Published in final edited form as:

J Mass Spectrom. 2013 November ; 48(11): 1178–1187. doi:10.1002/jms.3295.

Mass Spectrometry Imaging as a Tool for Surgical Decision-Making

David Calligaris^{1,u}, Isaiah Norton^{1,u}, Daniel R. Feldman³, Jennifer L. Ide¹, Ian F. Dunn¹, Livia S. Eberlin^{4,†}, R. Graham Cooks⁴, Ferenc A. Jolesz², Alexandra J. Golby^{1,2}, Sandro Santagata³, and Nathalie Y. Agar^{1,2,4,*}

¹Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

²Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

³Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115

⁴Department of Chemistry and Center for Analytical Instrumentation Development, Purdue University, West Lafayette, IN 47907

⁵Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, 02115

Abstract

Despite significant advances in image-guided therapy, surgeons are still too often left with uncertainty when deciding to remove tissue. This binary decision between removing and leaving tissue during surgery implies that the surgeon should be able to distinguish tumor from healthy tissue. In neurosurgery, current image-guidance approaches such as magnetic resonance imaging (MRI) combined with neuro-navigation offer a map as to where the tumor should be, but the only definitive method to characterize the tissue at stake is histopathology. While extremely valuable information is derived from this gold standard approach, it is limited to very few samples during surgery and is not practically used for the delineation of tumor margins. The development and implementation of faster, comprehensive and complementary approaches for tissue characterization are required to support surgical decision-making – an incremental and iterative process with tumor removed in multiple and often minute biopsies. The development of atmospheric pressure ionization sources makes it possible to analyze tissue specimens with little to no sample preparation. Here, we highlight the value of desorption electrospray ionization (DESI) as one of many available approaches for the analysis of surgical tissue. Twelve surgical samples resected from a patient during surgery were analyzed and diagnosed as glioblastoma (GBM) tumor or necrotic tissue by standard histopathology, and mass spectrometry results were further correlated to histopathology for critical validation of the approach. The use of a robust statistical approach reiterated results from the qualitative detection of potential biomarkers of these tissue types. The correlation of the MS and histopathology results to magnetic resonance images brings significant insight into tumor presentation that could not only serve to guide tumor resection, but that is worthy of more detailed studies on our understanding of tumor presentation on MRI.

*Corresponding author: Dr. Nathalie Y.R. Agar Departments of Neurosurgery and Radiology, Brigham and Women's Hospital, and Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Nathalie_Agar@dfci.harvard.edu, +1617/525-7374.

^uThese authors contributed equally to this work.

[†]Current address: Department of Chemistry, Stanford University, Stanford, CA, 94305-5080, USA.

Keywords

DESI-MSI; brain tumors; real-time diagnosis; surgery; image-guided therapy

Abbreviations

MALDI; DESI; MS; MSI; H&E; MRI; SVM; pLSA; PCA; GBM

INTRODUCTION

Surgery is typically the first step for the treatment of brain tumors. To minimize the removal of functional healthy tissue, brain mapping techniques are often used prior to and during surgery. During the procedure, surgeons can use intraoperative ultrasound^[1] and MRI^[2–5] in centers where the technology is available, but these tools still provide limited temporal resolution (MRI) and discriminative capability (ultrasound). In addition, neither ultrasound nor MRI directly samples the tumor to determine the molecular characteristics of the tissue, thereby providing only an indirect assessment of the tumor.

Over several decades, various methods have been proposed to provide tissue discrimination including infrared or Raman spectroscopy^[6–8], flow-cytometry^[9–11], *in vivo* labeling techniques coupled with spectroscopy^[12,13], and scintillation counting^[14] for the characterization of tissues in an operating room. Due to issues of complexity, limited sensitivity for properly discriminating tissues, or limited compatibility with the surgical environment none of these techniques has yet gained widespread use.

