Mass spectrometry imaging as a tool for surgical decision-making

David Calligaris,† Isaiah Norton,† Daniel R. Feldman, Jennifer L. Ide, Ian F. Dunn, Livia S. Eberlin,‡ R. Graham Cooks, Ferenc A. Jolesz, Alexandra J. Golby, Sandro Santagata and Nathalie Y. Agar

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 magnetic resonance imaging (MRI) combined with neuronavigation offer a map as to where the tumor should be, but the only definitive method to characterize the tissue at stake is histopathology. Although 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 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 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 mass spectrometry and histopathology results to MRI brings significant insight into tumor presentation that could not only serve to guide tumor resection, but that is also worthy of more detailed studies on our understanding of tumor presentation on MRI. Copyright © 2013 John Wiley & Sons, Ltd.

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however, they would permit the precise molecular characterization of tissue and serve, as an analytical tool in image-guided therapy. Different 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.\textsuperscript{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\textsuperscript{19} and has recently been increasingly employed for the analysis of small molecules such as lipids, drugs, and their metabolites.\textsuperscript{22–30} MALDI mass spectrometry imaging (MALDI-MSI) analyses of tissue have become an extremely promising tool to support decision-making in histopathology evaluation of tissue.\textsuperscript{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.\textsuperscript{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 desorption electrospray ionization (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.\textsuperscript{31,32} In DESI, a pneumatically assisted electrospray produces charged droplets that are directed to collide with the surface of a sample.\textsuperscript{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.\textsuperscript{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.

Desorption electrospray ionization 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 environment.\textsuperscript{34} Examples include methods in which the energetic beam is metastable gas-phase atoms and reagent ions (i.e. DART\textsuperscript{35}, DAPCI\textsuperscript{36–38}, FAPA\textsuperscript{39}, LTP\textsuperscript{40,41}), energetic droplets (i.e. DESI\textsuperscript{42,43}, EASI\textsuperscript{44}, JeDI\textsuperscript{45}), and combinations of laser radiation and electrospray ionization (i.e. ELDI\textsuperscript{46}, MALDES\textsuperscript{47,48}, LAESI\textsuperscript{49,50}). Ambient methods have many applications including imaging biological tissue\textsuperscript{51} and thin layer chromatography plates\textsuperscript{52} as well as the direct analysis of pharmaceutical tablets\textsuperscript{53} and inks on banknotes\textsuperscript{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.\textsuperscript{55,56} Because 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-dimension and three-dimension (3D). 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.\textsuperscript{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.\textsuperscript{31,32}

Desorption electrospray ionization has successfully been employed for the study of small molecules\textsuperscript{60} including the investigation of lipid distributions in a variety of healthy and diseased animal and human tissues,\textsuperscript{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. Although very high spatial resolution is desirable for research and development, for example the nanometer range resolution achieved by technologies such as secondary ion MS, 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.\textsuperscript{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. Although 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.\textsuperscript{58}

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,\textsuperscript{69} Fourier transform infrared spectroscopy imaging,\textsuperscript{70} diffusion tensor imaging,\textsuperscript{71} positron emission tomographic/single-photon emission computed tomography,\textsuperscript{72} electrocoritical stimulation,\textsuperscript{73} and functional MRI\textsuperscript{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 developed at Brigham and Women’s Hospital in Boston, Massachusetts is a highly accurate method and is increasingly being used in neurosurgery to improve surgical decision making. However, this methodology is time-consuming and requires significant post-processing to finalize the data. Intraoperative DESI-MSI is expected to provide similar diagnostic information but in real-time and with significantly reduced post-processing time.
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. Although 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 min 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 with a well-defined and well-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 glioblastoma (GBM) tumor. We first established correlation between histopathological staining and DESI-MS to distinguish viable from nonviable 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 MS 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 MS into the operating suite, we push this validation further by correlating MS and histopathology results to preoperative and intraoperative 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 in the succeeding text. 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 MS could play a significant role in the near-time 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 protocols. Samples were obtained in cooperation with the BWH Neurooology 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 sequences included full T2 (1 × 1 × 2 mm, 100 × 100 slice matrix) and post-contrast T1 (1 × 1 × 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]

**Desorption electrospray ionization 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 μm 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 min 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 μm. MS instrumental parameters used were 200 °C heated capillary temperature, 5 kV spray voltage, and 41 min⁻¹ dry gas. MS data were acquired from m/z = 50 to 1100 in UltraScan mode (32 500 m/z 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 μl 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 min), (2) rinse in water (10 dips), (3) stain in Harris modified hematoxylin solution (1.5 min), (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 s), (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.[160] New support vector machine classification models were calculated to classify spectra for each surgical sample (‘GBM’ multiforme 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.\textsuperscript{[81]} With PCA, important information that is present in the data is retained, whereas the dimensionality of the data set is reduced. For DESI-MSI, each mass spectrum presents a series of \textit{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 principal components (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.\textsuperscript{[82]} For each tissue, a distinct distribution can be considered and mass spectra acquired from this tissue are analyzed as a specific combination of \textit{m/z} values. In contrast to PCA, pLSA allows to directly visualize the discriminating peaks for a specific tissue type within a mass spectrum.

