Abstract

MALDI Imaging is a technique that allows the direct detection of proteins, lipids, drugs and metabolites in tissue and when integrated with histology becomes a powerful tool for molecular histology. In contrast to other molecular histological techniques, it does not require a molecular probe and can therefore be used for the discovery of new biomarkers. In recent years, MALDI Imaging has been used successfully to identify biomarkers for a number of clinical cases. However, there has been discussion in the field whether the resulting protein markers are merely surrogate markers for disease states or if they also have functional implications.

This mini-review focuses on recent imaging studies that use validation strategies to demonstrate a functional role of identified markers within the respective disease pathways. We include also one example of a recently published drug-safety study with important implications for human safety.

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MALDI Imaging reveals mechanism of neoadjuvant chemotherapy resistance in Barretts carcinoma

In oesophageal adenocarcinoma the patient is usually treated with a neoadjuvant chemotherapy consisting of Cis-Platin and 5-Fluorouracil to shrink the tumor prior to surgery. However, a large percentage of patients do not respond to this treatment. Aichler et al. [1] has used MALDI Imaging to compare protein profiles from tissue samples from 10 patients who responded to treatment and 13 patients who were non-responders. They found that 22 of the 150-250 proteins detected were differentially regulated between responders and non-responders. Four of the proteins down regulated in responders were identified as mitochondrial proteins, COX7A2, COX6B1, COX6C and complex I-MLRO. From this observation it was hypothesised that mitochondria might be involved in the drug resistance. Using electron microscopy to examine the mitochondria of responders and non-responders it was found that the mitochondria of non-responders presented a normal phenotype while the mitochondria of the responders appeared abnormally large and with damaged cristae.

The authors further hypothesized that mitochondrial damage might lower the threshold of the cancer cells for cell death and consequently pre-dispose the cells to respond positively to the chemotherapy. The protein COX7A2 exhibited the strongest correlation between chemotherapy response and the MALDI Imaging and so COX7A2 knock-out cells were cultured and treated with the chemotherapeutic drugs. The knock-out tumor cells showed a distinct change in mitochondrial phenotypes to a “cup-shape” similar to that seen in the responder cohort after neoadjuvant chemotherapy, confirming the hypothesis. Moreover, these cells exhibited a significantly lower threshold to cell death which also confirmed the hypothesis on the role of mitochondria in the response to chemotherapy.

This study stands out because it linked the protein changes seen in MALDI Imaging to a functional understanding of a drug resistance. It is also a prime example for a “reverse-translational” study, where the initial findings were made in patients and the mechanism was confirmed by model experiments, and it shows how knowledge gene-rated by MALDI Imaging can have potential implications for personalized medicine.

MALDI Imaging reveals posttranslational changes in histones are linked to microvascular invasion in hepatocellular cancer

Hepatocellular carcinoma is a leading cause of cancer-related deaths. Long-term prognosis is poor due to high recurrence rates and microvascular invasion (MiVI) is a major risk factor for recurrence and mortality. Usually, MiVI cannot be assessed in pre-operative biopsies, because the sampled volume is too low for clinical assessment using known gene expression signatures. MiVI can only be determined after surgical resection and histological analysis of the complete tumor. There is a need for predictive proteomic markers that can be assessed with immunohistochemical staining of routine biopsies prior to resection to better direct therapeutic strategy. Poté et al [2] used a MALDI Imaging strategy to investigate a discovery set of hepatocellular carcinoma tissues that presented both with and without MiVI with the aim of finding diagnostic protein biomarkers. The discovery set consisted of 30 patient samples presenting MiVI and 26 patient samples without MiVI. Comparative analysis of image data from these patient groups identified 30 mass spectrometric peaks that were differentially expressed between the groups. Two of the signals were identified as modified forms of Histone H4: both acetylated at the N-terminus and dimethylated at Lysine 20 and one with an additional acetylation at Lysine 16. The modified histone markers were confirmed in an independent validation cohort of 23 samples by immunohistochemistry and western blot analysis.

Histones have a regulatory function in transcription, and post-translational modifications such as acetylation and methylation play a role in this regulation. Furthermore, the modification of histones leads to less tight wrapping of the DNA in the nucleus and therefore to an increased transcription of the regulated genes into RNA. To our knowledge, this is the first study that linked MALDI Imaging data of post-translationally modified proteins to a histological disease state.

