COMBINING MASS SPECTROMETRY IMAGING AND TOP-DOWN PROTEOMICS TO PREDICT IMMUNOTHERAPY RESPONSE IN NON-SMALL-CELL LUNG CANCER PATIENTS

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State of the art

Advanced non-small-cell lung cancer (NSCLC) is generally linked with a poor prognosis and is one of the leading causes of cancer-related deaths worldwide. Immunotherapy might be a valid alternative in the lung cancer treatment field, as immunotherapy attempts to strengthen the body's own immune response to recognize and eliminate malignant tumor cells. However, positive response patterns to immunotherapy remain unclear, so it is of great importance to determine **which and where immune-related proteins/peptides** are expressed within the lung tumor microenvironment to provide crucial insights into the interplay between tumor cells and adjacent immune cells.

Objectives

Clustering into NSCLC patient subgroups based on individual expression pattern (MSI)

Response pattern to immunotherapy

Non-responder to immunotherapy

MSI as compagnion diagnostic for pre-treatment biopsies

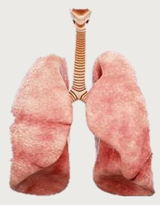
- minimize the toxicity for the patient
- maximum therapy response

→ Better quality of life of NSCLC patient

Linkage MSI and top-down proteomics for biomarker discovery

MALDI Mass spectrometry imaging (MSI)

MALDI mass spectrometry imaging generates an unbiased molecular profile of the lung tumor microenvironment, as MSI allows us to produce spatially resolved mass spectrometric data directly from a tissue without destroying the tissue morphology. This makes a correlation with histological data possible. The main advantage of MALDI MSI over traditional peptidomics and/or proteomics is that the spatial information of the peptides and proteins is retained throughout the tissue.



Fresh frozen human lung tissue (Biobank@UZA)

Tissue sectioning

MSI analysis

Laser ablation
Tissue scanning

Matrix application
2,5-DHB

Lipid removal
Carnoy's washing procedure

Tissue homogenization
Methanol extraction

Lipid removal
n-hexane

Extracted endogenous peptides

LC-MS/MS analysis of intact peptides and/or proteins

Alcohol wash to remove matrix

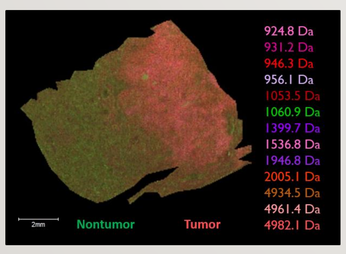
HE staining

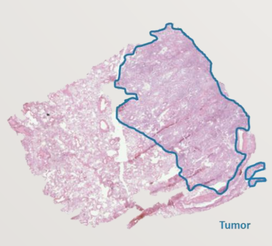
Top-down peptidomics/proteomics

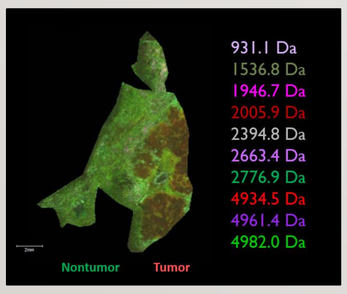
By linking MSI data directly with top-down peptidomics and/or proteomics from consecutive tissue homogenates, it is possible to reliable identify MSI targets with an interesting distribution observed with MALDI MSI. Top-down experiments have the advantage to retain information about possible post-translational modifications (PTMs) and the obtained m/z value of a single molecule corresponds the m/z value of the intact molecule observed in mass spectrometry images.

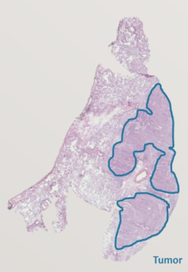
MSI results of lung tissue

MALDI MSI (rapifleX; Bruker) has been used to study the peptidomic differences in fresh frozen non-small-cell lung cancer (NSCLC) tissues. This allows to distinguish the cancerous lung tissue from adjacent normal lung tissue in lung periphere tissues.







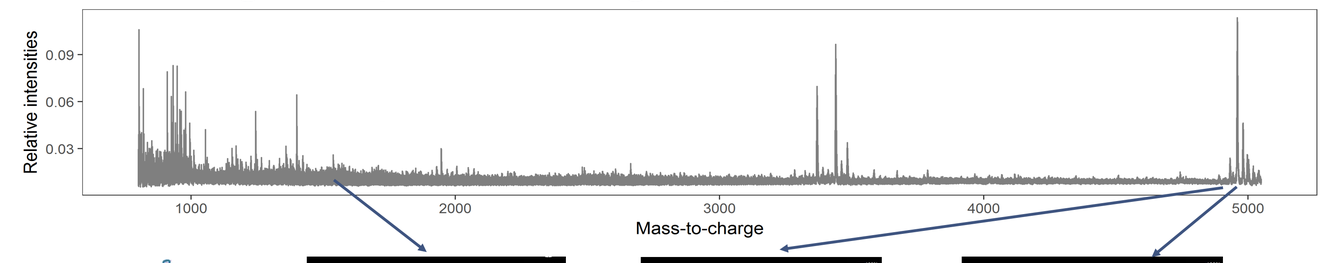


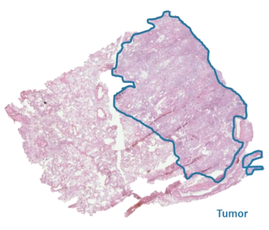
Results MSI data linked with top-down peptidomics

