alterations of histone PTMs from 9 CRC patients and 9 healthy donors. Among 54 histone proteoforms identified and quantified in plasma samples, 13 histone PTMs were distinctive in CRC. Notably, methylation of histone H3K9 and H3K27, acetylation of histone H3 and citrullination of histone H2A1R3 were upregulated in plasma of CRC patients. A comparative analysis of paired samples identified 3 common histone PTMs in plasma and tumor tissue including the methylation and acetylation state of lysine 27 of histone H3. Moreover, we highlight for the first time that histone H2A1R3 citrulline is a modification upregulated in CRC patients. This new method presented herein allows the detection and quantification of histone variants and histone PTMs from circulating nucleosomes in plasma samples and could be used for biomarker discovery of cancer.

#### 1) Nu.Q<sup>®</sup> Capture—Mass spectrometry



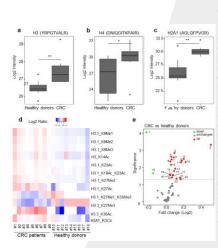
450ng 225ng 112.5ng

- 900 µl of plasma samples 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 liquid а 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.

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



Box plots showing the abundance of unmodiable histone peptides H3 (a), H4 (b), H2A1 (c) detected by Nu.Q® Capture-MS in plasma samples from healthy donors (n = 9) or CRC patients (n = 9).

were determined by p-values . student's t- Test (\*p < 0.05; \*\*p < 0.01)

(d) Heat map showing the histone differentially PTMs peptides abundant between CRC patients (n =9; #1 to #9) and healthy donors (n = 9 ; #10 to #18); p < 0.05. Data are shown as Log2 ratio of histone PTM levels (log 2 intensity) for a given sample based on average PTM levels across all samples. (e) Volcano plot representing the signicance (expressed as -log10(pvalue))as a function of the magnitude of fold change between CRC patients (n = 9) vs healthy donors (n = 9) of the 54 histone proteoforms identied in plasma using the Nu.Q<sup>®</sup> Capture-MS. 23 were signicantly dysregulated including 2 downregulated (green) and 21 upregulated (red) (p < 0.05).

### 4) Histone PTMs on circulating nucleosomes reflect the epigenetic signature of the tumor

(a) Heat map showing the histone PTMs peptides differentially abundant between tumor resection tissue and normal adjacent tissue (NAT) from the same patients (b) Volcano plot representing the signicance (expressed as log10(p-value)) as a function of the magnitude of fold change between CRC tissues vs NAT paired samples) of the 79 histone proteoforms identied in plasma. 26 were signicantly dysregulated including 4 downregulated (green) and 22 upregulated (red) (p < 0.05). Venn diagram (c) analysis showing the logical relation between the histone PTMs differentially peptides in tumor tissues expressed (blue) and plasma (red). (d) Table with log2 intensity value of the

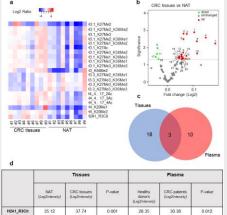
common histone PTMs 3 similarly dysregulated in plasma and tumor tissue

H3.1 K27Ac

H3.1\_K2

29 18

31.60



(b) Anti-histone H3 Western blot analyses on the immunoprecipitated material from H3.1 recombinant nucleosome or plasma samples from CRC patients (n = 3; #1 to #3) or a plasma sample

(c) Nu.Q® H3.1 ELISA results showing the depletion of nucleosomes after Nu.Q® Capture immunoprecipitation (anti-H3.1\_IP) in comparison to the level present in the initial plasma (plasma). (d) Representative blood proteins, peptides and histone proteins detected by MS (log2 intensity) in unprocessed plasma samples or processed with the Nu,Q<sup>®</sup> Capture protocol (anti-H3.1 IP).

**Enrichment of nucleosomes from plasma** 

### Contact

(a) Coomassie blue staining

2)

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without detectable level of circulating nucleosomes.

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29.1

18.7

28

0.004