A wealth of reports have been published over the past decade on the ability of mass spectrometry to discern and characterize biological tissues with increasing sensitivity and specificity^[15–17]. It therefore becomes very natural to return mass spectrometers back into the operating room where they were routinely used in the 1980s to sample airway gases from anesthetized patients.^[18] Now, however, they would permit the precise molecular characterization of tissue and serve, as an analytical tool in image-guided therapy. Different mass spectrometry (MS) platforms will likely find themselves interfacing with surgical decision-making at various points in the clinical workflow. MS has already proven to be useful for the characterization of intact biological tissues.^[19–21] For over a decade, matrix-assisted laser desorption/ionization (MALDI) mass spectrometers have successfully been used for the profiling of peptides and proteins from tissues and cells in the research setting^[19] and has recently been increasingly employed for the analysis of small molecules such as lipids, drugs and their metabolites.^[22–30] MALDI mass spectrometry imaging (MSI) analyses of tissue have become an extremely promising tool to support decision-making in histopathology evaluation of tissue.^[20] With its ability to capture essentially a complete mass range of biomolecules that include accepted biomarkers such as proteins, MALDI MSI should assist in diagnosis providing enhanced discriminating power over visual inspection of tissue.^[19] A higher level and certainty of diagnosis provided during frozen section analysis would certainly benefit surgical decision-making in better understanding the disease faced by the surgeon. Typically, one or two samples are sent for frozen section analysis during a surgical case, and MALDI MSI could find a way to fit within comparable timelines to standard analysis. For the delineation of tumor margins though, multiple minute specimens would need to be analyzed, and the analysis should result in real-time feedback. Currently, the sample preparation steps required for MALDI MSI would not be compatible with such a workflow.

With the development of ambient ionization methods such as DESI, it is possible to perform MS analysis with essentially no sample preparation, hence making such methods compatible with the time restrictions required for intraoperative tumor diagnosis and margin delineation.^[31,32] In DESI, a pneumatically assisted electrospray produces charged droplets that are directed to collide with the surface of a sample.^[33] As the charged droplets collide with the sample surface they create a thin liquid film into which analytes are extracted; the impact of subsequent primary droplets releases secondary microdroplets in a process termed droplet pick-up.^[33] Following this pick-up mechanism, the standard electrospray solvent evaporation processes occur, followed by the production of dry ions of analyte either by the field desorption or charge residue process.

DESI is one of multiple atmospheric pressure ionization sources. Aimed at ease of implementation and execution, these enabling technologies produce instantaneous results from solids, aerosols, vapors and liquids situated externally to the MS, in their native environment³⁴. Examples include methods in which the energetic beam is metastable gas-phase atoms and reagent ions (i.e. DART^[35], DAPCI^[36–38], FAPA^[39], LTP^[40,41]), energetic droplets (i.e. DESI^[42,43], EASI^[44], JeDI^[45]), and combinations of laser radiation and ESI (i.e. ELDI^[46], MALDESI^[47,48], LAESI^[49,50]). Ambient methods have many applications including imaging biological tissue^[51], and thin layer chromatography plates^[52], as well as the direct analysis of pharmaceutical tablets^[53] and inks on banknotes^[54] and many other surfaces. DESI is readily implemented on existing commercial instruments that have a direct interface with the atmosphere and on small, field portable MS systems.^[55,56] Since sampling occurs outside the vacuum system of the instrument, a broad range of samples and sample forms can be presented to the mass spectrometer.

Another critical feature of DESI is that it allows MSI of sections of tissue. MSI enables to record spatially-defined biochemical information in two- and three-dimensions. DESI-MSI analysis is commonly performed by rastering the sample surface with respect to the stationary continuous flux of spray-charged droplets through an array of predefined coordinates while collecting a mass spectrum at each position containing mass-to-charge (m/z) and relative abundance information. The resulting data are concatenated into an array and selected m/z values are plotted to assess spatial distribution of intensity at specific m/z values. DESI coupled with MSI is particularly valuable in the field of tissue diagnosis for comparison with standard clinical diagnosis performed on hematoxylin and eosin (H&E) stained histological tissue sections.^[31,57–59] In contrast to extractive techniques such as liquid chromatography MS, tissue sections that have been imaged with DESI-MSI are relatively well preserved and can still be stained after the MS sampling, therefore allowing MSI data to be correlated to the exact area of tissue that was analyzed.^[31,32]

DESI has successfully been employed for the study of small molecules^[60] including the investigation of lipid distributions in a variety of healthy and diseased animal and human tissues^[51,58,61–66] exemplifying the utility of the method for determining diagnostically-relevant information by MS with minimal sample preparation. In comparison to existing MS and optical imaging modalities, the ambient ionization methods show only modest spatial resolution. Despite this limitation, these methods have considerable benefits: they facilitate measurements outside the vacuum of the instrument, require no contrast agents or chemical-tags, and do not require further sample treatment. While very high spatial resolution is desirable for research and development, for example the nanometer range resolution achieved by technologies such as secondary ion mass spectrometry, the modest spatial resolution and fast analysis time provided by ambient MS technologies is ideal for applications in the clinical setting, especially during surgery. The miniaturization of mass spectrometers could also eventually facilitate clinical implementation.^[67]

