Circulating Nucleosomes in Lung Cancer Diagnosis following low-dose computed tomography

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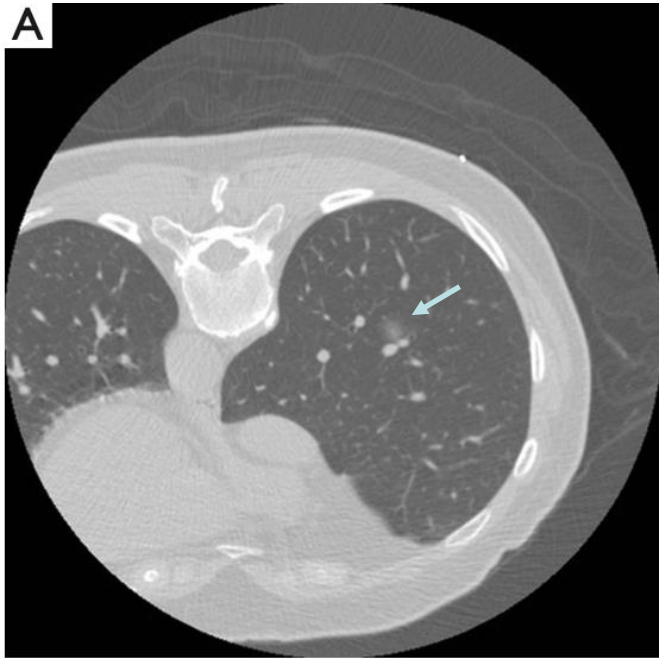
DISCLOSURES

Commercial Interest	Relationship(s)

This work was done in collaboration with the company Volition

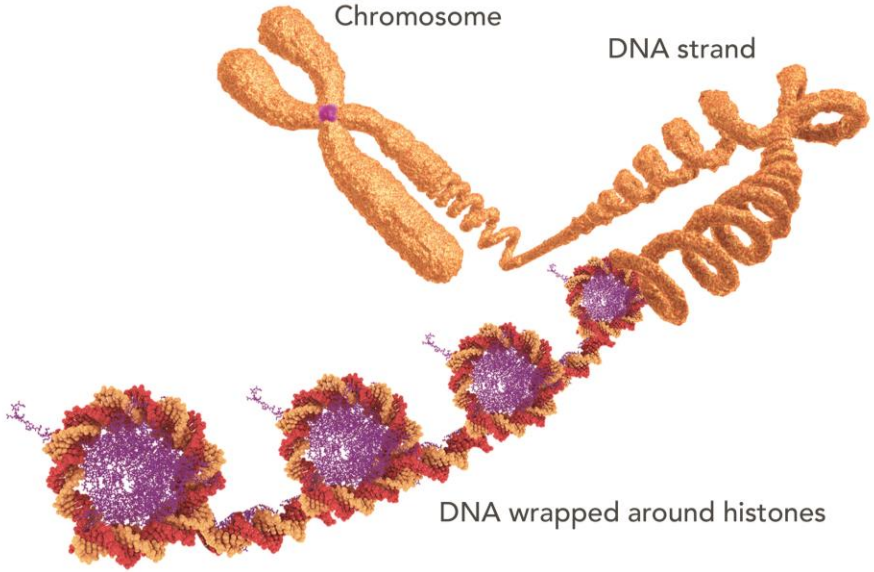
Lung Cancer Diagnosis: How to better discriminate between malignant and non-malignant nodules?



Tsai et al., *Ann Transl Med* 2019;7(2):31

- Low-Dose Computed tomography (LDCT) is the widely accepted standard for screening of individuals at high risk of lung cancer (LC).
- However, LDCT has several limitations including the **high prevalence of non-malignant nodules** detected leading to **overdiagnosis**, the potential harms of **cumulative radiation dose** and **poor adherence** to recommended follow-up
- Therefore, a **novel blood-based tests** could offer a simple follow confirmation approach to help to discriminate between lung cancer and non-malignant nodules.

