Circulating H3K27 methylated nucleosome concentration in lung cancer improves the contributive value of ctDNA molecular profiling result at diagnosis

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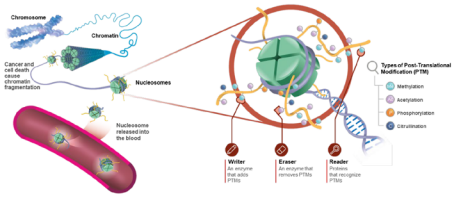
Introduction

Nucleosomes are small fragments of chromosomes released into the blood during cell death and consist of a histone octamer core with DNA wrapped around it. Histones proteins could be modified by a variety of post-translational modifications (PTMs) also called epigenetic modifications such as methylation, acetylation, phosphorylation or citrullination.



References: Li X and Li XD. Integrative Chemical Biology Approaches to Deciphering the Histone Code: A Problem-Driven Journey. Acc Chem Res 2021 54(19), 3734-3747; Regnier FE, Kim J. Proteins and Proteoforms: New Separation Challenges. Anal Chem 2018 Jan 2;90(1):361-373

In patients with cancer, a large portion of cfDNA/nucleosomes are released from tumor cells and may harbor tumor DNA (ctDNA) and a variety of histone PTMs. Histone methylation is dynamically controlled by various lysine histone demethylase (HDM) or histone methyltransferase (HMT) enzymes that remove or add the methyl group(s) from the lysine residue. Mis-regulation of the HDM and HMT leads to aberrant levels of histone methylation and have been associated with a variety of cancer types including breast, prostate, **lung** and brain. Quantification of tumor-specific mutations in ctDNA is part of the clinical practice for molecular profiling at diagnosis and targeted therapy selection.
In addition to ctDNA molecular profiling, analysis of circulating nucleosomes and their histone PTMs could provide a wider view of the genetic and epigenetic landscape of a tumor.

In this retrospective non-interventional study, we evaluated, at diagnosis, the role of circulating nucleosome-containing methylated histone and especially H3K27Me3-nucleosome as potential biomarker in patients with stage IV NSCLC.

A parallel study was performed on NSCLC at progression, during or after treatment (Published Abstract Number: 2591)

Patients & Methods

Study Population: Patients were recruited from the pulmonology department at Lyon University Hospital from 2015 to 2022 (Etude RNIPH 22-5065 NUCLEO_CIRCAN avis CSE n°22-5065). Inclusion criteria were: >18 years old with histologically proven NSCLC lung cancer. It was done in routine use. K2EDTA plasma samples from 319 patients at diagnosis defined by a clinical or CT-scan alteration during their treatment from the CIRCAN study were analyzed.

For custom-validated NGS library preparation, 10–100 ng cfDNA were used, using a custom capture-based technology provided by SOPHiA GENECTICS (Lausanne, Switzerland) and performed according to the manufacturer's instructions. The custom panel covered 78 genes involved in cancer (such as EGFR, TP53 or KRAS).

Nu.Q® assay: Quantitative measurement of circulating H3 methylated-nucleosomes in K2-EDTA plasma samples was performed using the Nu.Q® immunoassay (Belgian Volition SRL, Isnes, Belgium) according to the manufacturer's instruction. Sandwich immunoassay was performed on an IDS-i10 automated chemiluminescence immunoanalyzer (Immunodiagnostic Systems Ltd (IDS) UK). Briefly, 50µl of plasma was mixed with an acridinium ester labeled anti-nucleosome detection antibody. Then, an anti-histone modification antibody coated beads were added. Finally triggers solution were added and the signal emitted was measured by the luminometer.

