COMMENTARY

Intraoperative mass spectrometry of tumor metabolites

Whitney B. Pope[1]
Department of Radiology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095

In oncologic surgery, establishing tumor margins while the surgery is in progress can be of paramount importance in ensuring maximal tumor resection and the sparing of normal tissue. This remains no easy task. Analyzing surgically resected tissue to determine the presence and type of cancer is a persistent challenge. Surgeons need information as quickly as possible to guide tumor resection, and the current method of tissue freezing, sectioning, and staining, followed by microscopic examination is not only time-consuming, limiting the number of samples that can be processed, but also not always sufficient in establishing the diagnosis. Is there a better way? Several recent papers (detailed below) have demonstrated the potential for a new form of mass spectrometry to be used in real-time to complement—or perhaps eventually replace—standard histopathological assessment of tumor tissue. In PNAS, Santagata et al. (1) present the use of intraoperative desorption electrospray ionization-mass spectrometry (DESI-MS) as a method of measuring a tumor-specific metabolite during brain cancer surgery. The metabolite signal provides diagnostic and prognostic information about the tumor and can also be used to help define tumor margins.

Approximately 50,000 new primary brain tumors are diagnosed each year in the United States. Of these newly diagnosed tumors, roughly 70% are malignant and 30% are lower-grade gliomas. Malignant gliomas are the second leading cause of cancer mortality in people under the age of 35 and kill ∼13,000 people per year (2). Standard treatment for malignant gliomas is maximal safe tumor resection followed by concurrent chemotherapy and radiation treatment (3). Total tumor resection (as defined by postoperative MRI) is associated with longer survival in both high- and low-grade tumors (4). Therefore, improved delineation of tumor margins could lead to more complete resections and improved outcomes.

Recently it has been discovered that a large proportion of gliomas have a mutation in the isocitrate dehydrogenase-1 (IDH1) gene. IDH catalyzes the reversible decarboxylation of isocitrate in the citric acid cycle to produce α-ketoglutarate. The *IDH1* mutation results in a gain-of-function leading to the production of the putative onco-metabolite 2-hydroxyglutarate (2-HG). Elevated levels (up to 100-fold) of 2-HG are present in tumors with mutant compared with wild-type *IDH1*, and 2-HG is not detectable in normal brain. 2-HG production has been implicated in alterations of metabolism that could affect cell growth and treatment susceptibility. *IDH1* mutations are also found in a variety of other cancers, including acute myeloid leukemia, cholangiocarcinoma, T-cell lymphoma, and chondrosarcoma (5).

DESI-MS was developed by Graham Cook and his colleagues at Purdue University in 2004 (6). It provides a means to ionize untreated surgical samples in open air at ambient conditions for analysis by MS. The method can be used to detect lipids, hormones, drugs, and other biological and pharmaceutical molecules (6–8). Ionization occurs when an electrically charged mist is targeted at the sample by applying a voltage to the sample holder. Desorbed ions from the sample then travel through the air and into the MS inlet (Fig. 1). Circumvention of laborious preprocessing methods required by standard MS brings the analysis to near-real time, allowing for its application in the intraoperative environment. A notable advantage of the technique is that many more tissue samples can be processed during a surgery than would be possible for standard histological assessment of frozen sections.

The ability of DESI-MS to identify target molecules from tumor tissue in near-real time has many potential clinical applications.

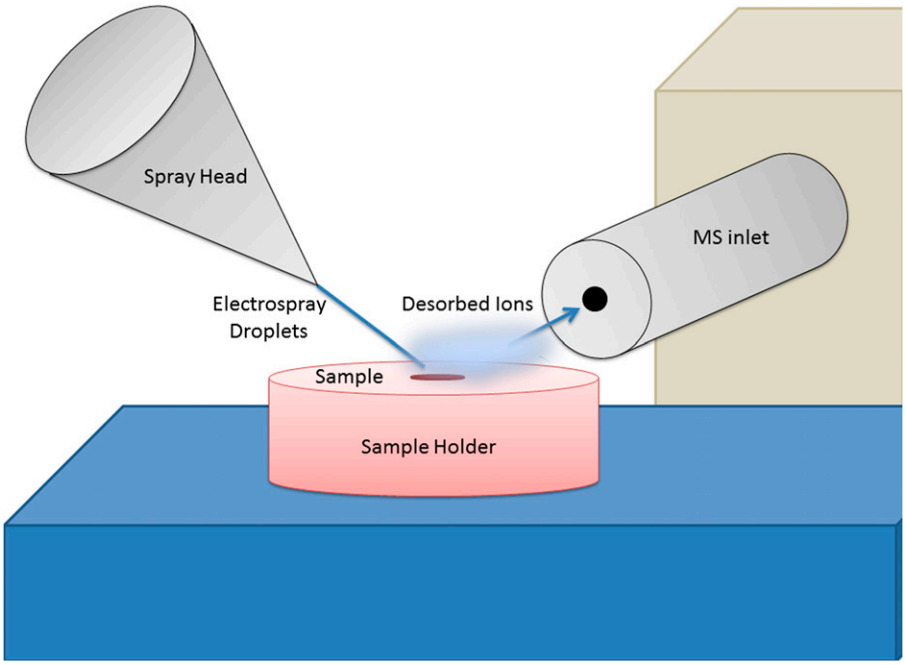

Fig. 1. Model of DESI-MS. Ionization occurs when electrospray droplets are targeted at the sample by applying a voltage to the sample holder. Desorbed ions from the sample then travel through the air and into the MS inlet for MS analysis.