General workflow

Surgery remains the most important and usually the first treatment modality for devastating brain tumors such as gliomas as well as other primary and metastatic tumors. While maximal surgical excision with the goal of gross total tumor resection is desirable, in practice, delineation of resection margins is very difficult because tumors can closely resemble normal tissue and frequently infiltrate into surrounding normal brain structures. In addition, tumors often abut or directly involve critical brain regions – too large a resection margin may increase the risk for postoperative neurologic deficits. Preoperative localization by MRI of brain tumors is used to plan the surgical intervention and to minimize postoperative deficits. But the shift in the position of brain structures that occurs following a craniotomy can lead to spatial inaccuracies.^[68]

Molecular information obtained rapidly during a surgical procedure could provide surgeons with a powerful tool for performing real-time, image-guided surgery. A variety of mapping techniques (i.e. Raman imaging^[69], Fourier transform infrared spectroscopy imaging^[70], diffusion tensor imaging^[71], positron emission tomographic/single-photon emission computed tomography^[72], electrocortical stimulation^[73] and functional magnetic resonance imaging^[74–77]) have been developed to provide surgeons with such understanding of the relationship of the tumor to surrounding key cortical areas for neurosurgery. Intraoperative MRI (iMRI) developed at Brigham and Women's Hospital (BWH) has provided unprecedented intraoperative visualization.^[2–5]

Histopathological evaluation of frozen sections from tumor biopsies is currently the only method available to provide surgeons with information about tumor type and grade. While customarily used, evaluating tumors with frozen sections has a number of significant limitations that are disruptive to the surgical workflow – in particular, the analysis of each sample requires 20 minutes or more, and typically no more than a few samples are practical to analyze during any one surgical procedure. Moreover, visual review of stained tissue sections does not provide any direct molecular information about a tumor. The use of DESI MS could help with some of these problems, by allowing continuous sampling of multiple areas within the surgical field, by providing specific information about tumor type, grade and possibly prognosis rapidly (within seconds) and by offering very specific molecular information about a sample including levels of biomarkers or therapeutic compounds. The imaging capabilities of DESI can be used to develop a well-annotated reference system correlating specific molecular signatures to standard histopathology information. For real-time applications though, the rapid profiling capabilities of DESI can be used.^[78] The data acquired in a seconds-scale profiling fashion can then be mathematically compared to a well-defined and validated reference system, providing the surgeon with critical information of the tissue at stake in a real-time.

We describe results highlighting the use of MS as a powerful tool in characterizing tissue for surgical-decision making. More specifically, we used DESI MS to distinguish necrotic tumor tissue from viable GBM tumor. We first established correlation between histopathological staining and DESI MS to distinguish viable from non-viable tumor tissue, and built a classification model representative of the histological evaluation. We then used a robust statistical method to validate the detection of potential biomarkers. Direct correlation of mass spectrometry and histopathology results offers a level of validation that cannot be bypassed for achieving the goals of introducing this promising analytical tool in the surgical decision-making workflow and of gaining widespread acceptance by medical teams. In our approach to implement mass spectrometry into the operating suite, we push this validation further by correlating mass spectrometry and histopathology results to pre- and intra-operative MRI. In doing so, we not only ensure the validity of the information acquired from our MS experiment and its data analysis, but we also enable clinicopathologic correlations

as presented below. The case presented here addresses the discrimination between necrosis and viable tumor which challenges pre-existing knowledge of the characteristics of such tissue on MRI. Our work demonstrates that mass spectrometry could play a significant role in the near- and real-time diagnosis of tumors, assist in tumor delineation, and complement MRI.

EXPERIMENTAL SECTION

Sample Collection

Research subject (surgical case 9) was recruited from surgical candidates at the neurosurgery clinic of the BWH, and gave written informed consent to the Partners Healthcare Institutional Review Board (IRB) protocols. Samples were obtained in cooperation with the BWH Neurooncology Program Biorepository collection, and analyzed under Institutional Review Board-approved research protocol.

