Nucleosome changes associated with Cancer and NETosis related diseases

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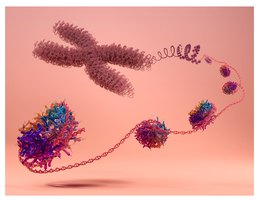
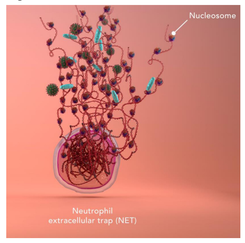
Introduction

- Nucleosomes are the repeating unit of chromatin
- Chromatin becomes decondensed in Cancer and NETosis related diseases resulting in nucleosomes being released into circulation as cell free (cfDNA)
- Nucleosome levels are elevated in patients with a variety of Cancers as well as COVID and Sepsis
- Nucleosomes contain important information about the cells from which they were derived
- Nucleosome modifications associated with Cancer have been studied for decades, but there is relatively less known about the nucleosome changes associated with NETosis and Sepsis

Cancer
Chromatin Decondensation



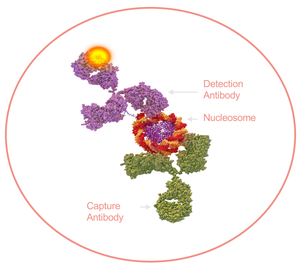
NETosis
Neutrophil Extracellular Traps



Methods

- Nucleosomes were measured using Volition's H3.1 Nu.Q® Assay
- Neutrophils were isolated using MACSxpress® Whole Blood Neutrophil Isolation Kit
- 100 bp paired end sequencing was performed (Illumina)
- Copy Number Analysis (CNA) was done using iChor CNA and default settings (https://github.com/broadinstitute/ichorCNA)
- Real-time Netosis was measured buy inclusion of Sytox green and continual fluorescent measurements using a SpectraMax plate reader

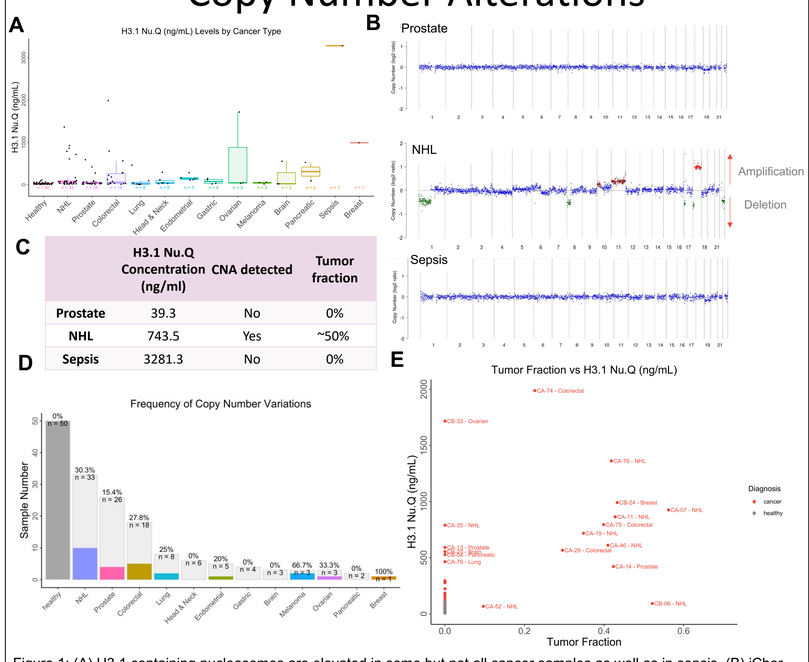
Nu.Q ® Assay



Next Generation Sequencing

Results

Correlation of Nucleosome Levels and Copy Number Alterations



	H3.1 Nu.Q Concentration (ng/ml)	CNA detected	Tumor fraction
Prostate	39.3	No	0%
NHL	743.5	Yes	~50%
Sepsis	3281.3	No	0%

