The epigentic fingerprint of asbestos-exposed lung cancer

Validation study

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Asbestos

Asbestos: A group of 6 naturally occurring, fibrous silicate minerals

Associated cancers: Mesothelioma, lung and ovarian cancer

Most important route of exposure: Inhalation

Uses: Found in roofing, thermal and electrical insulation, cement pipe and sheets, friction materials (such as automobile brakes), and other products

Occupational exposures: construction sector

Environmental exposures: Via buildings that have deteriorating asbestos insulation or have undergone poorly performed asbestos removal

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Asbestos-induced lung diseases

- Pleural disease

- Mesothelioma

- Asbestosis

- **Lung cancer**

  Comparable distribution of histological subtypes as in population not exposed to asbestos
  - 45% adenocarcinoma
  - 40% squamous cell carcinoma
  - 5% small cell carcinoma
  - 5% other histological cancer types including sarcoma

  → No histological characteristics that enable distinction of asbestos-related from asbestos-unrelated lung cancer.
  → Huge (90%) overlap between exposure to asbestos and cigarette smoking.
  → *KRAS* mutations but not other mutations commonly see in non-exposed lung cancers

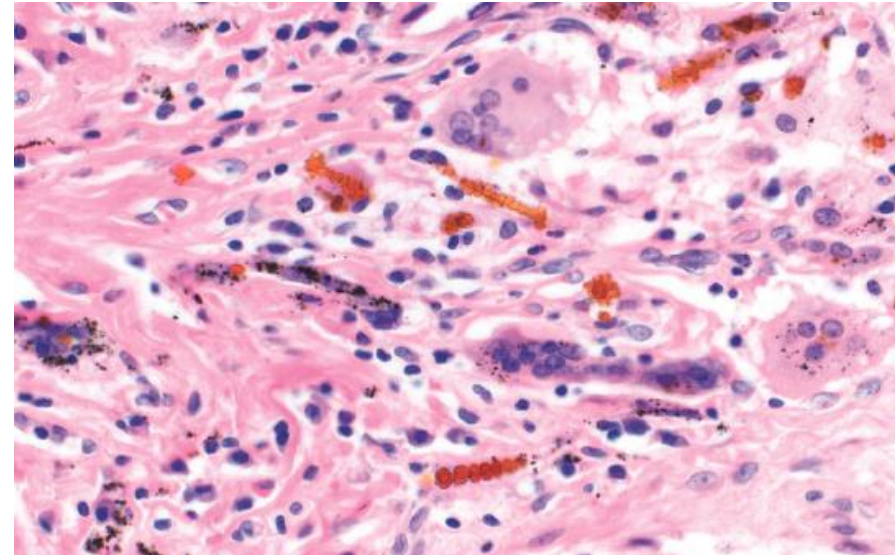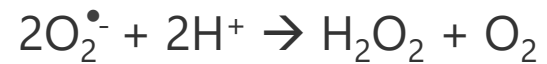# Diagnosis of asbestos-induced lung cancer

Country-specific diagnostic criteria for asbestos-related lung cancer include:

- Medical criteria
  - Histological confirmation of malignancy

  - Presence of asbestos-related lung disease: asbetosis or pleural plaques
    often not documented

  - Quantification of asbestos fibres per g dry lung tissue or in alveolar lavage
    Not routinely determined

- Exposure criteria
  - Documented fibre years

- Latency

→ Lack of biomarkers

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Carcinogenic effect of asbestos

Asbestos causes reactive oxygen and nitrogen species (ROS, RNS)

Asbestos types	Chemical composition
Chrysotile	$Mg_3[Si_2O_5](OH)_4$
Amosite	$Fe^{2+}_7[Si_8O_{22}](OH)_2$
Crocidolite	$Na_2Fe^{3+}_2Fe^{2+}_3[Si_8O_{22}](OH)_2$
Tremolite	$Ca_2Mg_2[Si_8O_{22}](OH)_2$
Anthophyllite	$(Mg, Fe^{2+})_7[Si_8O_{22}](OH)_2$
Actinolite	$Ca_2(Mg,Fe)_5Si_8O_{22}(OH)_2$