Image-Guided Neurosurgery

All surgeries were performed with auxiliary image guidance of the BrainLab Cranial 2.1 neuronavigation system (BrainLab). Preoperative MRI-imaging sequences included full T2 ($1 \times 1 \times 2$ mm, 100×100 slice matrix) and post-contrast T1 ($1 \times 1 \times 1$ mm, 256×256 slice matrix, 176 slices), processed in the BrainLab iPlanNet 3.0 software. Standard clinical protocols were observed to obtain primary diagnosis from stained frozen sections.

Stereotactic Sample Acquisition

After clinical frozen-section diagnosis was confirmed, additional samples were acquired during the course of clinical resection and stored at -80°C . Each sample site was localized by the neurosurgeon using the neuronavigation system pointer, and the locations were transferred for offline visualization using the OpenIGTLink protocol (client: open-source 3D Slicer software on www.Slicer.org; server: BrainLab Cranial 2.1 with OpenIGTLink license option).^[79]

DESI Mass Spectrometry Imaging

Tissue sections were prepared on a Microm HM 550 (Thermo Scientific, USA) with the microtome chamber chilled at -21°C and the specimen holder at -20°C . $10\text{ }\mu\text{m}$ thickness coronal sections were prepared and thaw mounted onto a glass slides. Following thaw mounting of tissue sections, slides were allowed to dry for 10 minutes in a desiccator. DESI-MSI was performed using an amaZon speed(TM) ion trap mass spectrometer (Bruker Daltonics) equipped with a commercial DESI ion source from Prosolia, Inc. DESI-MSI was performed in a line-by-line fashion with a lateral spatial resolution of $200\text{ }\mu\text{m}$. MS instrumental parameters used were 200°C heated capillary temperature, 5 kV spray voltage and $4\text{ L}\cdot\text{min}^{-1}$ dry gas. MS data were acquired from m/z 50 to 1100 in UltraScan mode ($32500\text{ }m/z\text{ s}^{-1}$) with a target mass of m/z 600 and trap drive level of 100%. Seventeen microscans were averaged for each pixel in the images for a scan time of 1 s. The spray solvent was 1:1 acetonitrile:dimethylformamide and the solvent flow rate was $0.7\text{ }\mu\text{L}\cdot\text{min}^{-1}$.

Hematoxylin and Eosin Staining

The following protocol for H&E staining was performed on sections previously analyzed by DESI-MSI: 1) fix in MeOH (2 minutes), 2) rinse in water (10 dips), 3) stain in Harris modified hematoxylin solution (1.5 minutes), 4) rinse in water (10 dips), 5) blue in 0.1% ammonia (a quick dip), 6) rinse in water (10 dips), 7) counterstain in Eosin Y (8 seconds), 8) rinse and dehydrate in 100% EtOH (10 dips), 9) rinse and dehydrate again in 100% EtOH (10 dips), 10) dip in xylene (6 dips), and 11) dip in xylene again (6 dips). Sections were

dried at room temperature in hood and covered with histological mounting medium (Permount[®], Fisher Chemicals, Fair Lawn, NJ) and a glass cover slide.

Statistical Analysis

Classification models for glioma subtype, grade, and tumor cell concentration of gliomas had been previously developed using Support Vector Machine analysis in Bruker ClinProTools 3.0.^[80] New SVM classification models were calculated to classify spectra for each surgical sample (glioblastoma multiforme 'GBM' Vs. necrosis). Principal component analysis (PCA) and probabilistic latent semantic analysis (pLSA) were also carried out using ClinProTools 3.0 software (Bruker Daltonics). PCA is a mathematical technique designed to extract, display and rank the variance within a data set.^[81] With PCA, important information that is present in the data is retained while the dimensionality of the data set is reduced. For DESI-MSI, each mass spectrum presents a series of m/z values with specific intensities. With PCA, we factorized the set of spectra such that the constituent principal component vectors are ranked in the order of variance. In MSI, the first three PCs generally differentiate the most the samples. PCA also provides loading values (comprised between -1 and 1), originating from the calculation of the PCs, that make it easy to select the contributing peaks of each PC for further analysis. pLSA has been introduced in the MS literature as a technique to divulge latent tissue-type specific molecular signatures.^[82] For each tissue, a distinct distribution can be considered and mass spectra acquired from this tissue are analyzed as a specific combination of m/z values. In contrast to PCA, pLSA allows to directly visualize the discriminating peaks for a specific tissue type within a mass spectrum.

