Early detection of stage I/II Lung Cancer by immunoassay of crosslinked plasma cell free nucleosomes

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Introduction

Cellular chromatin immunoprecipitation (ChIP) studies commonly involve stabilization of nucleoproteins by crosslinking with formaldehyde to preserve protein-DNA interactions.

Sandwich immunoassay methods for plasma cell free nucleosome (cf-nucleosome) measurement are similarly based on ChIP. However, immunoassay of crosslinked plasma cf-nucleosomes has not previously been described.

We hypothesized that crosslinking might stabilize cf-nucleosome conformations, potentially preserving diagnostic information that might otherwise be lost during sample processing.

Here, we report results for the measurement of crosslinked cf-nucleosomes in the plasma of lung cancer patients and healthy volunteers by automated magnetic chemiluminescent immunoassay.

Methods

Plasma samples were collected from 30 treatment naïve patients diagnosed with Lung Cancer, 100 healthy control subjects and 10 subjects with an inflammatory disease (not in a flare). Samples were collected in EDTA plasma tubes containing a formaldehyde releasing agent (Streck Cell-Free DNA BCT). Samples were processed according to the manufacturer's instructions and stored frozen at -80ºC until assayed for the level of crosslinked cf-nucleosomes containing histone isoform H3.1 using the Volition Nu.Q® H3.1 assay.

Declaration of Interest

J Micallef is an employee and shareholder of Volition.

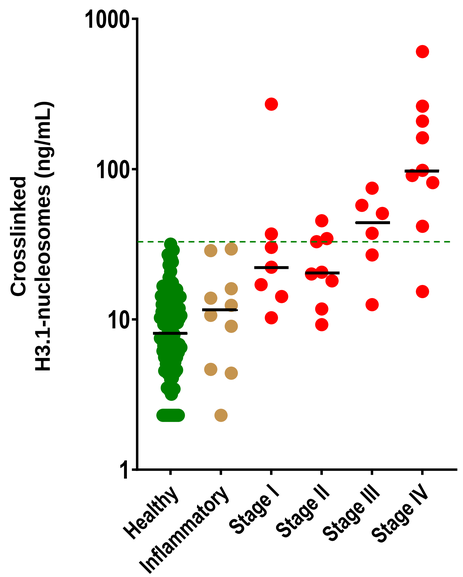

Figure 1

Dot plot of crosslinked H3.1-nucleosome levels measured in plasma samples from asymptomatic healthy volunteers (green, n = 100), non-hospitalized patients diagnosed with an inflammatory disease (beige, n = 10) or lung cancer (red, n = 30)

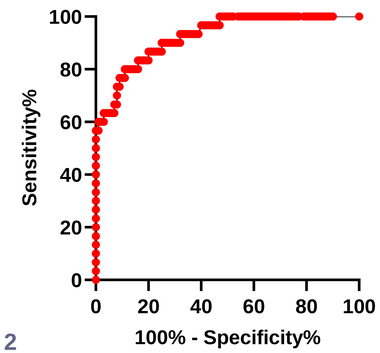

Figure 2

ROC curve for lung cancer (n=30) vs healthy control subjects (n=100). AUC = 92.5%

Results

We set a cut-off level for healthy subjects at the highest crosslinked cf-nucleosome level observed for any healthy subject to obtain elevated cf-nucleosome levels in lung cancer patients at 100% observed specificity.

All 10 inflammatory samples tested negative.

We observed that 17 of the 30 lung cancer samples tested were positive for elevated crosslinked cf-nucleosome level, including 5 of 15 stage I/II lung cancer patients, at 100% observed specificity.

Sensitivity for lung cancer (all stages) = 57%

Sensitivity for early stage I/II lung cancer = 33%

Observed specificity = 100%

Disease Stage	Sensitivity at 100% specificity
I	29% (2/7)
II	38% (3/8)
III	67% (4/6)
IV	89% (8/9)

Table 1

Assay sensitivity by disease stage (% positive (number positive/number tested)).

Conclusions

Crosslinked plasma cf-nucleosome measurements show promise for the early identification of subjects at high risk of lung cancer by rapid, low-cost immunoassay.

The next stage of research will also include the assessment of additional nucleosome biomarkers.

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