Circulating Nucleosomes and Epigenetic Modifications



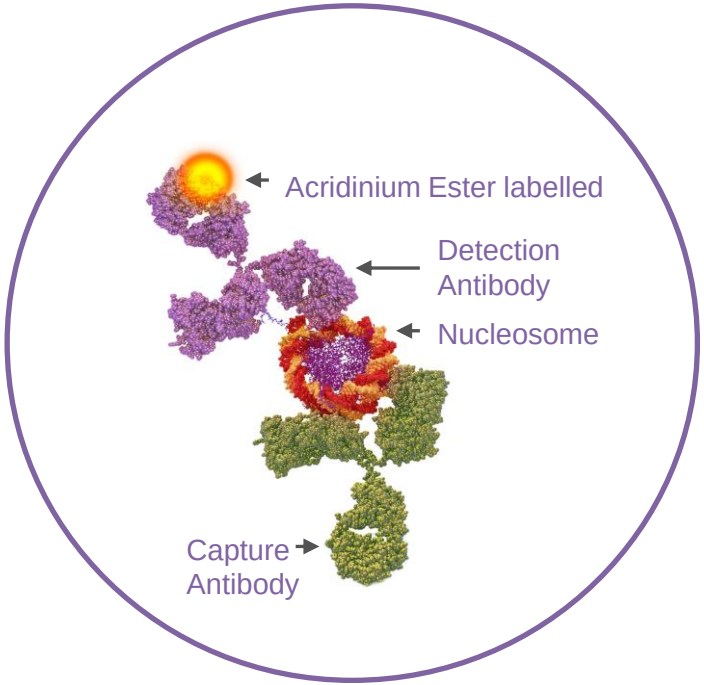
- In the nucleus of eukaryotic cells, **DNA** is wrapped around eight histone proteins to form the fundamental repeating unit of chromatin called the **nucleosome**.
- Each nucleosome octamer is composed by two copies of each core **histone protein**: H2A, H2B, H3 and H4.
- All core histones could also have a variety of **epigenetic modification**: histone post-translational modifications (PTMs).
- Cancer leads to cell death which results in fragmentation and **release of nucleosomes into the blood**.

Methods: Prospective collection of 220 patients:

- **220 patients referred for CT scans** at National Taiwan University Hospital.
- The patients were later confirmed to have either a **Lung Cancer (LC)**, or non-malignant nodules (**Benign Nodule (BN)**) or **no-nodules**.
- Whole blood was collected in EDTA plasma tube
- Interim report on 1,200 patient study

Diagnosis	Patients (n)	Age (median, IQR)	Male:Female	Smoker:Nonsmoker	Family History: No Family History
Lung Cancer	100	62 (53-70)	39:61	20:80	32:68
Stage 0-I	67	60 (50-67)	16:51	4:63	23:44
Stage II	3	66 (52-68)	2:1	1:2	1:2
Stage III	15	69 (55-73)	10:5	9:6	5:10
Stage IV	15	66 (55-71)	11:4	6:9	3:12
Benign Nodules	50	58 (50-66)	20:30	9:41	15:35
Healthy	70	57 (45-66)	23:47	12:58	32:38

