Developing MALDI Imaging Technology for Cancer Profile

Normal and diseased tissues are complex mixtures of different cell populations. A better understanding of protein expression changes that occur during diseases needs sensitive and specific technologies for each of these cell types. Mass spectrometry based tissue imaging (MALDI-MSI) is a newly developed technique, allowing the visualization of proteins, peptides, lipids and small molecules directly on thin sections cut from fresh frozen or fixed paraffin embedded tissues. The major breakthrough is the possibility to study spatial localization of molecules without long and tedious steps of separation and extraction. This technology is being used to characterize tumors at molecular level leading to the improved classification of neoplastic and normal sample and new biomarkers discovery.

Introduction

To study molecular markers associated with human pathologies, tissue obtained both for diagnostic and therapeutic purposes represent one of the best sources. However, the great heterogeneity of cancer tissues represents a limit in the investigation of biomarkers. Accumulated evidences demonstrated that altered cellular pathways in cancer cells and interactions between surrounding stroma cells are both critical to the onset and progression of neoplastic diseases [1]. This close association leads to alterations in the cellular structure as well as modifications in the proteomic pattern [2]. In this picture, every cellular type has a pivotal role and distinct information can be obtained at the proteomic level for each of these components. The current proteomics approach used to investigate cancer biomarkers, show some limitations which must be overcome to address the problem of protein localization.

Classical approaches for routine diagnostics in pathology, such as immunohistochemistry, can provided information of protein localization, but are limited by the presence of antibody reagents and by the quantification of the staining that is subjective.

Isolation techniques, such as two dimensional electrophoresis (2-DE) or surface-enhanced laser desorption/ionization mass spectrometry (SELDI), have been coupled to laser capture microdissection to enrich sub-populations of cells [3, 4].
Despite their suitability these techniques are limited by several shortcomings, including the number of cells required for the analysis [5]. Compared to these methods, MALDI-MSI has the potential to overcome these drawbacks, providing information about the relative abundance and spatial distribution of molecules. MALDI-MSI combines the specificity and sensibility of mass spectrometry with the ability to obtain information about their spatial localization in tissues, without sophisticated steps of sample preparations, while maintaining cellular and molecular integrity (fig. 1).

Since its introduction by Caprioli et al., over ten years ago [6], MALDI-MSI has been applied to the study of biochemical changes of proteins, peptides, lipids and small molecules, associated with several diseases. Here, we review some recent applications of MALDI-MSI in cancer research.

**Technological Aspects**

In a typical MALDI-MSI experiment, a focused laser beam is used to analyze a tissue slice placed on a conductive indium tin oxide (ITO) glass slide. Basically, for sample preparation the tissue is cut on a cryo-microtome, washed several time with organic solvents, covered with a chemical matrix and allowed to dry to form a crystalline material. Obviously, sample handling during all these processes is extremely important to avoid any type of tissue modification, which can adversely affect MALDI results [7].

Notably, direct analysis of both, frozen tissue section, and formalin-fixed paraffin-embedded tissue (FFPE), usually 10 μm thick, can be performed. Many investigators reported the use of FFPE tissue analysis by MALDI-MSI. Ronci et al. described a procedure, that combines heat induced antigen retrieval, EDTA treatment and on target trypsin digestion [8]. Additionally, proteomic analysis of FFPE tissue microarrays (TMA) and in situ enzymatic digestion were proposed [9]. In our laboratory, we have previously developed a method for the analysis of longer stored (>1 year) FFPE tissue, which is based on in situ enzymatic digestion with in
situ extraction of the tissue section. We also used a reactive matrix, 2,4-dinitrophenylhydrazine, together with HCCA for FFPE tissues stored less than 1 year [10].

The intensity of the signal can be improved by washing tissue with organic solvents to remove salts and other contaminants that make the spectra more complex and also cause suppression of ionization. It has been described that the treatment with chloroform, acetone, hexane, toluene, or xylene makes it possible to investigate peptides and proteins more in depth by removing lipids from the tissue surface [11]. Alternative approaches to reduce ion suppression have been described in the literature or are under investigations in our laboratory [12, 13].