DESI-MSI data was converted for import to ClinProTools 3.0 using in-house software. Extracted DESI mass spectra were internally recalibrated on common spectra alignment peaks within ClinProTools 3.0. An average mass spectrum created from all single spectra was used for peak selection using the ClinProTools 3.0 internal method (based on vector quantization). For statistical analyses, mass spectra were selected from the tissue from representative areas (GBM Vs. necrosis). Extracted DESI MS spectra acquired from D43 surgical sample were imported into ClinProTools 3.0 software. Normalization, baseline subtraction, peak peaking and spectra recalibration were automatically performed using the software. The initial peak integration windows were manually verified against the average spectrum to ensure that no over- or under-calculation were present.

Visualization of MRI and MS Data

MRI data obtained were plotted in 3D Slicer (www.Slicer.org) (version 4.1). The results of MS data subjected to the described classification system were overlaid as stereotactic points rendered in color scales representing the different tissue types.

RESULTS AND DISCUSSION

Mass Spectrometric Evaluation of a Glioblastoma Resection

Twelve surgical samples (D32 to D43) were taken from a brain tumor. After a full pathologic evaluation, a final report was issued that diagnosed the tumor as a glioblastoma. This report was issued nine days following the operation. Stereotactic information was registered for ten of the biopsies (D32 to D41). Frozen sections from these surgical samples were analyzed by DESI-MSI and subsequently stained with H&E. Review of the H&E stained sections by light microscopy revealed some of these surgical samples were entirely composed of viable tumor while others were entirely composed of nonviable tumor tissue (i.e. necrotic GBM tissue) (Table 1). Because GBM tumors are composed of rapidly proliferating cells, these tumors will frequently display regions of necrosis, either focally or in large regions (termed geographic necrosis).

H&E stained tissue sections of surgical sample D40 showed typical histological features of GBM with a high concentration of viable tumor cells (inset of Figure 1A) while sample D38 was entirely composed of necrotic tissue (inset of Figure 1B). In negative-ion mode, mass spectra acquired from D40 and D38 frozen tissue sections demonstrated distinct profiles (Figure 1) with certain ions exclusively observed in viable GBM (e.g. m/z 279.0 and m/z 391.3 from D40, Figure 1A) and others in the necrosis region (m/z 544.5, m/z 626.6 and m/z 650.6 for D38, Figure 1B). We also noted some ions were present with a higher relative abundance in one of the two surgical samples (e.g. m/z 437.3 and m/z 491.3 for D40, Figure 1A and m/z 572.7 for D38, Figure 1B). Corresponding ion images indicate that these ions are present throughout the tissue sections of D40 (m/z 279.0, m/z 391.3, m/z 437.3 and m/z 491.4 ions, Figure S1A) and D38 (m/z 544.5, m/z 626.6, m/z 650.6 and m/z 572.7 ions, Figure S1B).

We have previously shown that tissue specimens can be discriminated based upon the presence of specific lipid patterns.^[31,57–59] To validate the ability to distinguish viable from necrotic GBM by DESI MS molecular profiling, we next turned to surgical specimens from this GBM resection that contain within the same tissue section both viable and necrotic tumor tissue. As shown in Figures 2 and S2, H&E staining revealed distinct boundaries between viable GBM and necrotic tumor (N) in both surgical samples D43 (Figure 2A) and D42 (Figure S2A). The DESI MS data revealed that both of the lipid patterns that we had observed in sample D40 and D38 (Figure 1) were now present in the same sample (Figures 2B, 2C, S2B and S2C; m/z values in red in each Figure) and were located in the appropriate histologic regions – the ion images in the insets of Figures 2 and S2 highlight both the areas of viable GBM (ion at m/z 279.0 Figures 2B and S2B) and the necrotic GBM (ions at m/z 572.7 and m/z 544.5 Figures 2B and S2B, respectively). We observed similar results for other ions that we had previously identified as discriminating viable and necrotic tumor (m/z 391.3, m/z 437.3, m/z 491.3 for GBM and m/z 626.6, m/z 650.6 for necrosis; ion images of Figure 4 for D43 and S4 for D42).