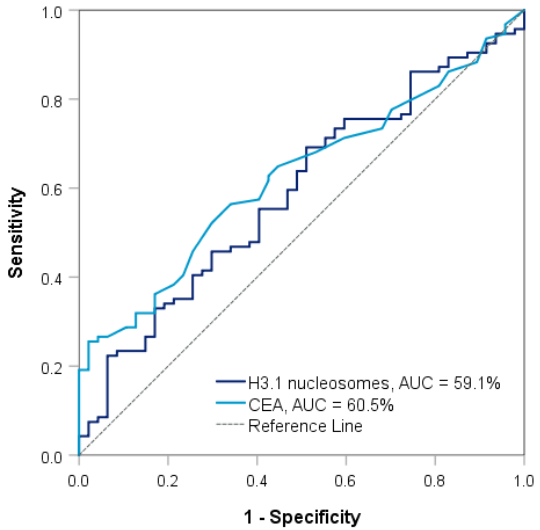
Methods: Nu.Q™ Immunoassays



- Volition **Nu.Q™ immunoassays** target nucleosomes in blood containing different variant or histone post-translational modifications (PTMs) and measure their circulating levels.
- sandwich immunoassays are based on magnetic beads and chemiluminescence technology and were performed on an automated platform
- Levels of circulating **H3.1 nucleosomes** (Nu.Q™ H3.1) or methylated lysine 27 of histone H3 (Nu.Q™ **H3K27Me3**) or methylated lysine 36 of histone H3 (Nu.Q™ **H3K36Me3**) were evaluated

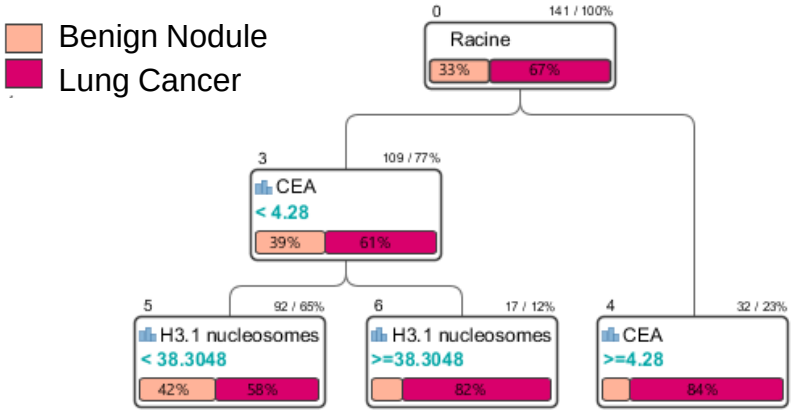
Combination of circulating H3.1 nucleosomes and CEA discriminates between Lung Cancer and Benign Nodule