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$$

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$$
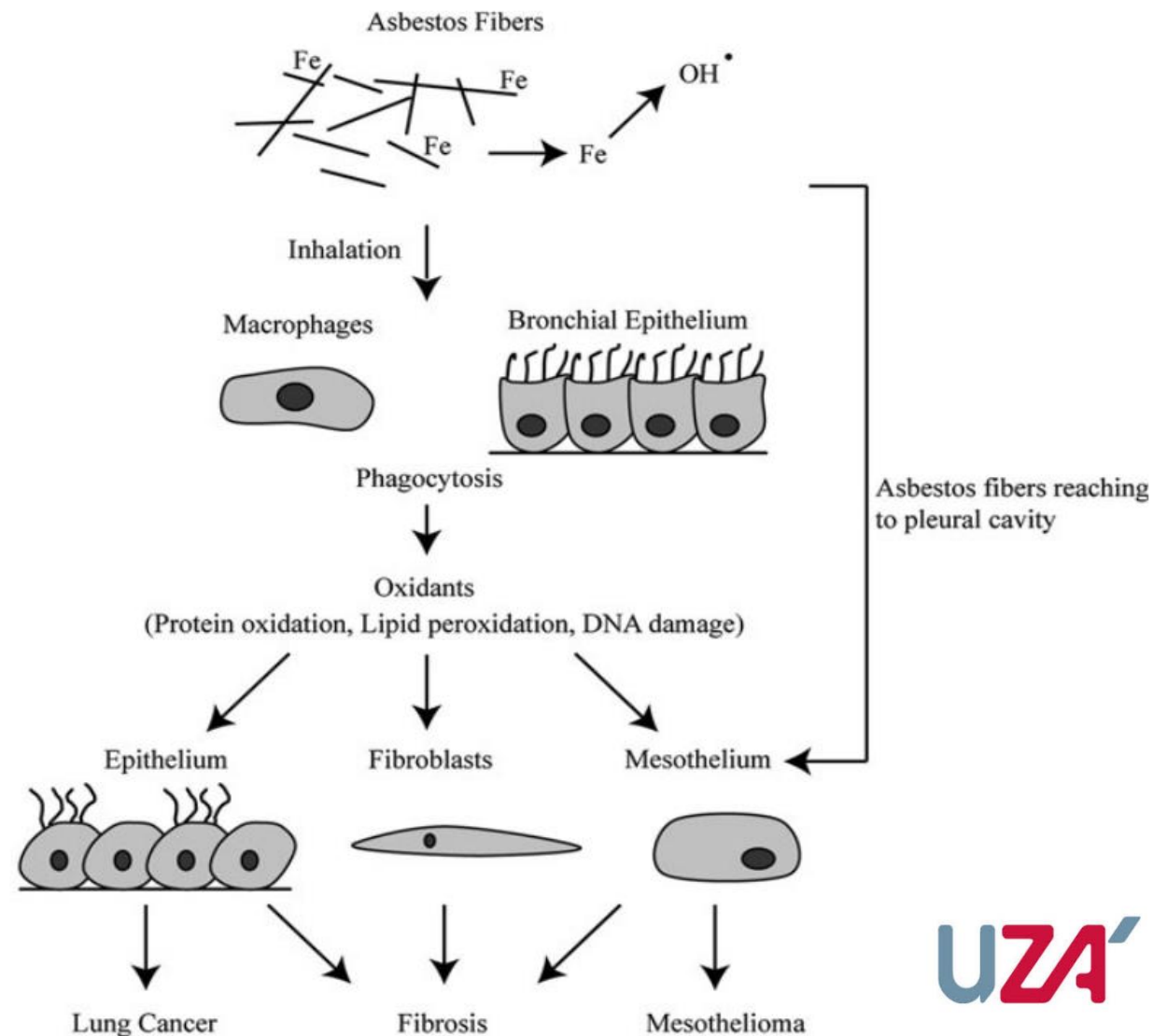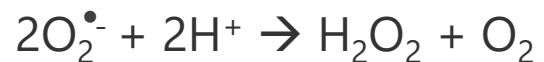
$$O_2^{\bullet -} + NO \rightarrow ONOO^-$$