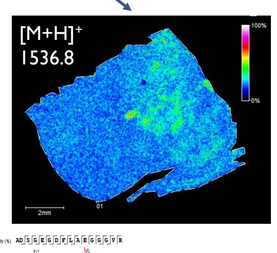
MSI data (rapifleX; Bruker)

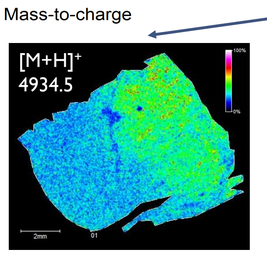
Targeted LC-MS/MS analysis for identification of MSI targets (Q Exactive Plus)

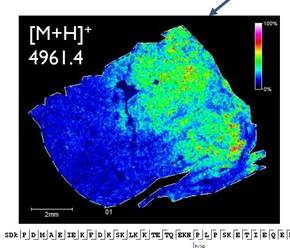
Overall average MSI spectrum of lung periphere tissue

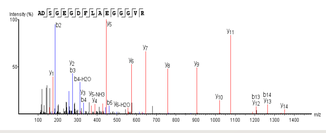




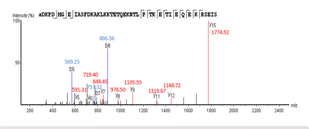






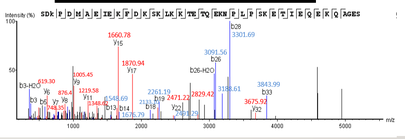


Fibrinopeptide A



Thymosin β10

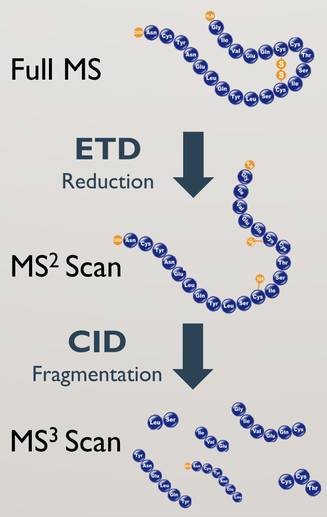
PTM: N-acetylated (+42.011 Da)



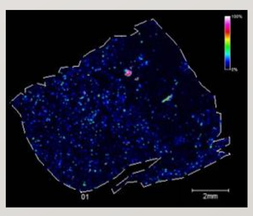
Thymosin β4

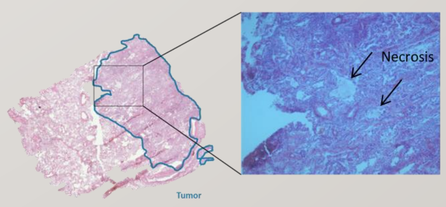
PTM: acetyllysine (+42.011 Da)

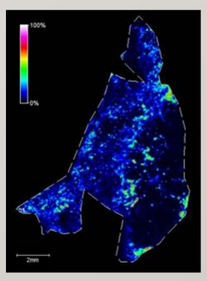
MSI for biomarker discovery

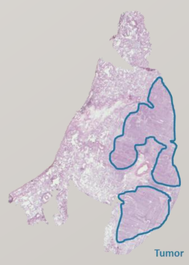


Differential expression analysis of peptides in tumor versus nontumor region, revealed extra peptides that show an interesting distribution and characterize different regions. With LC-MS/MS, corresponding fragmentation spectra could not be acquired, likely due to the presence of disulfide bridges. We developed a method where the peptides are reduced by electron-transfer dissociation (ETD), directly followed by fragmentation of the reduced peptide with collision-induced dissociation (CID) (LTQ Velos). These identified peptides are described as potential biomarkers for many cancers, although their definitive contribution to NSCLC need to be confirmed. Physiological studies to determine their biological activity against NSCLC are ongoing.









Conclusion

To study the molecular tumor microenvironment of lung cancer, mass spectrometry imaging (MSI) linked with top-down proteomics has been recognized as a powerful tool to accurately identify differential features in lung cancerous tissues. With MSI, lung cancer patients can be clustered into subgroups, based on their individual protein/peptide expression profile in pre-treatment biopsies, for whether or not immunotherapy will be beneficial. In addition, this study is another example where combining information on distribution obtained with MSI and proteomics for identification of potential targets are used for biomarker analyses.

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