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Recent Advances of MALDI-Mass Spectrometry Imaging in Cancer Research

Joohee Jung[1,2]*

[1]*College of Pharmacy, Duksung Women's University, Seoul 01369, Korea*
[2]*Duksung Innovative Drug Center, Duksung Women's University, Seoul 01369, Korea*

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Abstract: For several decades, cancer has been the primary cause of mortality worldwide. New diagnosis and regimens have been developed to improve the chemotherapeutic efficacy and the quality of life of the patients. However, cancer tissues are complex and difficult to assess. Understanding the various properties of the tumor and its environment is crucial for cancer and pharmaceutical research. Several analytical techniques have been providing new insights into cancer research. Recently, matrix-assisted laser desorption ionization (MALDI)-mass spectrometry imaging (MSI), an advanced analytical technique, has been applied to translational research. Proteomic and lipidomic profiling obtained by MALDI-MSI has been critical for biomarker discovery and for monitoring heterogenous tumor tissues. In this review, we discuss technical approaches, benefits and recent applications of MALDI-MSI as a valuable tool in cancer research, namely for diagnosis, therapy, prognosis.

Key words: MALDI imaging, cancer, biomarker, diagnosis

Introduction

Cancer is the major cause of mortality worldwide for several decades. To control cancer progression, many researchers have studied cancer cells and their environment (e.g. metastasis, hypoxia, acidic condition).[1-5] Finding molecular biomarkers is critical for cancer diagnosis and prognosis. However, cancer cells have different characters although they included in same tumor tissue. On the other hand, commercial cancer cell lines are established for research, so they are homogenous. Thus, our results obtain from commercial cancer lines are often inconsistent with clinical data. This being so, we need the understand of cancer cell heterogenous properties for diagnosis and therapy.[6-9] Various technical approaches are required to obtain sufficient information from the cancer tissues.

Generally, positron emission tomography (PET) scan, computerized tomography (CT) scan, magnetic resonance imaging (MRI) scan and endomicroscopy are the imaging techniques used to assess cancer volume and its site in the body. However, the molecular characterization of the cancer specimens required the analysis by immunohistochemistry (IHC) using antibodies against specific biomarkers. Thus, finding specific cancer biomarkers is critical for cancer diagnosis, treatment, and prognosis.

Among the analytical techniques, matrix-assisted laser desorption ionization (MALDI) has been used to identify molecular biomarkers (DNA, peptides, and so on) in cancer tissues. Recently, MALDI-mass spectrometry imaging (MSI), as an advanced analytical technique, is noticeable and its applications have expanded. MALDI-MSI provides a resourceful data set, which may include the lipidomics and proteomics of the tumor tissues, depending on the sample preparation. The application of MALDI-MSI in cancer research is critical to understand the tumor tissues and its environment. Thus, the use of this technique could be an advantage for diagnosis, drug monitoring and prognosis in cancer.

In this review, published data was collected from the PubMed database using the keywords 'MALDI', 'cancer', 'imaging', 'biomarker', or 'diagnosis'. Total 450 articles were researched until May 2019. However, only 66 articles until 2009 were published according to Gemoll's report.[10] From 2010 to 2019, related articles have increased approximately 6 times, which indicates that the use of MALDI-MSI has rapidly increased in the field of oncology (Figure 1). Thus, the recent advances in MALDI-MSI were introduced and its prospect was discussed.

*Reprint requests to Joohee Jung
E-mail: joohee@duksung.ac.kr

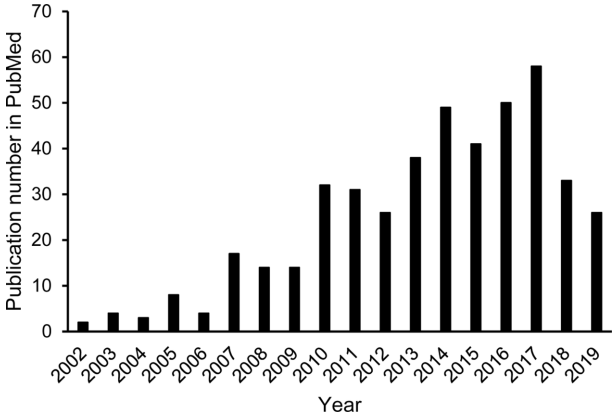

Figure 1. Histogram of published data in PubMed.

