EXPERT REVIEWS

Mass spectrometry imaging: linking molecule profiles to tissue spatial distribution

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Advances in Bioanalytical Mass Spectrometry: 5th Symposium of the Göttingen Proteomics Forum

Mass Spectrometry with Spatial Resolution: MALDI-Imaging and Laser Capture Microscopy

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MALDI mass spectrometry imaging (MSI) combines the speed and molecular specificity of MALDI-MS detection with information on spatial organization. In the last years, MSI found large application in proteomics research for determining the spatial distribution of compounds in biological tissues and started to draw increasing interest in clinical research. To shed light on the new developments in the field of MSI, the Göttingen Proteomics Forum organized a symposium that was held in Göttingen as part of the series of regular symposia organized by the members of the Göttingen Proteomics Forum. The symposium was on 22 November 2012, with more than 80 delegates that attended the event entitled 'Mass spectrometry with spatial resolution: MALDI-imaging and laser capture microscopy'. The one-day agenda consisted of nine oral presentations from renowned experts in the field with subsequent discussion sessions. As usual, the meeting was fruitful and offered a good platform for discussion between the delegates and proteomics specialists.

The Göttingen Proteomics Forum (GPF) was established in 2004 as a local network of scientists of the Georg-August University, the University Medical Center Göttingen and the Max-Planck Institutes of Experimental Medicine and Biophysical Chemistry (Göttingen, Germany) with the common interest in the analysis of proteins by mass spectrometry (proteomics). In the meantime, the GPF enhanced its cooperation with the local corporate data processing facility of the University and the Max-Planck-Society (GWDG). In 2010, the GPF became a member of Göttingen Research Campus. The idea of the forum is the pooling of local proteomics expertise and tools to generate synergism and provide mutual support for the proteomics community in Göttingen. Members meet on a regular basis to exchange information, discuss scientific projects and plan joint activities such as seminars and symposia. Among the activities of the GPF is the well-established regular symposium under the title of 'Advances in Bioanalytical Mass Spectrometry'. This year, the meeting's main topic was mass spectrometry (MS) with spatial resolution: MALDI-imaging and laser capture microscopy. The speakers were from five different countries and the talks were grouped into three different sessions.

MS imaging in clinical proteomics

MALDI–TOF MS has been widely used in the last two decades and contributed to revolutionizing the ability to analyze proteins. As a soft ionization technique, MALDI allows the detection of intact proteins and qualifies the technique for molecular imaging. MALDI MS imaging (MSI) offers the possibility of profiling and imaging molecules directly from thin tissue sections, allowing the correlation of molecular signatures with sample phenotypes [1]. The samples are thin sections of tissue, either cut from a fresh frozen sample or from a formalin fixed paraffin embedded (FFPE) specimen, and are mounted directly onto a MALDI target plate or compatible glass microscope slide [2]. The section is then generally covered with matrix dissolved in acidified organic solution with the help of a robotic spotter or spray-coating device. The molecule's mass patterns are then obtained using a mass spectrometer, most commonly, a MALDI–TOF instrument.

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The visualization of the spatial distribution of the analyzed molecules (proteins, peptides, drugs and their metabolites or other chemicals) within thin slices of samples such as animal or plant tissue opens new possibilities in targeting biomedical questions [3–5]. Compared with other imaging technologies, MSI offers the major advantage that the samples can be directly analyzed without the need of additional steps, such as extraction and labeling. The first session of the meeting included talks from Marc Baumann (Medical Faculty, University of Helsinki, Helsinki, Finland), Isabelle Fournier (Université Lille Nord, Lille, France) and Detlev Suckau (Bruker Daltonics, Bremen, Germany). The lectures introduced the various MSI techniques by giving a detailed overview on the methods used and their underlying principles. For a successful MSI experiment, tissue preparation, storage and processing are of great importance. An overview on the tissue processing and preparation for MSI was given and the critical steps were highlighted, in particular the influence of the quality of the samples on the outcome of the analysis was clearly demonstrated. In general, the MSI workflow is straightforward, rapid and inexpensive compared with alternative imaging techniques. A drawback is that sample preparation protocols are optimized for specific substance classes, resulting in the necessity of a previous selection of the mass range of interest and the type of molecules to be analyzed (e.g., lipids, peptides or proteins).