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[1]Email: wpope@mednet.ucla.edu.

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For example, lipid or other metabolite signatures detected by DESI-MS and that are specific for tumor tissue can be used to help identify the margin between tumor and normal brain (7, 9). DESI-MS can therefore confirm the presence of tumor and potentially spare resection of normal neural tissue. This ability could prove highly useful when the accuracy of image-guided navigation is degraded by shifts in the position of the brain that can occur following craniotomy and tumor debulking. If, in the future, the probe could be used in situ, it would be possible to establish regional tumor infiltration before any tissue is removed, and thus maximize the preservation of normal brain, which is critical to maintaining patient neurological functioning. Currently, intraoperative MRI is used by some neurosurgeons to assess residual tumor and account for this brain shift, but the required equipment is expensive and not universally available. An additional drawback is the time that it takes to acquire intraoperative imaging, which is on the order of 1 h. These limitations provide additional impetus for the use of DESI-MS or other techniques that can deliver a cheaper and quicker method of identifying unresected tumor.

Although to date untested, DESI-MS may have the ability to distinguish tumor recurrence from areas of enhancement that mimic tumor, but are actually necrotic regions associated with treatment effect. This phenomenon of pseudoprogression, in which therapy-related increase in contrast-enhancement mimics tumor growth, cannot be reliably distinguished from true tumor progression without histopathologic analysis (10). Identifying areas of pseudoprogression at the time of surgery may help reduce the size of the resection required to remove viable tumor.

The pace of translating DESI-MS to a clinically useful intraoperative method appears to be accelerating (1, 7, 11, 12). A recent report demonstrates the ability of DESI-MS to establish tumor margins in gastric cancer specimens (13). Previous work has used lipid profiles from tumor tissue followed by DESI-MS to discriminate different types of brain tumors, and also provide information on glioma grade (7, 11). In PNAS, Santagata et al. (1) identify a MS peak corresponding to 2-HG, thereby allowing for the intraoperative identification of *IDH1* mutant tumors, something that was previously not possible. The method proved to be quick and reliable, and as mentioned, required no processing of the surgical samples before analysis. Identifying *IDH1* mutant tumors at surgery may impact clinical decision making, as recent reports have suggested that more aggressive surgical resection of *IDH1* mutant tumors is associated with improved outcomes (14). Santagata et al. (1) demonstrate that the DESI-MS technique is remarkably sensitive; the detection limit is ∼3 μmol 2-HG per gram of tissue and 2-HG is detectable in samples where the tumor concentration is as low as 5%. This detectability suggests that DESI-MS could identify areas of tumor infiltration that may not be readily apparent on MRI. Indeed, the authors illustrate a case in which DESI-MS was used to guide resection where the MRI showed imaging changes (T2 hyperintensity without enhancement) that were equivocal for tumor. 2-HG was detected by DESI-MS, the tissue was resected, and the presence of tumor was confirmed histopathologically.

Santagata et al. identify a MS peak corresponding to 2-HG, thereby allowing for the intraoperative identification of *IDH1* mutant tumors.

Although the intraoperative use of DESI-MS to improve the chance of complete tumor resection could be a significant advance in the treatment of brain tumors, the fact remains that even complete surgical excision is rarely curative for these aggressive neoplasms (15). However, several other exciting possibilities for the application of MS for brain tumors exist; in particular, the technique raises the possibility of real-time monitoring of drug treatment effects. For example, in the future it might be possible to deliver drugs to the tumor during an operation and use a metabolic response detected by DESI-MS or similar MS techniques as a surrogate marker for treatment effect, essentially in real time. Multiple drugs could be tested specifically in each patient and combinations of effective drug therapies could be optimized to provide the best, personally tailored antiglioma treatment. Response of oncogenic signaling pathways based on protein phosphorylation states could be interrogated as well to provide a better understanding of which pathways are inhibited by targeted therapies. Drug distribution and metabolism would yield information on, for example, whether a drug was effectively crossing the blood-brain barrier (16), whether the drug was hitting its target, and potentially whether a prodrug is being converted into the active drug within the tumor. These uses could greatly accelerate development of drug therapies, which to date has been painfully slow for gliomas, with only three drugs of limited efficacy being developed over the past 30 y (17). Thus, it seems clear that bringing the highly powerful analytic tool of MS into the operating room will provide an entirely new information stream that could guide a variety of clinical decisions by providing real-time assessment of tissue state and potentially treatment response.

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