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Improved assay for quantifying citrullinated histones as markers of disease

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Abstract

Neutrophil extracellular traps, or NETs, are stringy chromatin networks that are ejected from immune cells to capture and neutralize harmful pathogens. But over-casting NETs can be indicative of disease, including cancer and thrombosis. As a result, NETs have become a promising non-invasive biomarker, and one way to quantify these networks is by detecting a critical component of NETs— histones whose arginine residues have been converted to citrulline, or H3Cit. These assays use antibodies to measure H3Cit in patient plasma, but a recent study shows that existing assays are limited by two important factors: Part of the problem is that available antibodies to H3Cit display low specificity, poor signal-to-noise, and lot variability. The second issue is that these assays are calibrated using enzymatically citrullinated, free histone peptides, which are unstable in human plasma and produce variable performance between batches. Application of modified recombinant nucleosomes, synthesized by EpiCypher®, addressed both of these problems, leading to a robust and quantitative assay for this emerging biomarker. First, nucleosomes were used to identify highly specific antibodies to H3Cit. The selected antibodies displayed improved specificity and lot consistency compared to leading antibodies against this target, highlighting the importance of the nucleosome context in antibody validation. Citrullinated nucleosomes were also developed as standards for assay quantification, leading to improved assay sensitivity and reliability. Together, these changes resulted in an assay with exquisite target selectivity, lower variability within and between samples, and the ability to differentiate patients with cancer from healthy individuals. Using these semi-synthetic nucleosomes for antibody validation and assay calibration could help standardize the detection of pathogenic NETs and other plasma-based biomarkers and may ultimately enable the delivery of precision medicine in the clinic.