Protein identification in MSI

Currently, a strong emphasis is put on increasing sensitivity for on-tissue measurements with parallel identification of the detected compounds. One of the limitations of MSI is the protein/peptide identification. The identity of the molecules behind the tissue-specific signature can deliver valuable information on the biological status, but can also reveal important candidates for the understanding of disease mechanisms and the improvement of therapy development. Fournier and Suckau presented different protein identification strategies in the case of MSI. Although on-tissue fragmentation can tentatively deliver information on the identity of the molecules, especially in case of peptides and lipids, proteins need adapted protocols with external identification steps. These usually require isolation of the protein of interest from the tissue and, depending on its size, the protein is then identified with (bottom-up) or without (top-down) previous enzymatic digestion through fragmentation in the mass spectrometer. In case of the bottom-up MSI strategy, the tissue digestion followed by MS analysis and protein identification can be used with different kinds of tissue samples, especially FFPE tissues conserved for a long time in hospital sample banks. In contrast, the top-down imaging approach cannot be applied to the analysis of FFPE tissue samples. To overcome the limitation of protein identification, the microextraction offers a promising strategy. In this case, on-tissue microdigestion by trypsin is performed using a microspotting device, then the samples are processed by microextraction and finally collected via five extraction cycles. Protein identification is then performed using routine nanoLC-MS/MS workflows.

MSI in understanding the pathophysiology of diseases

In the majority of the diseases, diagnostic decisions are made by pathologists based on tissue morphology and histology. Because of its practical simplicity and ability to gain reliable information even from the smallest tissue amounts (e.g., from endoscopic biopsy sections), MSI may have the potential to complement histopathologic evaluation. It can help the pathologist to correlate molecular details to morphological and physiological changes, thus offering the ability to localize disease-specific molecular changes in tissues and to support pathologists in overcoming critical processes in diagnostics, risk assessment or prediction of response to therapy. Moreover, MSI provides a tool to identify novel pathogenesis-related biomarkers for earlier diagnosis of diseases and their personalized treatment.

The second session of the meeting highlighted successful examples of using MSI in pathological questions. Axel Walch (Helmholtz Centre München, Institute for Pathology, Neuherberg, Germany) presented interesting data illustrating the use of MSI in investigating cancer diseases. To identify a disease-specific pattern, MSI was applied to investigate the outcome in gastric cancer after surgical resection. Tumor tissue sections were analyzed using MSI, and a protein signature that correlates with unfavorable survival was established [6]. The protein pattern could serve as a new indicator of patient survival, complementing the previously known clinical parameters in terms of prognostic relevance. Tissue imaging using MS may provide clinically relevant information that may be beneficial in improving risk stratification for gastric cancer patients. Imaging studies performed in other type of cancer, for example, breast cancer, confirmed that tissue proteome profiling can reflect tumor biology and can deliver valuable information for therapy [7]. In case of biomarker identification using MSI, validation is an important and challenging step to confirm the true importance of the identified molecules. One common way is to perform targeted *in situ* experiments, such as immunohistochemistry for proteins, on larger, independent sample collections. Because of the low amount of human samples available, the validation will stay a challenging step.

The diagnosis of the majority of CNS diseases is often not satisfactory. Therefore, there is a need to discover new biomarkers for both diagnosis and prognosis of these diseases, which include the large group of neurodegenerative disorders. As they can reproduce the clinical manifestation of human diseases at least in part, animal models have been used extensively to better understand the pathomechanisms behind neurodegenerative diseases. The talk of Andreas Pich (Institute for Toxicology, Hannover Medical School, Hannover, Germany) from the second session focused on the use of MSI in scanning brain tissue from animal models for neuronal diseases. MSI has been applied in different studies to such animal models of neurodegenerative disorders, particularly to establish disease-specific peptide and protein patterns [8,9]. MSI revealed an altered protein pattern in an animal model of Parkinson's disease, in which alterations in metabolic processing were identified as well [10]. Moreover, similar alterations were also found in both Parkinson's disease patients and the respective animal models [11,12]. Brain sections from an animal model for Usher

syndrome were also investigated using MSI and the results were promising. The CNS peptide and protein patterns are complex, and MSI can help by delivering information on changes in the patterns to help identify specific proteins involved in brain diseases. In this, it will provide significant insight into the pathogenesis of many neurodegenerative diseases. Neuropeptides are used by the CNS for communication between neurons. As neuronal signaling molecules, they are important for brain function and activity. One of the most challenging aspects in the investigation of brain function is the detection of endogenous neuropeptides due to their low *in vivo* concentrations [13,14]. Per Andrén (Uppsala University, Uppsala, Sweden) showed the advantage of MSI in overcoming the limitation in neuropeptides investigation. It offers a tool for investigating these neurosignals directly on the brain tissue, and this enables the study of the physiological and disease-related metabolic processing of neuropeptides.

Another field of use of MSI is in drug applications [15,16]. The talk of Malcolm Clench (Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK) presented studies demonstrating that MSI technology can also be used to investigate the efficacy of new therapeutics and to monitor therapy response. It may provide deeper insight into drug distribution in tissue and disclose their potential toxicological processes, revealing the mechanism of efficacy or side effects at the molecular level.