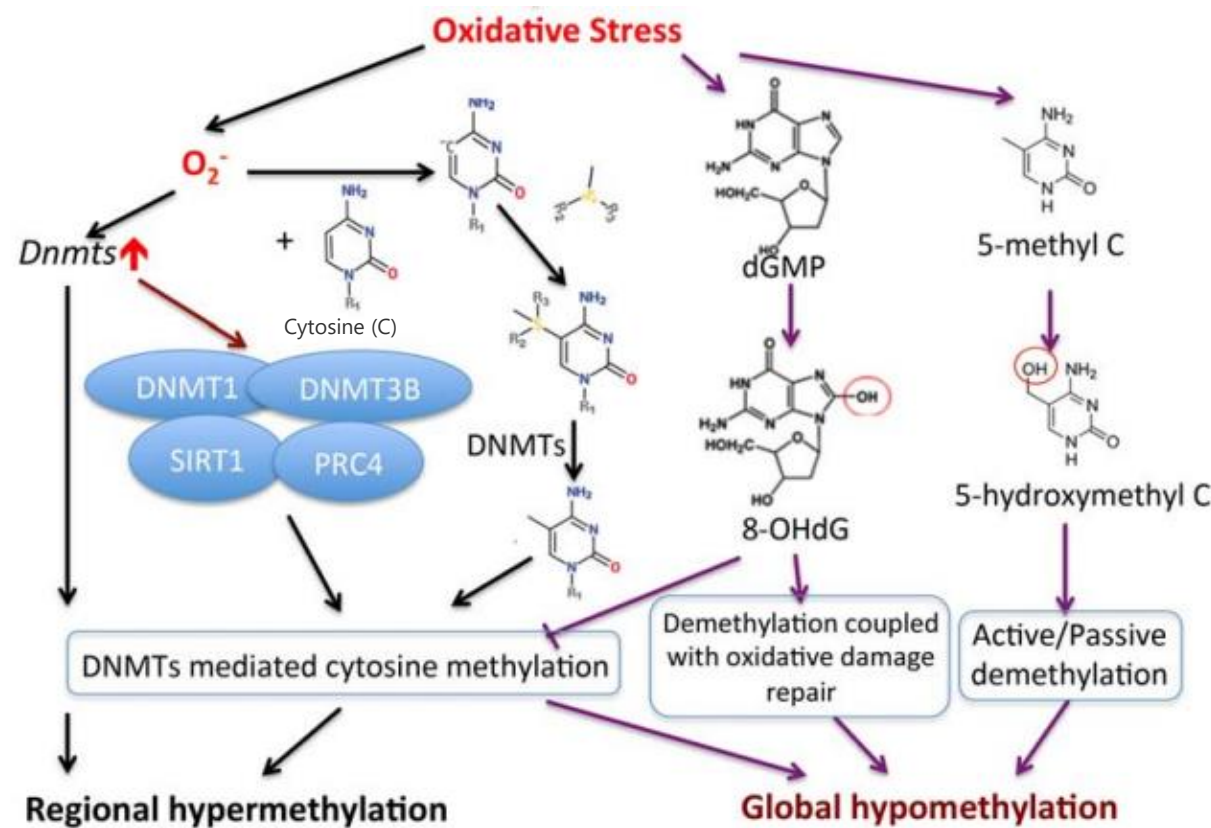
Butt and Tazelaar. Atlas of Pulmonary Pathology 2022