Matrix application and matrix solvent conditions greatly affect tissue analysis (fig. 2). For peptide direct tissue analysis, ionic matrixes, in particular CHCA/ANI, should be preferred in respect to classical matrixes such as CHCA [14]. The use of detergents like Triton X-100 has also been demonstrated to increase the detection of higher m/z proteins in tissue [15]. In addition, the ability of matrix to access to tissue interior and extract analyte molecules to the tissue surface is influenced by tissue slice thickness and matrix solvent evaporation [16].

Matrix deposition can be performed either manually using an automatic pipette or by automated devices. In the second approach, using robotic spotter or spraying devices, the matrix is deposited uniformly on the tissue section to achieve high-reproducible spectra, without protein delocalization. Recently, a new method that employs acoustics to eject droplets of matrix onto the tissue has also been proposed [17].

Other important advancements include the possibility to visualize lipids and peptides at a resolution of 15 μm in mammalian tissues, using amorphous small nanoparticles (fNP) with a diameter of 3.7 nm [18], and the unique opportunity to extend the range of molecules that can be studied by MALDI-IMS to nucleic acids, using molecular probe, which binds to a molecule of interest [19].

Finally, data are analyzed by specific software-tools to pre-process all these information for statistical analysis [20]. Advances in bioinformatic tools have facilitated the analysis of spectra of a given tissue specimen and several software-solutions are now commercially available. However, most of this software is specific to an application or a particular instrument. Validated software was developed in our laboratory for the analysis of MALDI-IMS data. This tool was compatible with all MALDI instruments, and is available on our web site (www.maldi-imaging.com) [21].

Application of MALDI-IMS to Cancer

IMS has been successfully used to identify subsets of markers useful for cancer diagnosis, as well as for improving proteomic classification of tumor samples. Oral
squamous cell carcinoma (OSCC) and prostate cancer have been imaged to
differentiate between tumor- and normal tissue [22, 23]. Principal component
analysis (PCA) and clustering algorithms were used to compare tissue samples of
gastric cancer by separating tumorous tissue from tumor-free mucosa from the
same patient [24].
For biomarkers discovery, MALDI-IMS analysis of human colon cancer liver
metastasis visualized two phospholipids as differentially expressed molecules
between the cancerous and normal areas [25]. Furthermore, an increased
expression of Thymosin β.4 (Tβ.4) was observed in the proliferating area of the
tumor glioblastoma [26] and in the stroma region of human breast carcinoma tissue
[27]. IMS was also applied to study the therapeutic-response of tumors treated with
erbB receptor inhibitors OSI-774 and Herceptin [28]. We used MALDI-IMS to study
ovarian cancer (stage III/IV) and benign ovarian biopsies.
Ovarian cancer is one of the most common cancers worldwide and the seventh-most
case of cancer related deaths in women. Widely asymptomatic, the great majority
of cancers are diagnosed as high stage, characterized by peritoneal spread and low
survival. For this reason the need of biomarkers for early cancer diagnosis and
prognosis remains a priority.
Using MALDI MSI we identified a fragment of PA28 alpha or immunoproteasome
11s protein as a new putative biomarker for ovarian cancer. This marker was
validated by immunocytochemistry and western blot. In addition, the protein
showed a nuclear localization in benign tumors and a cytoplasmic localization in
carcinoma cells with possible application in the correct diagnosis of borderline
ovarian tumors [29].

Future Outlook

Our studies clearly demonstrate the impact of MALDI-IMS in cancer research.
Imaging of tumor- and non-tumor areas could improve the understanding of cancer
onset and progression and the possible application to FFPE tissues, a still
unexplored resource for biomarker research, can enlarge drastically the number of
analyzed samples.
Although certain advancements in sample preparation and automation have been
introduced, some limitations still remain, including the detection of low-abundance
and high molecular weight proteins. Many of these drawbacks are actually under
investigation in our laboratory with the aim to translate these results into clinical
research.

References are available from the authors.

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