The last session of the meeting sheds light on new applications and technologies in tissue MS. Theo Luider (Clinical and Cancer Proteomics, Department of Neurology, Rotterdam, the Netherlands) gave an overview on combining MS with laser capture microscopy to identify proteins on cellular level. He illustrated the advantage of the method in investigating the proteome of neurodegenerative diseases plaques. In the last talk, Ian Edwards (Waters Corporation, Manchester, UK) presented the advantage of the integration of ion mobility in MSI.

Conclusion

From the presentations during the GPF symposium, it became clear that in the last 10 years a lot of effort was put in to improve MSI, which is a rather young technology. But despite the huge progress achieved, there is still further improvement needed to move from bench to bedside. Among the critical steps in MSI is the standardization of sample collection, storage protocols and data acquisition. From an instrumental side, an improvement in the resolution down to single cell level will boost MSI and make it suitable for clinical settings. Many tentative studies proved the usefulness of MSI for pathological questions, but further clinical studies with large sample cohorts are still required. With MSI, a new tool was developed for molecular classification of tissues with emphasis on the improvement of diagnosis, therapy and drug development.

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References

1 Goodwin RJ, Pennington SR, Pitt AR. Protein and peptides in pictures: imaging with MALDI mass spectrometry. *Proteomics* 8(18), 3785–3800 (2008).

2 Schwartz SA, Reyzer ML, Caprioli RM. Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: practical aspects of sample preparation. *J. Mass Spectrom.* 38(7), 699–708 (2003).

3 Cazares LH, Troyer D, Mendrinos S *et al.* Imaging mass spectrometry of a specific fragment of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase 2 discriminates cancer from uninvolved prostate tissue. *Clin. Cancer Res.* 15(17), 5541–5551 (2009).

4 Djidja MC, Francese S, Loadman PM *et al.* Detergent addition to tryptic digests and ion mobility separation prior to MS/MS improves peptide yield and protein identification for *in situ* proteomic investigation of frozen and formalin-fixed paraffin-embedded adenocarcinoma tissue sections. *Proteomics* 9(10), 2750–2763 (2009).

5 Franck J, Arafah K, Elayed M *et al.* MALDI imaging mass spectrometry: state of the art technology in clinical proteomics. *Mol. Cell Proteomics* 8(9), 2023–2033 (2009).

6 Balluff B, Rauser S, Meding S *et al.* MALDI imaging identifies prognostic seven-protein signature of novel tissue markers in intestinal-type gastric cancer. *Am. J. Pathol.* 179(6), 2720–2729 (2011).

7 Balluff B, Rauser S, Ebert MP, Siveke JT, Höfler H, Walch A. Direct molecular tissue analysis by MALDI imaging mass spectrometry in the field of gastrointestinal disease. *Gastroenterology* 143(3), 544–549. e1–2 (2012).

8 Pierson J, Norris JL, Aerni HR, Svenningsson P, Caprioli RM, Andrén PE. Molecular profiling of experimental Parkinson's disease: direct analysis of peptides and proteins on brain tissue sections by MALDI mass spectrometry. *J. Proteome Res.* 3(2), 289–295 (2004).

9 Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM. Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues. *Nat. Med.* 7(4), 493–496 (2001).

10 Klintenberg R, Andrén PE. Altered extracellular striatal *in vivo* biotransformation of the opioid neuropeptide dynorphin A(1–17) in the unilateral 6-OHDA rat model of Parkinson's disease. *J. Mass Spectrom.* 40(2), 261–270 (2005).

11 Johansson PA, Andersson M, Andersson KE, Cenci MA. Alterations in cortical and basal ganglia levels of opioid receptor binding in a rat model of l-DOPA-induced dyskinesia. *Neurobiol. Dis.* 8(2), 220–239 (2001).

12 Nisbet AP, Foster OJ, Kingsbury A *et al.* Preproenkephalin and preprotachykinin messenger RNA expression in normal human basal ganglia and in Parkinson's disease. *Neuroscience* 66(2), 361–376 (1995).

13 Kendrick KM. Microdialysis measurement of *in vivo* neuropeptide release. *J. Neurosci. Methods* 34(1–3), 35–46 (1990).

14 Strand FL. Neuropeptides: general characteristics and neuropharmaceutical

potential in treating CNS disorders. *Prog. Drug Res.* 61, 1–37 (2003).

15 Nilsson A, Fehniger TE, Gustavsson L *et al*. Fine mapping the spatial distribution and concentration of unlabeled drugs within tissue micro-compartments using imaging mass spectrometry. *PLoS One* 5(7), e11411 (2010).

16 Shariatgorji M, Nilsson A, Goodwin RJ *et al*. Deuterated matrix-assisted laser desorption ionization matrix uncovers masked mass spectrometry imaging signals of small molecules. *Anal. Chem.* 84(16), 7152–7157 (2012).