Desorption electrospray ionization mass spectrometry imaging data were 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-calculation or under-calculation were present.

**Visualization of magnetic resonance imaging and mass spectrometry data**

Magnetic resonance imaging 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 GBM. This report was issued 9 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, whereas 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).

Hematoxylin and eosin stained tissue sections of surgical sample D40 showed typical histological features of GBM with a high concentration of viable tumor cells [inset of Fig. 1(a)], whereas sample D38 was entirely composed of necrotic tissue [inset of Fig. 1(b)]. In negative-ion mode, mass spectra acquired from D40 and D38 frozen tissue sections demonstrated distinct profiles (Fig. 1) with certain ions exclusively observed in viable GBM [e.g. \textit{m/z} = 279.0 and \textit{m/z} = 391.3 from D40, Fig. 1(a)] and others in the necrosis region [\textit{m/z} = 544.5, \textit{m/z} = 626.6, and \textit{m/z} = 650.6 for D38, Figure 1(b)]. We also noted some ions were present with a higher relative abundance in one of the two surgical samples [e.g. \textit{m/z} = 437.3 and \textit{m/z} = 491.3 for D40, Fig. 1(a) and \textit{m/z} = 572.7 for D38, Fig. 1(b)]. Corresponding ion images indicate that these ions are present throughout the tissue sections of D40 [\textit{m/z} = 279.0, \textit{m/z} = 391.3, \textit{m/z} = 437.3, and \textit{m/z} = 491.4 ions, Figure S1A] and D38 (\textit{m/z} = 544.5, \textit{m/z} = 626.6, \textit{m/z} = 650.6, and \textit{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.\textsuperscript{[31,37–59]} To validate the ability to distinguish viable from necrotic GBM by DESI-MSI 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 Figs. 2 and S2, H&E staining revealed distinct boundaries between viable GBM and necrotic tumor (N) in both surgical samples D43 [Fig. 2(a)] and D42 (Figure S2A). The DESI-MS data revealed that both of the lipid patterns that we had observed in sample D40 and D38 (Fig. 1) were now present in the same sample [Figs. 2(b), 2C, S2B, and S2C; \textit{m/z} values in red in each Figure] and were located in the appropriate histologic regions – the ion images in the insets of Figs. 2 and S2 highlight both the areas of viable GBM [ion at \textit{m/z} = 279.0 Figs. 2(b) and S2B] and the necrotic GBM [ions

<table>
<thead>
<tr>
<th>Name</th>
<th>Histopathology diagnostic</th>
<th>Tissue type (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D32</td>
<td>GBM/necrosis</td>
<td>GBM 12, Necrosis 88</td>
</tr>
<tr>
<td>D33</td>
<td>necrosis</td>
<td>GBM 2, Necrosis 98</td>
</tr>
<tr>
<td>D34</td>
<td>necrosis</td>
<td>GBM 1, Necrosis 99</td>
</tr>
<tr>
<td>D35</td>
<td>necrosis</td>
<td>GBM 0, Necrosis 100</td>
</tr>
<tr>
<td>D36</td>
<td>GBM/necrosis</td>
<td>GBM 91, Necrosis 9</td>
</tr>
<tr>
<td>D37</td>
<td>necrosis</td>
<td>GBM 4, Necrosis 96</td>
</tr>
<tr>
<td>D38</td>
<td>necrosis</td>
<td>GBM 0, Necrosis 100</td>
</tr>
<tr>
<td>D39</td>
<td>GBM</td>
<td>GBM 99, Necrosis 1</td>
</tr>
<tr>
<td>D40</td>
<td>GBM</td>
<td>GBM 100, Necrosis 0</td>
</tr>
<tr>
<td>D41</td>
<td>GBM</td>
<td>GBM 96, Necrosis 4</td>
</tr>
<tr>
<td>D42</td>
<td>GBM/necrosis</td>
<td>GBM 86, Necrosis 14</td>
</tr>
<tr>
<td>D43</td>
<td>GBM/necrosis</td>
<td>GBM 42, Necrosis 58</td>
</tr>
</tbody>
</table>

Results indicate the percent of pixels within each image that were assigned to a given class. Surgical samples used as reference to build the support vector machine classifier are in boldface (D38 and D40). GBM, glioblastoma.
at \( m/z = 572.7 \) and \( m/z = 544.5 \) Figs. 2(b) 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 Fig. 4 for D43 and S4 for D42).

**Figure 1.** Desorption electrospray ionization mass spectrometry imaging lipid profiles of surgical samples D40 and D38.

**Figure 2.** Histological evaluation and desorption electrospray ionization mass spectrometry imaging analyses of surgical sample D43.
Toward the validation of desorption electrospray ionization mass spectrometry imaging 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 oligodendroglia from low-grade astrocytoma).