Figure 1: (A) H3.1 containing nucleosomes are elevated in some but not all cancer samples as well as in sepsis. (B) iChor CNA shows genomic amplifications and deletions across the genome in plasma from a non-Hodgkin's lymphoma patient, but not a prostate cancer or sepsis sample. (C) Representative samples and the corresponding nucleosome level and CNA and tumor fraction characterization. Nucleosome levels are correlated with copy number alterations in cancer but not sepsis. (D) Number of samples characterized and percentage that contained copy numbers alterations using default iChor CNA settings. (E) Correlation of Nucleosome level, measured using H3.1 Nu.Q® assay, and tumor fraction, as assessed by iChorCNA across a variety of healthy and cancer samples.

Whole Blood NETosis Induction

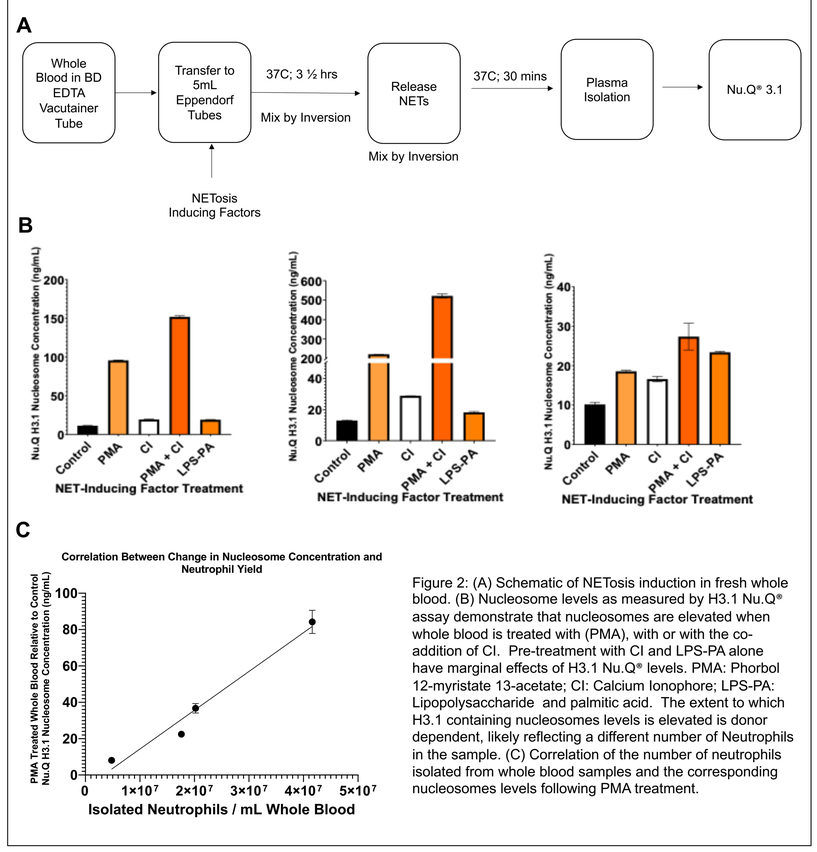


Figure 2: (A) Schematic of NETosis induction in fresh whole blood. (B) Nucleosome levels as measured by H3.1 Nu.Q® assay demonstrate that nucleosomes are elevated when whole blood is treated with (PMA), with or with the co-addition of CI. Pre-treatment with CI and LPS-PA alone have marginal effects of H3.1 Nu.Q® levels. PMA: Phorbol 12-myristate 13-acetate; CI: Calcium Ionophore; LPS-PA: Lipopolysaccharide and palmitic acid. The extent to which H3.1 containing nucleosomes levels is elevated is donor dependent, likely reflecting a different number of Neutrophils in the sample. (C) Correlation of the number of neutrophils isolated from whole blood samples and the corresponding nucleosomes levels following PMA treatment.