Toward the Validation of DESI-MSI for Real-Time Molecular Diagnostic

We are developing DESI-MSI as a platform for intraoperative diagnostics. In prior studies we were able to discriminate tumors of the central nervous system. This was possible not only for tumors that are highly distinct from one another (e.g. glioma from meningioma) but also for tumors that are histologically similar (e.g. discriminating low grade gliomas such as oligodendroglioma from low grade astrocytoma).^[31,57–59]

Here, we further demonstrate that we can build a classification method as a proof of concept based on a small training set for discriminating viable from non-viable tumor tissue. This was readily achieved by building a classification model based on machine learning and then determining the rate of cross-validation and recognition capability between GBM and necrotic tissues in other samples. The cross-validation and recognition capability of the classifier was 98 % and 100 % in the training dataset. The results for the test dataset are reported in Table 1. For D43 and D42 surgical samples, each mass spectra contributing to classify tissues as GBM or necrosis were mapped on binary images in figures 3A and S3A.

PCA (Figures 3B and S3B) and pLSA (Figures 4 and S4) are two statistical tools that were used in addition to the machine learning approaches to further identify discriminating peaks between tissue types. According to the 2 first principal components, PCA results show that mass spectra acquired in each region belong to the same tissue type delimited in Figure 3A (left panel of Figure 3B). Moreover, the loading model of the Figure 3B (right panel) and the statistical data of Table 2 clearly indicate that m/z values presented in Figure 2B and 2C are specific of each tissue type according to the Wilcoxon/Kruskal-Wallis test. Finally, pLSA data confirm the relevance of these m/z values to discriminate the two tissue types (Figures

4A and S4A). Regarding the statistical study of DESI MS data of surgical case 9, we can assume that potential markers of GBM and necrosis could have been defined and further studies should be undertaken to specifically identify the nature of these biomolecules and assigned targeted peaks as previously described.^[31,57–59]

DESI-MSI and MRI: is the Whole Greater than the Sum of its Parts?

Samples from surgical case 9 were classified as GBM or necrotic tissue based on mass spectral information and the results were validated by histopathology evaluation of each specimen. Although lipid profiling provides highly specific data to discriminate tissues and define boundaries between tumor and healthy brain tissue, DESI-MSI is still an invasive technique requiring direct contact with the tissue of interest. Conversely, MRI is a non-invasive technique that may supply mm-scale localization of the tumor, but with limited information on the tumor's chemistry. As shown in Figure 5, 3D MR structural scans can delineate the tumor volume (Figure 5A) and axial gadolinium-enhanced T1-weighted MR images demonstrate the spreading of this bilateral GBM across the hemisphere boundary (Figure 5B). The majority of images in Figure 5B show a hypodense central core, commonly associated with necrosis. This core is circled by a thick irregular ring with a shaggy inner margin typical of GBM. GBM has prominent neovascularity with abnormal blood-brain barrier, and breakdown of this barrier is thought to cause leakage of the contrast agent (i.e. gadolinium) into tissues and to be responsible for a ring-enhanced signal on enhanced T1-weighted MR images.^[83] The highest neovascularity and therefore viable tumor concentration is typically associated with the enhancing tumor ring.

Using stereotactic data about the location of the biopsies from surgical case 9, we mapped information derived from our classifiers (GBM or necrotic tissue) onto the MR images (Figure 5). The 3D MR rendering of the segmented tumor in Figure 5A shows the relative distribution of surgical samples as they relate to tumor presentation, while individual axial MR images more specifically correlate tissue characteristics with the uptake of contrast (Figure 5B). As shown in Figure 5B, DESI MS data mapping indicates that the tumor presents necrotic components both in the central and peripheral portions of the tumor. Some studies have reported that necrosis is present in 85% of cases diagnosed as GBM^[84–86], but it is mainly associated with the central region of the tumor. Previous studies have also reported the propensity of radiation-induced necrosis that is the result of inflammatory cascades activated by radiation injury and exacerbated by the chronic hypoxia from endothelial remodeling.^[87] In GBM, this radiation-induced necrosis is generally observed in the periphery of the tumor, however, the patient (case 9) had not received prior radiotherapy.