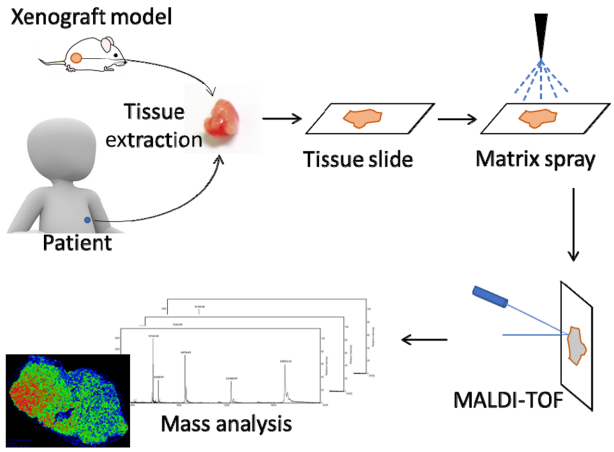

Figure 2. Flowchart for MALDI-MSI in tumor tissues.

Properties and merits of MALDI-MSI

MALDI is an ionization technique of large molecules with laser energy absorbing matrix. The matrix is composed of small organic acidic materials that are sprayed over the specimen, absorbs laser light, converts it into heat energy, and is vaporized together with the specimen. With MALDI, time of flight mass spectrometry (TOF MS) is an analytical method, in which the materials are separated in the specimen because the time of ion flight differs depending on the mass-to-charge ratio (*m/z*) value of the ion. MALDI-MSI, as one of the high-end mass spectrometric technique, improve the resolution and sensitivity.[11] It provides a molecular distribution map generated from the great amount of data obtained from each point of the tissue sample and converts into an image (Figure 2). The images obtained are visually appealing and provide a wide range of molecular information of tissues. This is a great advantage of MALDI-MSI over that of 2-dimensional polyacrylamide gel electrophoresis (2-DE PAGE) because the data correlates the protein expression with the tissue histology. Only a small amount (about 10 ~ 20 μm thickness) of tissue is required for analysis of MALDI-MSI.[12,13] Furthermore, MALDI-MSI could detect speedy in < 1 h[14,15] as well as IHC (< 1 h).[16] Appropriate conditions for the matrix supply are required for lipidomics, as well as for the peptidomis/proteomics of tumor tissues. MALDI-MSI also provides information about drug metabolism in tissues.[17] Additionally, the data generated by this technique can be simultaneously represented through a quantitative analysis.

The aforementioned technical advantages of MALDI-MSI confer to this technique a versatile utilization. The technical processing of MALDI-MSI and its application have been developed and improved (Table 1). Currently, in the cancer research, MALDI-MSI is one of the promising technical tools for diagnosis, prognosis, and metabolism.

Diagnosis and prediction

Classification of tumor stage

The classification of the tumor stage using cancer tissues was validated by several peaks of MALDI-MSI. The distribution of the peaks is calculated and converted into a mass spectra image depending on the tumor progression. Paraffin-embedded tissues and frozen tissues[13,18] can be used as resources for diagnosis by MALDI-MSI. Currently, the classification of the tumor stage relies on the identification of specific molecular markers detected by IHC.[19,20] Thus, the sensitivity and specificity of the antibodies are important limiting factors that affect the diagnosis accuracy. MALDI-MSI, however, compares peak changes between tumor tissue and normal tissue, and its result could classify tumor stage without antibodies, which is a considerable advantage over IHC. MALDI-MSI has been used for the diagnosis of pituitary adenomas,[21-23]

Table 1. Summary of application using MALDI-IMS in cancer research.