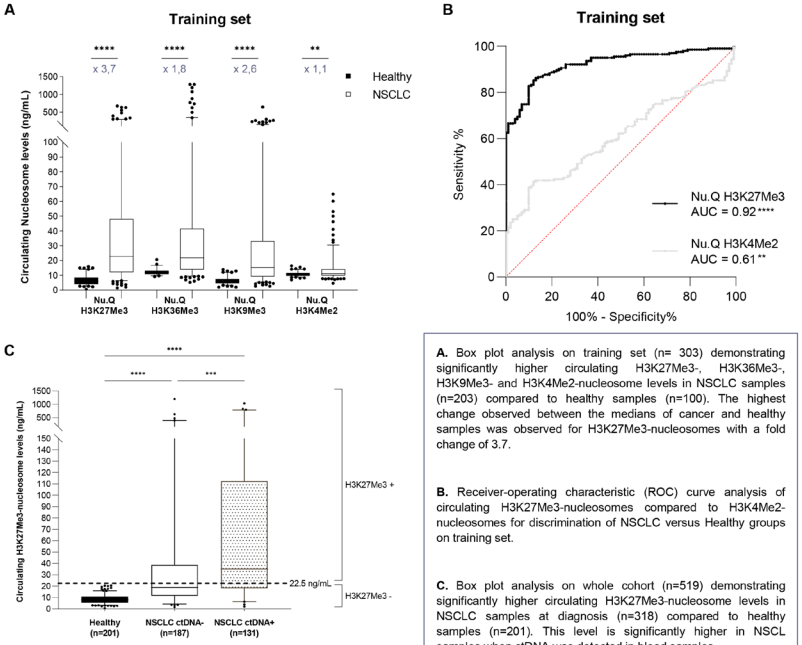
Statistical analyses were performed using GraphPad Prism (GraphPad Prism software version 9.5.0, San Diego, CA, USA). Descriptive statistics were used, and results are reported as mean, median, 2.5^{th}-25^{th}-75^{th} and 97.5^{th} percentiles. Data were subjected to Kolmogorov–Smirnov normality test. As data are not normally distributed, non-parametric methods were used : Mann-Whitney U test and Kruskal-Wallis H test for groups comparison and Spearman's rank correlation for measuring the dependence between variables. Significance values are represented by *: *p-value* < 0.05; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$. Chi Square and related crosstables statistics were applied for testing the hypothesis of equal or different behavior for H3K27Me3 TMA immunostaining in function of the cancer grade nominal variables classified in three groups "weak", "heterogeneous" or "strong").

Funding

We thank AstraZeneca and Volition for financial support for the manpower.
Institutional Review Board Statement: Medical data were collected through a mandatory prescription sheet and edited by the prescribing physician.
The patients were orally informed by physicians of the testing useful for their clinical management.
Informed Consent Statement: All included patients were fully orally informed.

Results

High levels of circulating H3K27Me3-nucleosomes are observed in NSCLC samples at diagnosis

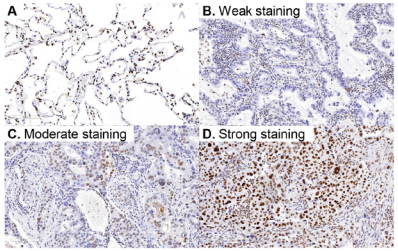


A. Box plot analysis on training set (n= 303) demonstrating significantly higher circulating H3K27Me3-, H3K36Me3-, H3K9Me3- and H3K4Me2-nucleosome levels in NSCLC samples (n=203) compared to healthy samples (n=100). The highest change observed between the medians of cancer and healthy samples was observed for H3K27Me3-nucleosomes with a fold change of 3.7.

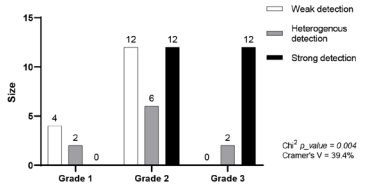
B. Receiver-operating characteristic (ROC) curve analysis of circulating H3K27Me3-nucleosomes compared to H3K4Me2-nucleosomes for discrimination of NSCLC versus Healthy groups on training set.

C. Box plot analysis on whole cohort (n=519) demonstrating significantly higher circulating H3K27Me3-nucleosome levels in NSCLC samples at diagnosis (n=318) compared to healthy samples (n=201). This level is significantly higher in NSCL samples when ctDNA was detected in blood samples.