Iron stain
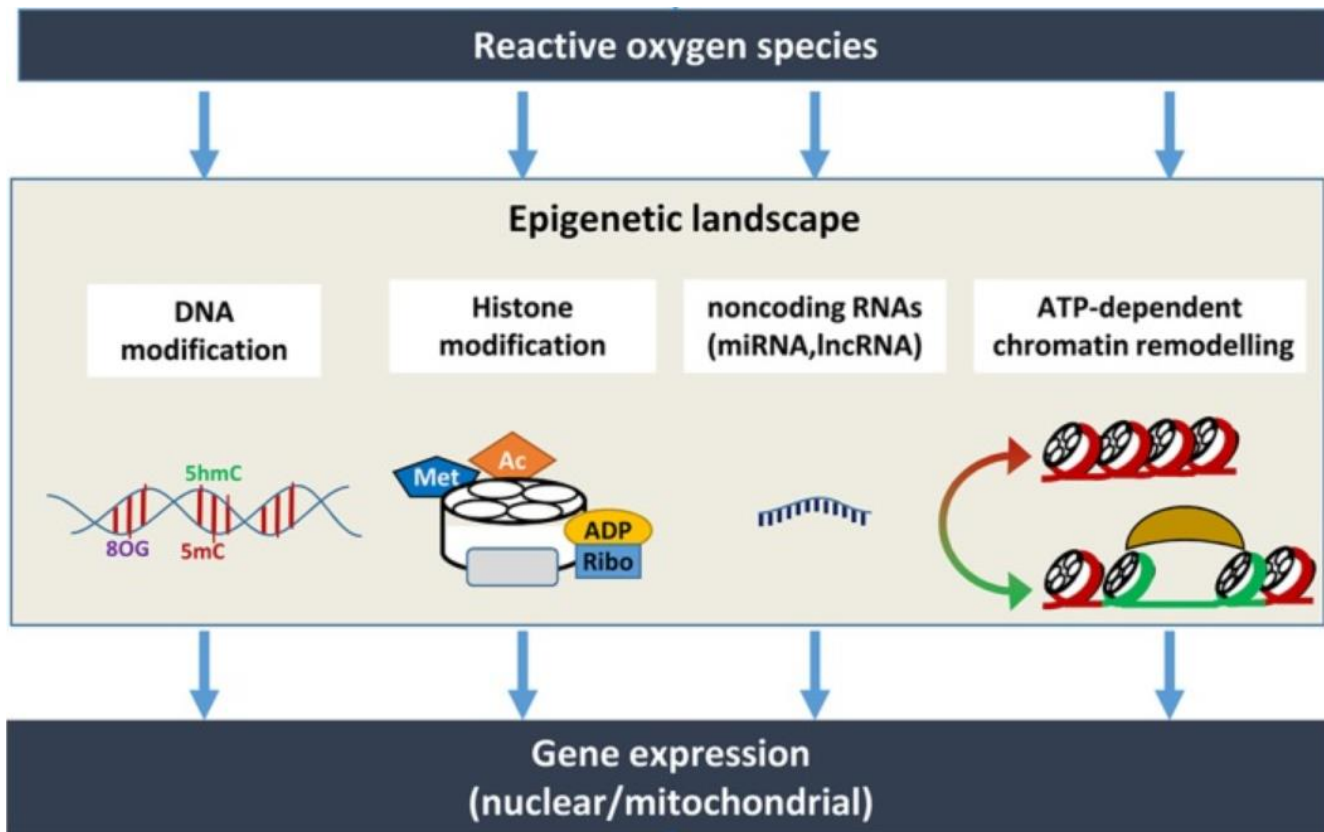
Carcinogenic effect of asbestos