ROC curve individual biomarker: circulating nucleosomes and CEA



Sensitivity at 90% specificity :
H3.1: 23%
CEA: 26%

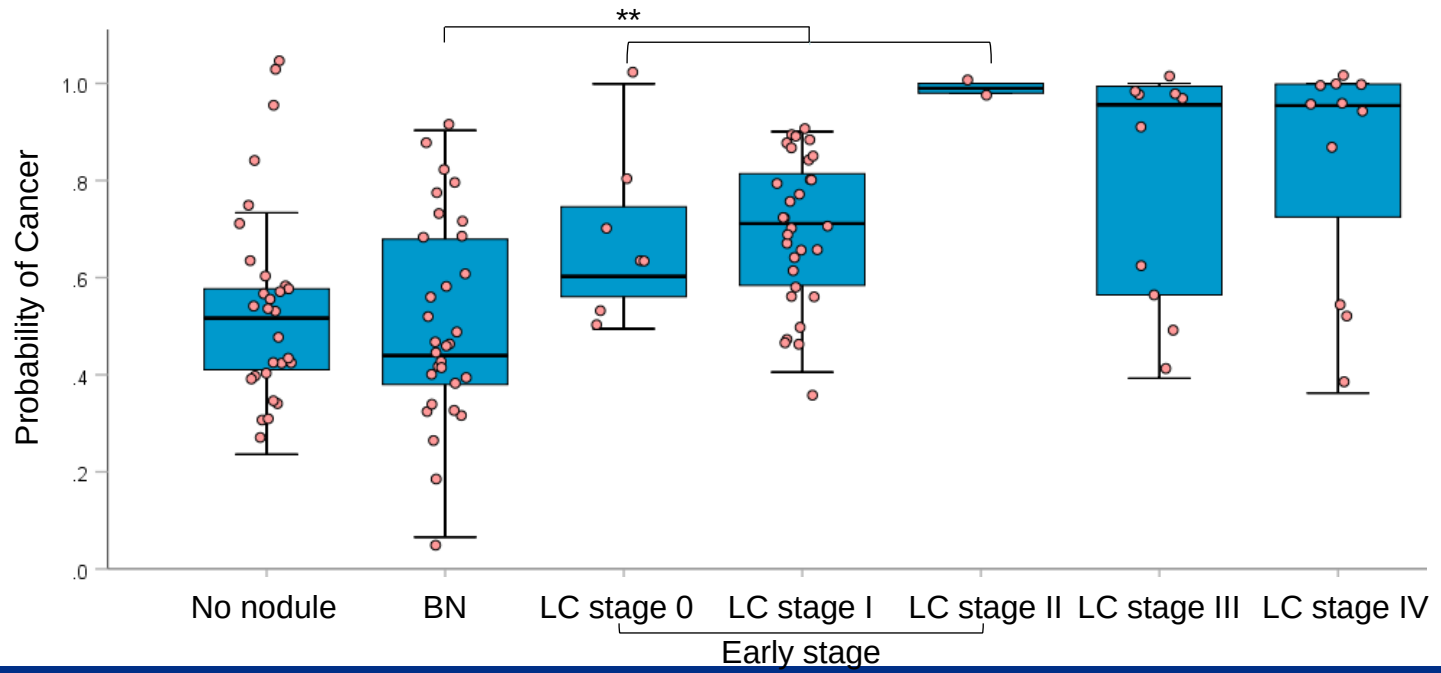
Decision Tree Method



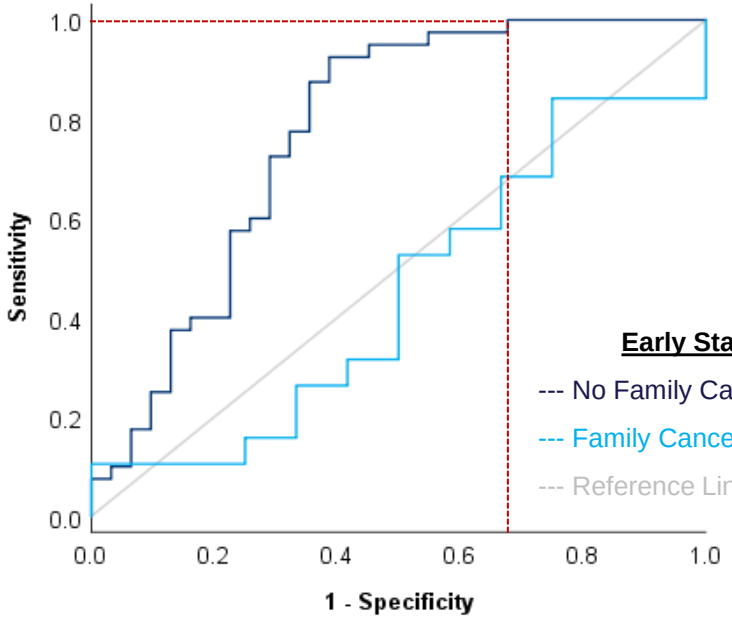
Sensitivity of 41% at 90% specificity

Use of Histone PTMs dramatically improves the detection of **early stage (0,I,II) non-familial Lung Cancer sub-group**

Combination of H3K27Me3, H3K36Me3, CEA



Potential *rule-out* method to reduce the number of unnecessary biopsy or repeated scan



Early Stage LC (Stage 0-I II)

--- No Family Cancer History; AUC = 77%

--- Family Cancer History; AUC = 45%

--- Reference Line

Combination of H3K27Me3, H3K36Me3, CEA

Specificity of 32% at 100% sensitivity for early stages lung cancer vs benign nodule for patients with no lung cancer family history

Take-Home Message

- **LD-CT scan detects pulmonary nodules, but it is known to not be specific.**

Data from this interim analysis suggest that Nucleosomes and Histone PTMs may:

- **discriminate between benign nodule vs early-stage LC from non-familial LC history patients.**
- **provide a non-invasive blood test to help rule-out lung cancer in cases of non-malignant nodules to reduce unnecessary biopsy and the frequency of radiation exposure.**

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