This study stands out, because it shows for the first time functional implications of post-translationally modified proteins observed directly by MALDI Imaging. In doing so it underpins the potential of MALDI Imaging as a true top-down technique.
MALDI Imaging was used to find a new prognostic marker in HER2 positive breast cancer

The HER2 receptor status is a well studied property in breast cancer. The overexpression of this receptor leads to an increased proliferation of tumor cells and is linked to an unfavorable outcome. There is a targeted individualized therapy available; the monoclonal antibody Trastuzumab can be used to treat HER2 overexpressing breast cancer. Previously, Rauser et. al [3] used MALDI Imaging to compare HER2 positive and negative breast cancer specimens. Among the differences they found an up regulation of the protein CRIP1 (Cysteine rich intestinal protein 1) in HER2 positive samples. This was the first time that this protein was found in a proteomic experiment in breast cancer (although an increased gene expression was linked to HER2 positive breast cancer earlier). Another notable aspect of this publication was also the direct identification of the protein by a top-down fragmentation with electron transfer dissociation in a spherical ion trap; an instrument that is particularly suited for this purpose. The CRIP1 marker could be validated by MALDI Imaging in an independent cohort, but at the time of the publication there was no antibody available to do a more comprehensive validation.

In the meantime, an antibody became available and allowed the analysis of the CRIP1 status in several types of tumors. In a follow-up to their previous MALDI Imaging study, Lydiga et al. [4] analyzed the CRIP1 status in breast cancer by immunohistochemistry. This study revealed that CRIP1 expression is indeed correlated with HER2 expression in breast cancer, Figure 1. Even more interesting is the fact that CRIP1 is an independent prognostic factor in Her2 positive breast cancer: Low expression of CRIP1 in HER2 positive breast cancer is associated with an unfavorable outcome while high expression of CRIP1 is linked to a good prognosis, Figure 2.

The important implication of this study is the proof that MALDI Imaging can find protein changes in diseases that not only are surrogate markers for known tissue states (such as HER2 status in this case), but can also be new markers for other important parameters, such as prognosis.

Figure 1: The co-expression of HER2 and CRIP1 in breast cancer tissue. Representative images of breast cancer tissues showing positive or negative immunohistochemical staining for HER2 and CRIP1, respectively. From [4], released under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0).
Figure 2: Kaplan Meier survival analysis of the distant metastases-free survival of patients. Patients with HER2-positive tumors were stratified according to their CRIP1 expression (negative vs. positive). Adapted from [4], released under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0).
Fosdevirine (FDV) is an antiretroviral drug that showed promising properties in early development. Pre-clinical toxicity studies on rodents and monkey showed no indication of risk of drug-related Central Nervous System (CNS) effects. One adverse reaction was observed in a test population of rabbit but this single example was later discounted. Results from a Phase I clinical study indicated that FDV was well tolerated and exhibited antiviral activity. During a Phase IIb clinical study a high number of seizures presented in a population of HIV patients who had previous antiretroviral therapy and the trial was placed on clinical hold. After which, results became available from a FDV study in minipigs in which a number of animals exhibited neurobehavioral signs, up to 25 days after final dose.

To gain better insight into a physiochemical cause for the adverse activity Castellino et. al [5] used LC-MS to characterize FDV and its metabolites in cerebrospinal fluid (CSF) from seizure patients, rabbit and monkey as well as brain tissue from rabbit, minipig and monkey. The authors supplemented the LC-MS data with a MALDI Imaging study of brain tissue from rabbit, monkey and minipig to map the distribution of the parent drug and several metabolites. These data showed that in the monkey the predominant drug-related material was the intact FDV which was observed by MALDI Imaging to be localized to the grey matter of the brain. LC-MS analyses of CSF in seizure patients and MALDI Imaging in rabbit and minipig revealed the predominant drug-related compound to be a cysteine-addition metabolite found in high concentrations in the corpus callosum. Together with results from other techniques the MALDI Imaging data provides valuable insight into the mechanism of the adverse side effect (the authors speculate a role of an altered blood-brain barrier in HIV patients) as well as the applicability of the animal models.

This paper is particularly noteworthy because it shows the potential for MALDI Imaging data to support classical workflows while offering a unique analytical perspective that is not otherwise possible. The authors make a strong case for including MALDI Imaging in the drug development process where it offers direct imaging of a drug and its metabolites.

Figure 3: Drug Imaging / Histopharmacology: Sagittal rabbit brain section showing distribution of a cysteine conjugate metabolite. (A) Monochromatic ion image showing brain distribution of cysteine conjugate metabolite (170 µm spatial resolution). (B) Same ion image as (A) but shown in rainbow intensity format. (C) Hematoxylin and eosin histology slide showing regions of the rabbit brain. From: Castellino et al. (2011) Bioanalysis 3(21),2427-41 with kind permission of the authors.
References


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