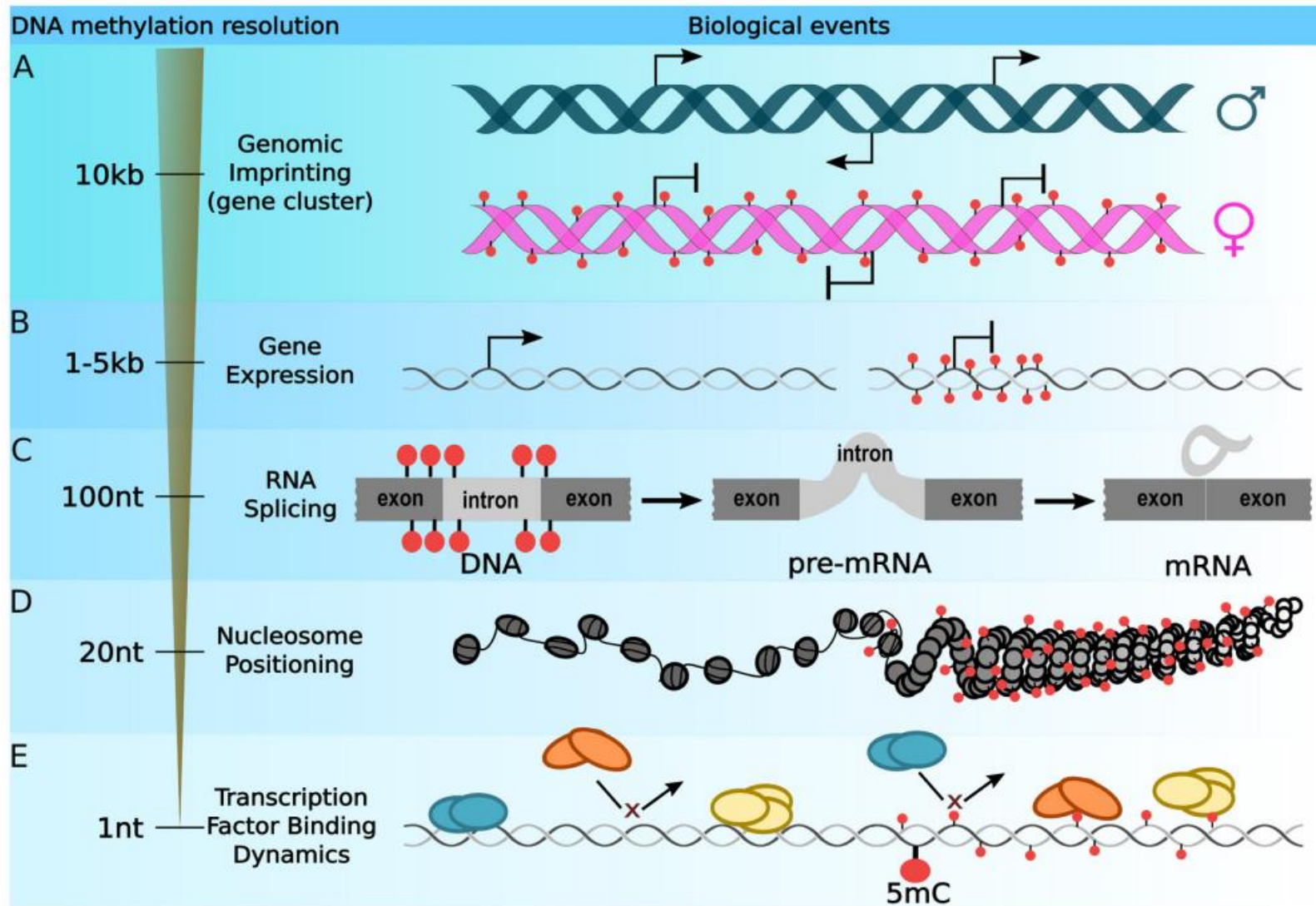Asbestos causes reactive oxygen and nitrogen species (ROS, RNS)