Results

Isolated Neutrophil NETosis Induction

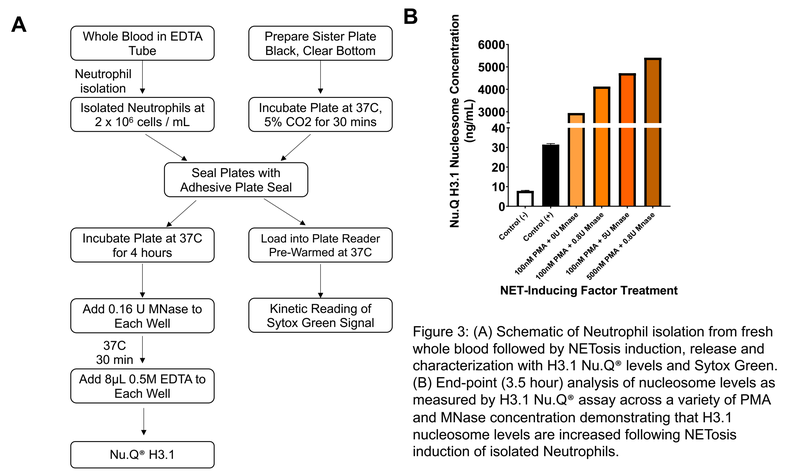


Figure 3: (A) Schematic of Neutrophil isolation from fresh whole blood followed by NETosis induction, release and characterization with H3.1 Nu.Q® levels and Sytox Green. (B) End-point (3.5 hour) analysis of nucleosome levels as measured by H3.1 Nu.Q® assay across a variety of PMA and MNase concentration demonstrating that H3.1 nucleosome levels are increased following NETosis induction of isolated Neutrophils.

Real Time NETosis Monitoring

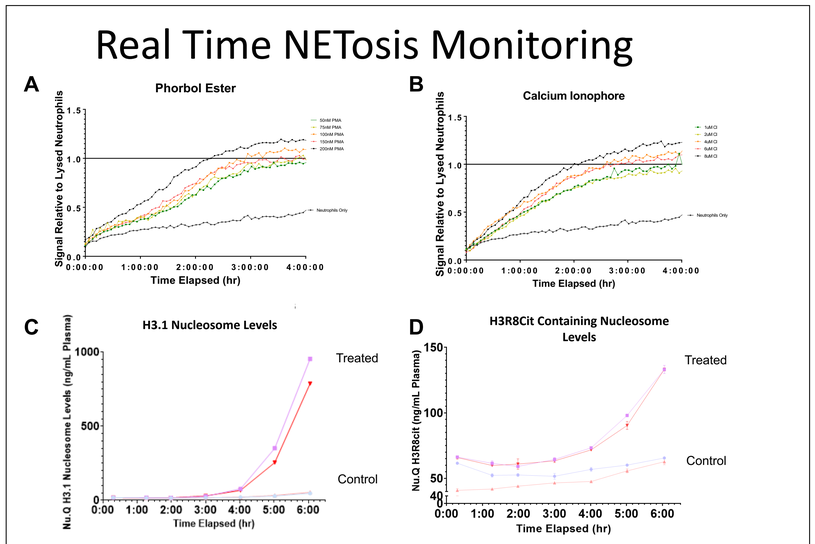


Figure 4: Time course of NET release in response to Phorbol Ester (A) and Calcium Ionophore (B) as measured by fluorescence from Sytox green intercalation normalized to total DNA content. Time course of H3.1 (C) and H3R8 Citrulline (D) nucleosome levels during NET release.

Summary & Conclusions

- H3.1 containing nucleosome levels and the presence of copy number alterations vary across cancer types and samples
- Elevated Nu.Q® levels correlate with copy number alterations for cancer but not sepsis samples
- NETosis can be chemically induced in whole blood and isolated neutrophils
- Nucleosome and H3R8 Citrullination levels increase during NETosis
- These models can be used to understand the underlying signaling cascades and chromatin mechanisms underlying NETs release

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