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 nonviable 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 Figs. 3(a) and S3A.

Principal component analysis [Figs. 3(b) and S3B] and pLSA (Figs. 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 first two principal components, PCA results show that mass spectra acquired in each region belong to the same tissue type delimited in Fig. 3(a) [left panel of Fig. 3(b)]. Moreover, the loading model of Fig. 3(b) (right panel) and the statistical data of Table 2 clearly indicate that m/z values presented in Fig. 2(b) and 2(c) 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 [Figs. 4(a) 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.

Figure 3. Spectral classification and principal component analysis from data acquired from desorption electrospray ionization mass spectrometry imaging analysis of surgical sample D43.

Figure 4. Probabilistic latent semantic analysis from desorption electrospray ionization mass spectrometry imaging analysis data from surgical sample D43.
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 Fig. 5, 3D magnetic resonance (MR) structural scans can delineate the tumor volume [Fig. 5(a)] and axial gadolinium-enhanced T1-weighted MR images demonstrate the spreading of this bilateral GBM across the hemisphere boundary [Fig. 5(b)]. The majority of the images in Fig. 5(b) 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.\(^8\)\(^3\) The highest neovascularity and therefore viable tumor concentration is typically associated with the enhancing tumor ring.

By 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 (Fig. 5). The 3D MR rendering of the segmented tumor in Fig. 5(a) shows the relative distribution of surgical samples as they relate to tumor presentation, whereas individual axial MR images more specifically correlate tissue characteristics with the uptake of contrast [Fig. 5(b)]. As shown in Fig. 5(b), 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,\(^8\)\(^4\)–\(^8\)\(^6\) 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.\(^8\)\(^7\) 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.

### Table 2. \(p\)-values obtained for the eight peaks from \(t\)-tests

<table>
<thead>
<tr>
<th>Index</th>
<th>Mass</th>
<th>PTTA</th>
<th>PWKW</th>
<th>PAD</th>
<th>Ave1</th>
<th>Ave2</th>
<th>StdDev1</th>
<th>StdDev2</th>
</tr>
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<tbody>
<tr>
<td>GBM</td>
<td>162</td>
<td>279.0</td>
<td>&lt;0.000001</td>
<td>&lt;0.000001</td>
<td>1.64</td>
<td>7.19</td>
<td>1.53</td>
<td>3.87</td>
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<tr>
<td></td>
<td>208</td>
<td>391.3</td>
<td>&lt;0.000001</td>
<td>&lt;0.000001</td>
<td>2.2</td>
<td>4.15</td>
<td>1.65</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>216</td>
<td>437.3</td>
<td>0.191</td>
<td>0.0141</td>
<td>&lt;0.000001</td>
<td>3.48</td>
<td>3.82</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>226</td>
<td>491.3</td>
<td>&lt;0.000001</td>
<td>&lt;0.000001</td>
<td>&lt;0.000001</td>
<td>4.33</td>
<td>6.28</td>
<td>2.36</td>
</tr>
<tr>
<td>Necrosis</td>
<td>228</td>
<td>544.5</td>
<td>&lt;0.000001</td>
<td>0</td>
<td>&lt;0.000001</td>
<td>9.55</td>
<td>2.62</td>
<td>4</td>
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<tr>
<td></td>
<td>232</td>
<td>572.7</td>
<td>&lt;0.000001</td>
<td>0</td>
<td>105.94</td>
<td>23.82</td>
<td>53.11</td>
<td>9.7</td>
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<tr>
<td></td>
<td>251</td>
<td>626.6</td>
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<td>17.68</td>
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<tr>
<td></td>
<td>259</td>
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<td>&lt;0.000001</td>
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<td>&lt;0.000001</td>
<td>24.4</td>
<td>7.35</td>
<td>9.95</td>
</tr>
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</table>

The \(p\)-values of the Wilcoxon/Kruskal–Wallis (PWKW) test and the Anderson–Darling Test (PAD) indicate a significant difference between the glioblastoma and the necrosis data sets for each \(m/z\) value of Figure 2B and 2C (\(\leq0.05\) 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 glioblastoma 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).

GBM, glioblastoma.

Figure 5. Label-free 3D molecular imaging of tumor presentation with desorption electrospray ionization mass spectrometry.
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 MS 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 MS into usable tumor classifiers that are ultimately useful for real-time applications. As more and more is carried out, 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 MS over optical approaches in characterizing tissue is that molecules need to leave the tissue for MS analysis, therefore, disrupting it. Because surgery innately exposes and disrupts tissue, MS-based approaches for real-time tissue characterization do not pose more risk to the patient. Some of the significant advantages of MS toward surgical decision-making applications include the following: (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 MS findings for a variety of surgical diseases tackled by the growing field of MSI and to continue technology development with the hope of improving patient care.

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References


Intraoperative mass spectrometry


Supporting information

Additional supporting information may be found in the online version of this article at the publisher’s web site.