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Asbestos Fibers
Fe, Fe, Fe → Fe, OH•

Inhalation

Macrophages / Bronchial Epithelium

Phagocytosis

Oxidants
(Protein oxidation, Lipid peroxidation, DNA damage)

Asbestos fibers reaching to pleural cavity

Epithelium / Fibroblasts / Mesothelium

Lung Cancer / Fibrosis / Mesothelioma

Oxidative stress alters the epigenetic landscape

Kietzmann et al Br J Pharmacol 2017, DOI 10.1111/bph.13792

Wu and Nie Current Drug Targets 2015 DOI: 10.2174/1389450116666150113121054

Methylation: Epigenetic regulation of gene expression

Tirado-Magallanes et al. Oncotarget 2017 DOI: 10.18632/oncotarget.13562

Creates chronic asbestos-induced oxidative stress a fingerprint ? → Potential biomarker

DNA methylation profiles in lung cancer (Kettunen et al, 2017)

Discovery set (genome-wide)

14 Lung cancer and adjacent non-tumor lung tissue

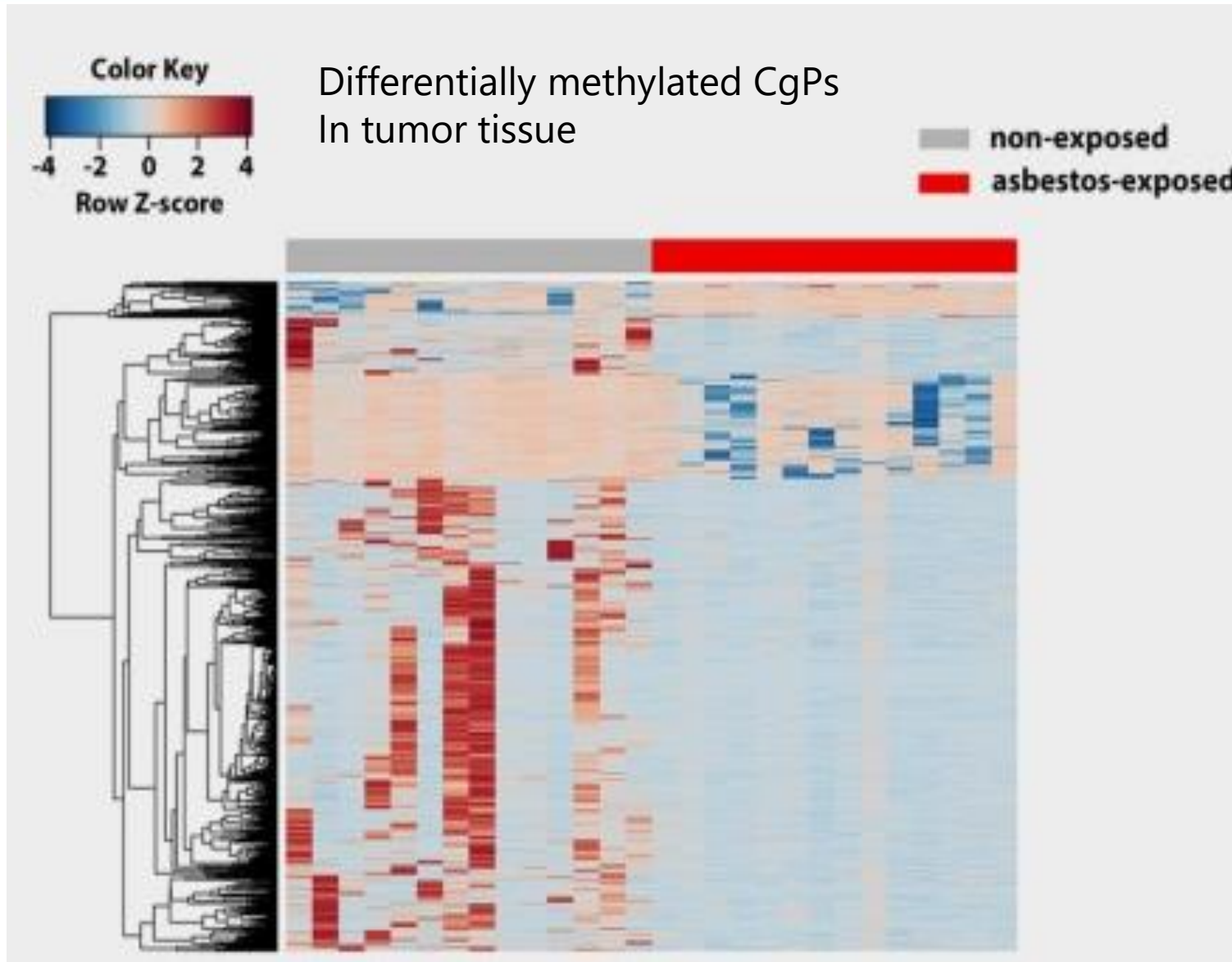14 Lung cancer asbestos-exposed and adjacent non-tumor lung tissue

6 Control (normal lung, non-cancer)

Validation set (targeted)

40 Lung cancer and adjacent non-tumor lung tissue

51 Lung cancer asbestos-exposed and adjacent non-tumor lung tissue

Differentially methylated CgPs
In tumor tissue

Color Key legend: Row Z-score, -4 -2 0 2 4

non-exposed (grey)
asbestos-exposed (red)

Discovery set
Top 999 CpG sites that discriminate asbestos-exposed from non-exposed tumors.
→ Global hypomethylation.