Application field		Application contents
Diagnosis and prediction	Tumor classification	normal *vs* cancer, primary *vs* metastasis, grades, non-recurrent *vs* recurrent
	Biomarker identification	peptides, lipids, glycoprotein
	Molecular histology	expression level
Metabolism	Cancer metabolism	proteomics, lipidomics
	Drug metabolism	pharmacokinetics, pharmacodynamics

classification of thyroid cancer[24,25] in fine needle aspiration biopsies,[26] and determination of the histological grades using peptide profiling[27-29] or lipidomic signature.[30,31] Additionally, MALDI-MSI has been used to differentiate cancer from normal cells in various cancer species[32-34] and primary cancer from metastasis,[35-37] as well as to differentiate between recurrent triple-negative breast cancer (TNBC) and non-recurrent TNBC,[38] meningiomas and glioma,[39] medulloblastoma and pineoblastoma,[40] and cancer and stroma cells.[41] Furthermore, the early diagnosis in ovarian cancer improved the 5-year survival rate, according to the International Federation of Gynecology and obstetrics (FIGO).[42] Thus, classification of ovarian cancer using MALDI-MSI technique could be a great advantage in the early diagnosis[43] and support the new prognostic parameters with high accuracy.[43]

Biomarker identification

MALDI-MSI technique distinguishes tumor tissues from normal tissues. The differences between peaks in MALDI-MSI allow to identify unknown or known peptides, proteins, lipids or glycans. Identified molecules in MALDI-MSI can compensate for the limitation of biomarkers detected by IHC.[44]

Some of the molecules detected by MALDI-MSI are specific for tumor cells and can be used as specific biomarkers (Table 2). In some cases, the expression level of the biomarkers correlates with the survival curve. Therefore, they can be monitored and used as prognostic markers. The post-translational modifications modulate protein functions. Among others, the N-linked glycans, which occur during tumorigenesis, are used as tumor biomarker. Drake's research suggested that the distribution of N-linked glycans was imaged using MALDI-MSI.[45]

For example, the monitoring of N-glycans were shown in gastric cancer,[46,47] hepatocellular carcinoma,[48] ovarian cancer,[49] and prostate cancer.[50] N-glycan polylactosamines indicates aggressive breast cancers.[35] Metallothioneins are also biomarkers of cancer progression in various cancer.[51] High LRP6 expression is correlated with prevalence, metastasis and survival curve of oral squamous cell carcinoma (OSCC).[52] In OSCC, NCOA7 could be used as a cell proliferation marker because NCOA7 activates aryl hydrocarbon receptor and induces the cell proliferation.[53] The overexpression of thymosin beta-4 showed low overall survival and is associated with recurrence of head and neck squamous cell carcinoma (HNSCC)[54] and colorectal cancer.[55] Furthermore, thymosin beta-4 expression is correlated with high grade of malignant intraductal pancreatic mucinous neoplasm.[56,57] Matrix metalloproteinases (MMPs) are well known biomarkers for metastasis. Among them, distribution of zinc and MMP-11 is used as diagnostic and prognostic biomarkers in breast cancer.[58] Changes in specific lipids were investigated and applied as biomarkers in colorectal cancer liver metastasis[59,60] and in human glioma, as well as in the rat brain.[61] Human neutrophil peptides (HNPs) are known to be overexpressed in various cancer types. Among the HNPs, HNP-1,[62] α-defensin 2, and 3[63] are highly expressed in gastric cancer. Furthermore, data extracted from MALDI-MSI experiments have shown that the expression of S100 calcium binding protein A6 (S100A6), S100A10, cytochrome c oxidase subunit 7 A2 (COX7A2), and transgelin-2 (TAGLN2) correlate with survival curve in gastric cancer.[64] Especially, S100A8 and S100A9 were identified as a biomarker of gastric cancer metastasis.[65] Human epidermal growth factor receptor 2 (HER2/neu)[66-68] is confirmed as a biomarker of various cancers by MALDI-MSI. Histones plays a role of gene expression. Thus, histone subtypes are also used as cancer biomarkers and are concerned about the anticancer drug target. In MALDI-MSI analysis, histone H2 in gastric cancer[69] and histone H4 in hepatocellular carcinoma[70] and gastric cancer[71] highly expressed. Phosphatidylinositol in breast cancer[72]

Table 2. Summary of molecular profiling using MALDI-IMS in cancer research.