H3K27Me3 expression in tissue is associated with the Tumor grade in adenocarcinomas



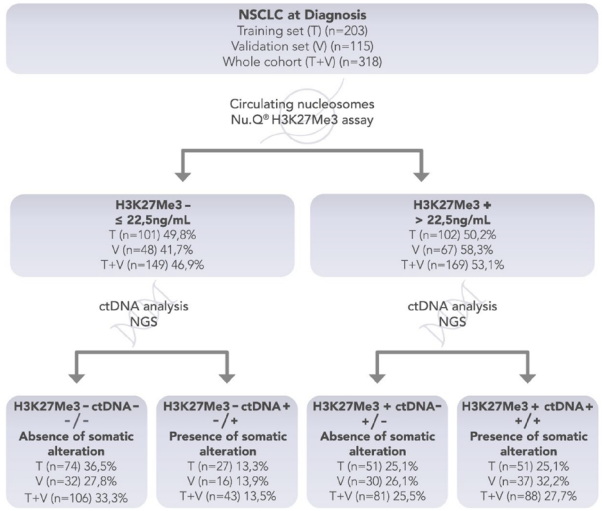
Representative H3K27Me3 immunostaining (10X objective).
(A) Normal lung tissue, the immunostaining is moderately to highly expressed by normal pneumocytes. **(B-D) For the cases of pulmonary adenocarcinoma**, we observed **(B)** in 42% of cases a weak expression of tumor cells (42/100), **(C)** in one third of cases a moderate expression of tumor cells (37/100), and **(D)** in 21% of cases a high expression of tumor cells (21/100).



Chi square statistic was significant ($p = 0.004$), demonstrating a relationship between H3K27Me3 expression and the Grade. We observed a weak immunostaining of H3K27Me3 in tissues in Grade 1 cancers, heterogeneous in grade 2 and strong in grade 3, independently from the Stage of Adenocarcinoma.

Same analyses were performed for TTF1 factor; however, no association was observed between TTF1 and H3K27Me3 immunostaining.

Relevant combined information from circulating H3K27Me3-nucleosome levels and ctDNA molecular profile in NSCLC at diagnosis.



Decision tree proposed for the classification of NSCLC samples at Diagnosis. The decision is based on H3K27Me3-nucleosomes levels below or above 22.5 ng/mL and on presence or absence of circulating tumor DNA (ctDNA) determined on EDTA plasma samples from NSCLC samples at diagnosis.

Take home message and Conclusions

- Circulating H3K27Me3-nucleosome levels are significantly higher in NSCLC samples at diagnosis
- This level is even higher in samples presenting a mutation in one of the 78 representative targeted oncodrivers.
- Expression of tissue level of H3K27Me3 is positively associated with the grade of the tumor.
- We propose to classify samples in 4 sub-groups depending on molecular profiling of the 78 major oncodrivers AND the H3K27Me3 status (below or above the cut-off at 22,5 ng/mL).

H3K27Me3 - / ctDNA - : meaning an absence of tumoral material containing ctDNA or epigenetic biomarkers released or decrease of expression of the H3K27Me3 in the tumor tissue

H3K27Me3 - / ctDNA + : indicating decreased tumor epigenetic remodeling processes in these NSCLC cancer samples

H3K27Me3 + / ctDNA - : indicative of the presence of tumoral epigenetic process

H3K27Me3 + / ctDNA + : pointing to both maintenance epigenetic and mutational burden processes.

Circulating H3K27Me3-nucleosome levels measured by Nu.Q® immunoassay is proposed to be a useful biomarker of the contributive value of circulating tumor DNA (ctDNA) molecular profiling in patient management at diagnosis. High levels of H3K27Me3 and the absence of detecting somatic alterations strongly support the presence of non-mutated ctDNA in the corresponding plasma. This greatly improves the confidence in the negative molecular results in cfDNA in lung cancer. This may reduce invasive tissue re-biopsies

Acknowledgments

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