CONCLUSION

Surgery is the primary treatment for most brain tumors. Surgical decision-making could be improved with tools that rapidly provide molecular information about multiple biopsies or continuous sampling at the time of surgery. Ambient mass spectrometry techniques that can provide near-real time molecular information from tissue samples hold great potential in this area, but they have to be carefully validated using well annotated histopathology evaluation of the tissue. With DESI MS, we have previously been able to classify tumors, define tumor subtypes, and identify tumor grade. Here we show that in surgical resection specimens we can readily identify necrotic tumor tissue, an indicator of a high-grade malignancy, and we can distinguish necrotic tumor tissue from viable tumor regions. As we apply DESI MS to a broad range of human malignancies we will be able to define the molecular correlates of a range of histologic features, many of which have become diagnostic hallmarks of cancer (such as necrosis in the diagnosis of GBM). Many of these insights will rely on the use of powerful machine learning and statistical tools to assist in turning the vast data sets acquired by mass spectrometry into usable tumor classifiers that are ultimately useful for real-time

applications. As more and more is done, DESI MS could have a significant role for a broad range of diagnostic applications including defining the boundaries between tumor and normal tissue, diagnosing image-guided needle biopsies and determining prognostic and predictive information for guiding patient care. One significant disadvantage of mass spectrometry over optical approaches in characterizing tissue is that molecules need to leave the tissue for mass spectrometry analysis, therefore disrupting it. Since surgery innately exposes and disrupts tissue, mass spectrometry-based approaches for real-time tissue characterization do not pose more risk to the patient. Some of the significant advantages of mass spectrometry toward surgical decision-making applications include 1) the ability to analyze any molecule, at least in principle, 2) acquire complex signatures that can increase specificity over a single biomarker paradigm, 3) no molecular labeling is required, and 4) rapidity of execution, especially when interfaced with ambient ionization methods. Our siting of a mass spectrometer into the AMIGO at BWH provides with invaluable opportunities to validate mass spectrometry findings for a variety of surgical diseases tackled by the growing field of mass spectrometry imaging and to continue technology development with the hope of improving patient care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to their patients and families who consent to participate in research. The authors would also like to acknowledge support from the Advanced Multimodality Image Guided Operating (AMIGO) suite team.

GRANT SUPPORT

The work received support from Daniel E. Ponton fund for the Neurosciences, the DFCI Pediatric Low-Grade Astrocytoma program, and the NIH Director's New Innovator Award (grant 1DP2OD007383-01 to N.Y.R. Agar); the Klarman Family Foundation (A.J. Golby). S.S. is supported by NIH grant K08NS064168. LSE, and RGC also thank the U.S. National Institute of Health (Grant 1R21EB009459). This project was supported by the National Center for Research Resources and the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health through Grant Numbers P41EB015898 and P41RR019703.

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Figure 1. DESI-MSI lipid profiles of surgical samples D40 and D38

Negative ion mode mass spectra from GBM surgical sample D40 (A) and necrotic surgical sample D38 (B). Insets show optical images of the sections stained with H&E after DESI-MSI analysis. In red, m/z values corresponding to lipid species exclusively detected in one of the two samples. In green, m/z values corresponding to lipids species having a higher relative abundance in one of the two surgical samples.

Figure 2. Histological evaluation and DESI-MSI analyses of surgical sample D43

(A) Optical images of a D43 section H&E stained after DESI-MSI analysis. Dotted lines on the section delineate areas of necrosis “N” and viable glioblastoma “GBM” tumor. (B) Negative ion mode mass spectrum acquired from the viable GBM area during DESI-MSI analysis (selected mass spectrum is indicated by an arrow in A). In red, m/z values corresponding to lipids species exclusively or preferentially detected in the GBM areas. Inset corresponds to a DESI-MSI ion image representing the repartition of an ion at m/z value 279.0. (C) Negative ion mode mass spectrum acquired from the necrotic area during DESI-MSI analysis (selected mass spectrum is indicated by an arrow in A). In red, m/z

values corresponding to lipids species exclusively or preferentially detected in areas of necrosis. Inset corresponds to DESI-MSI ion image representing the repartition of ion at m/z value 572.7.

Figure 3. Spectral classification and PCA analysis from data acquired from DESI-MSI analysis of surgical sample D43

(A) Binary image indicating spectral classification using the SVM based classifier. Mass spectra corresponding to red pixels were classified as viable GBM, while green pixels were classified as necrosis. (B) The left panel represents the separation of mass spectra corresponding to viable GBM (red dots) and necrosis (green dots) according to the first two principal components (PC1, contribution of 19% and PC2, contribution of 5%). The right panel shows the loading plot generated from PCA analysis (Load 1 and Load 2). Dots correspond to m/z values. Results define three groups from these data. Each m/z value highlighted in red in Figure 2B belong to the group circled in red (GBM) whereas those ones highlighted in red in Figure 2C to the group circled in green (necrosis). Additional m/z values are present in these two groups and imply that additional species could be specifically detected in GBM or necrosis tissue by DESI MS.