Cancer	Identified markers	References
Breast cancer	HER2	68
	MMP-11 and zinc	58
	N-glycan polylactosamine	37
	phosphatidylcholine	74
	phosphatidylinositol	72
Colorectal cancer	thymosin beta-4	55
Gastric cancer	COX7A2	64
	HER2	67
	histone H2A, H4	71
	HNP1, HNP2, HNP3	62, 63
	lysophosphatidylcholines acyltransferase 1	76
	N-glycans	46
	S100A6, S100A8, S100A9, S100A10	64, 65
	TAGLN2	64
Head and neck cancer	thymosin beta-4 (34)	54
Liver cancer	lysophosphatidylcholines acyltransferase 1	76
	N-glycans	48
Oral squamous cell carcinoma	LRP6 (33)	52
	NCOA7	53
Ovarian cancer	N-glycan	49
Pancreas cancer	thymoxin beta-4	56
Prostate cancer	lysophosphatidylcholine	78
	N-glycans	50
	phosphatidylinositols	73

and prostate cancer,[73] and phosphatidylcholine in breast cancer,[74] colorectal cancer[75] are also identified as cancer biomarkers of MALDI-MSI. Lysophosphatidylcholine acyltransferase 1 regulates hepatoma progression[76] and gastric cancer progression.[77]

Metabolism and drug metabolism

Cancer metabolism

Tumor cells reprogram their metabolic pathways to meet the bioenergetic demands of malignancy. Therefore, the studies on the resulting metabolites could be used to investigate cancer progression. In tumor tissues, *de novo* lipodogenesis related to cancer progression were assessed by MALDI-MSI. Metabolism of octadecenoic acid and lysophosphatidylethanolamine is distinguished between tumor area and normal tissues. Thus, the detection of metabolites is important for the early-stage diagnosis of gastric cancer.[78] From the lipid profiles obtained by MALDI-MSI, the arachidonic acid, lipoxygenase and COX pathways are upregulated in cancer cells and are associated with Myc activation and expression. Deactivating Myc decreased the expression of arachidonic acid and its eicosanoid metabolites.[79] Mitogen-activated protein kinase/ extracellular signal-regulated kinase (MEKK2),[80] biliverdin reductase B (BVRB), a cytoprotective and growth promoting protein),[81] were identified as metabolites in prostate cancers by MALDI-MSI and could be used as potential diagnostic markers. Erythroblast transformation-specific related gene (ERG) and transmembrane protease serine 2 (TMPRSS2) were identified by MALDI-MSI as metabolites of PC and used to evaluate targeted therapy and diagnosis.[82-84]