Validation set :
30 CpG sites were selected for validation
5 sites displayed ~80% reduction in methylation:
RARB, *GPR135*, *MYT1L*, *TPO*, *RPTOR* in asbestos-exposed tumors.

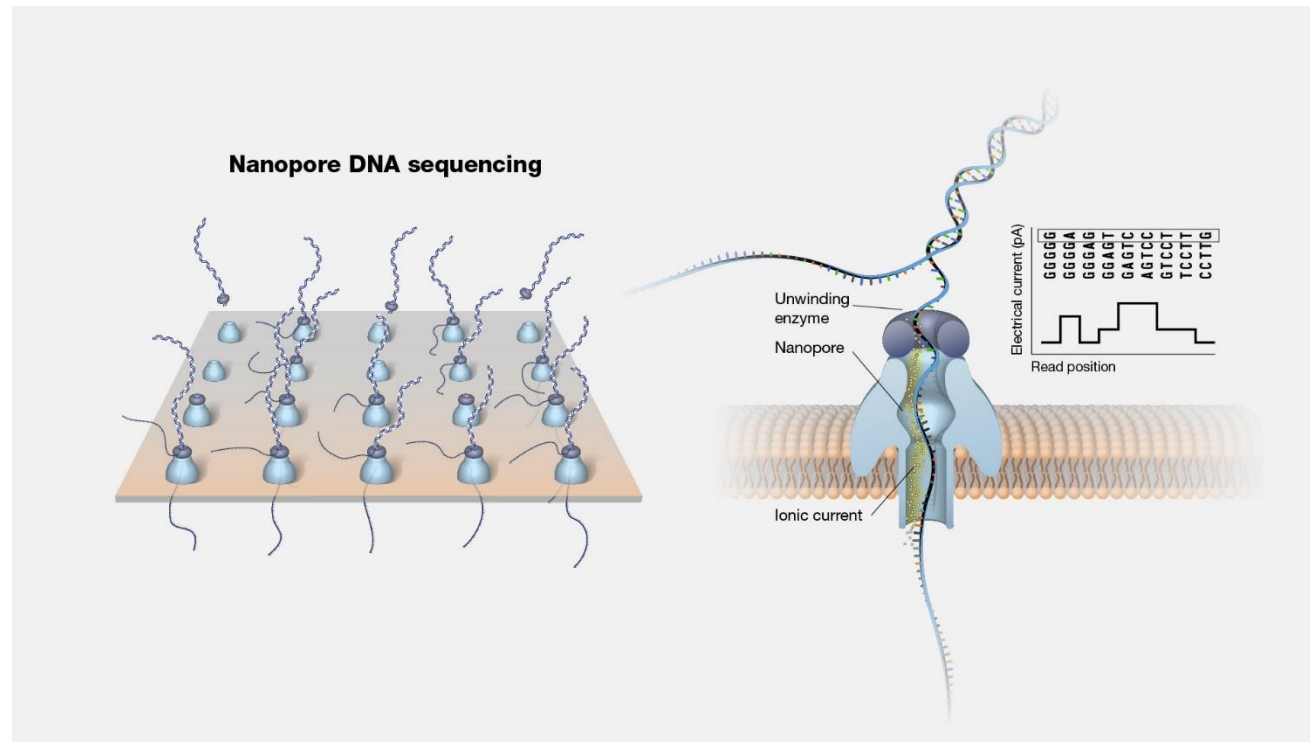Not a black & white difference.