Figure 4. pLSA analysis from DESI-MSI analysis data from surgical sample D43

(A) Excerpts of the m/z range showing pLSA results for peaks at m/z values 279.0, 391.3, 437.3 and 491.3. Blue and red bar plots correspond to the analysis of two components, with the blue bars corresponding to lipid species localized in viable GBM areas. At these m/z values, the blue and red bar plots have unequal intensity for the two component spectra, indicative of a discriminatory power from the m/z values. Ion images obtained by DESI-MSI for each of these m/z values are presented below each corresponding plot. (B) Excerpts of the m/z range of the DESI data set showing bar plots for the first two components obtained with pLSA for peaks at m/z values 544.5, 572.7, 626.6 and 650.6. The red bars here correspond to lipid species localized in areas of necrosis. Corresponding ion images to plotted m/z values are shown below each plot.

Figure 5. Label-free 3D molecular imaging of tumor presentation with DESI-MS

(A) 3D visualization of DESI-MSI results over MRI segmented tumor volume for surgical case 9. The MRI was acquired preoperatively, and the tumor segmented and modeled using Slicer 4.0. The overall tumor volume is represented in light green. The position of each stereotactic sample was digitally registered to the pre-operative MRI using BrainLab iplan cranial 3.0, and the corresponding 3 dimensional coordinates used to render the distribution of the DESI-MSI analyses in the 3D tumor volume. The warm color scale from yellow to red represents the classification results from each sample between viable GBM tumor and necrosis. (B) Classification results are further visualized on axial sections of post-contrast T1 MR images. This view allows the correlation of viable GBM and necrosis areas, with areas of contrast enhancement. S, superior, A, anterior, L, lateral, P, posterior.

Table 1
Classification results for samples from surgical case 9

Results indicate the percent of pixels within each image that were assigned to a given class. Surgical samples used as reference to build the SVM classifier are in boldface (D38 and D40). GBM, glioblastoma.

Name	Histopathology diagnosis	Tissue type (%)	
		GBM	Necrosis
D32	GBM/necrosis	12	88
D33	necrosis	2	98
D34	necrosis	1	99
D35	necrosis	0	100
D36	GBM/necrosis	91	9
D37	necrosis	4	96
D38	necrosis	0	100
D39	GBM	99	1
D40	GBM	100	0
D41	GBM	96	4
D42	GBM/necrosis	86	14
D43	GBM/necrosis	42	58

Table 2
p-values obtained for the eight peaks from t-tests

The p-values of the Wilcoxon/Kruskal-Wallis (PWKW) test and the Anderson-Darling Test (PAD) indicate a significant difference between the GBM and the necrosis data sets for each m/z value of Figure 2B and 2C (0.05 and > 0.05, respectively). All the average intensity values for the m/z values 279.0, 391.3, 437.3 and 491.3 are also increased in the GBM average mass spectrum (Ave2 values) and the others (m/z values 544.5, 572.7, 626.6 and 650.6), in the necrosis average mass spectrum (Ave1 values).
Index, sequence of peak; Mass, m/z ; PTTA, p value of t-test (two classes).

	Index	Mass	PTTA	PWKW	PAD	Ave1	Ave2	SthDev1	SthDev2
GBM	162	279.0	<0.000001	<0.000001	<0.000001	1.64	7.19	1.53	3.87
	208	391.3	<0.000001	<0.000001	<0.000001	2.2	4.15	1.65	2.22
	216	437.3	0.191	0.0141	<0.000001	3.48	3.82	2.11	1.61
	226	491.3	<0.000001	<0.000001	<0.000001	4.33	6.28	2.36	2.63
Necrosis	228	544.5	<0.000001	0	<0.000001	9.55	2.62	4	1.06
	232	572.7	<0.000001	0	<0.000001	105.94	23.82	53.11	9.7
	251	626.6	<0.000001	0	<0.000001	17.68	6.29	7.36	1.98
	259	650.6	<0.000001	0	<0.000001	24.4	7.35	9.95	2.45