Drug metabolism

In drug development, pharmacokinetics and pharmacodynamics are important to evaluate the drug efficacy and its side effects. The distribution of the drug in cancer tissues can predict the efficacy of the target therapy. The autoradiography method has been used as a detection method of drug in tissue sections.[85] This method which detects the distribution of isotope-labelled drug in tissue sections, is very sensitive. Target drug is easy to detect through measurement of radioactivity.[85] However, the digested drug metabolite distributions are difficult to distinguish to parent drug distribution. To treat the quantitative data, other tools (MS or HPLC) are needed.[86] Additionally, specific facilities and specialists are required for making isotope-labelled drug. In contrast to the autoradiography, MALDI-MSI technique combines the spatial resolution and quantity. Thus, MALDI-MSI is useful to show the distribution of drug and its metabolites in the tissues. Distribution of paclitaxel in tumor tissues was shown by MALDI-MSI technique and its efficacy was evaluated in colon cancer, breast cancer, malignant pleural mesothelioma,[87] as well as in angiogenesis models of ovarian carcinoma and colon carcinoma.[88] In gastric cancer, the pharmacodynamic response of HDAC inhibitor (hydroxamic acid panobinostat) was evaluated histone H4 overexpression indicates a poorly differentiated cancer tissue.[89] In mice model of gastric cancer, inhibitors of RHO-associated protein kinases 1 and 2 (ROCK 1/2) were evaluated for their distribution and effectiveness. Furthermore, its metabolite, hydroxyfasudil were detected.[90] In colorectal tumor organoids, infiltration of irinotecan and its metabolite was identified, but their efficacy awaits further evaluation.[91,92] In a liver cancer model, sunitinib and its metabolites were mapped and their imaging data gave information about pharmacokinetic profile with complementary fluorescence imaging.[93] In pancreatic cancer, the pharmacokinetic profile of erlotinib was evaluated.[94] In melanoma, vemurafenib and its prodrug were imaged to assess BRAF protein expression.[95] The inhibition of angiogenesis was evaluated by the localization of YCG185 and sunitinib in tumor tissues.[95] For epigenetic profile, UNC1999-treated multicellular tumor spheroids could be monitored during histone post-translational modification.[96]

Furthermore, the penetration of drugs affects pharmacokinetics and pharmacodynamics because several barriers protect each organ. Among them, the brain-blood barrier is known to be an obstacle of the effective drug delivery. To overcome the low penetration rate in brain, drug-carriers using drug delivery system should be produced and applied. In the development of drug-carriers, the comparative evaluation of original drug and drug-carrier is required.[97] The imaging profiling of MALDI-MSI as a good evaluation tool is utilized. For example, pharmacokinetics and pharmacodynamics information was supplied by MALDI-MSI in a glioblastoma-derived xenograft model[98,99] and in murine brain.[100] Even though anticancer drugs apply without some target molecule or carrier, leaky blood vessels around tumor induce enhanced permeability and retention of drugs into cancer cells. The enhanced permeability and retention effect (EPR effect) of drugs as a passive targeting could be monitored by MALDI-MSI. The penetration and distribution of platinum-based anti-cancer drugs and their metabolites could be imaged in 3D tumor model.[101] MALDI-MSI technique distinguishes cancer tissue from normal tissues using the EPR effect of silver nanoparticles in kidney cancer tissues without specific biomarker.[102] These results indicate that MALDI-MSI could provide essential information for pharmacological and toxicological profiling of drug candidates.

Overcoming the limitations of MALDI-MSI

Until now, we discussed that MALDI-MSI is a useful technique for identification of biomarkers, classification of

tumor grade, and biomonitoring of drugs in the tissues. Although MALDI-MSI is used in a broad number of fields, it has several drawbacks. First, other complementary analytical tools such as LC-MS/MS are needed for the identification of target molecules (proteins or drug/metabolites) in MALDI-MSI. Second, a quantitative assessment of the target molecules should be adjusted because of the spot-to-spot variation of ion intensity. Specific ion suppression effect is occurred by the matrix deposition method. Thus, standard spike of control tissues is measured to compensate it.[85] Third, the machine consumes an enormous amount of electric power and endures burden. Fourth, this technique is unsuitable for high molecular weight proteins. Finally, it demands high skilled experts for the analysis of a vast database.[83] Notwithstanding its limitations, MALDI-MSI is an advantageous imaging tool for cancer research.

Conclusions

This review on the current literature suggests a broad application of MALDI-MSI technique in the field of oncology. The data provided by MALDI-MSI can support the vast information about tissues (drug distribution, metabolomics, proteomics, lipidomics, and gene profiling) with imaging. MALDI-MSI technique can be used for cancer diagnosis and prognosis through the identification of cancer biomarkers.

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