Validation approach

- Validation of individual sites is time consuming (e.g. PCR). Individual data points.

- Genome-wide validation (e.g. array, genome-wide DNA methylseq) is cost prohibitive

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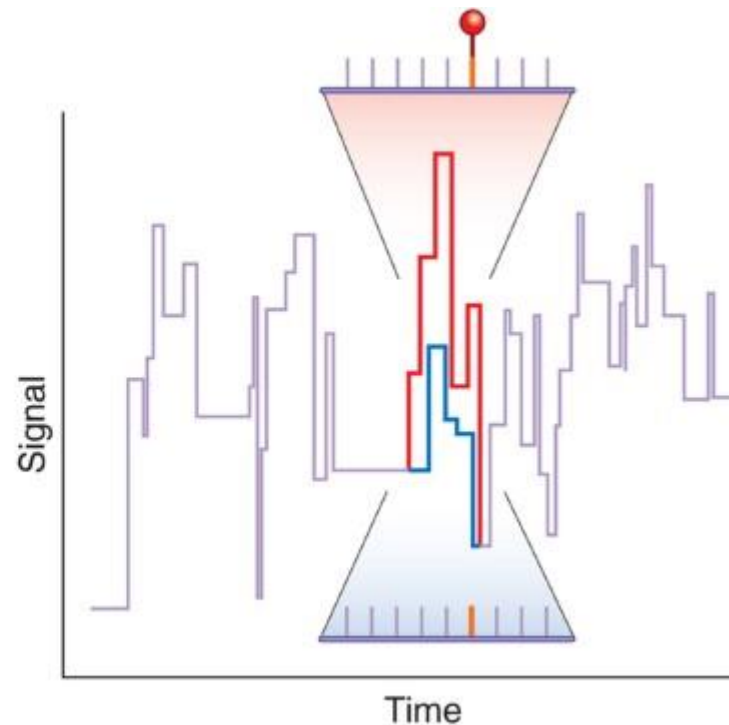
Nanopore sequencing

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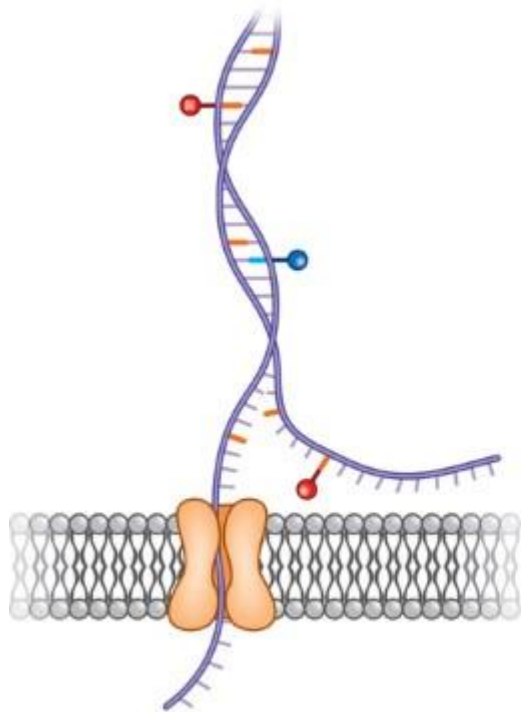
Nanopore methyl sequencing

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Nanopore methyl sequencing

Ultra-fast deep-learned CNS tumour classification during surgery

Transformation of the array-based glioma methylation classifier into a rapid nanopore DNA methylation sequencing test

Vermeulen et al. Nature 2023, 10.1038/s41586-023-06615-2

Cohort UZA biobank:

40 asbestos-exposed lung cancers : tumor and adjacent non-involved tissue

40 non-exposed lung cancers : tumor and adjacent non-involved tissue

Methodology Oxford nanopore targeted methyl DNA sequencing at UZA dept of Pathology

'Kettunen methylation signature'

Endpoint *Primary*

Lung cancer methylation signature associated with asbestos exposure

→ molecular biomarker

Secondary

Evolution of methylation signatures in relation to exposure / fiber load

Confirmation of the signature in an independent series of samples

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