CANCER BIOLOGY & THERAPY
2018, VOL. 19, NO. 11, 953–955
https://doi.org/10.1080/15384047.2018.1456610

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Tissue preservation with mass spectroscopic analysis: Implications for cancer diagnostics

O. Morgan Hall, Cody J. Peer, and William D. Figg

Clinical Pharmacology Program, National Cancer Institute, Bethesda, MD

ABSTRACT

Surgical intervention is a common treatment modality for localized cancer. Post-operative analysis involves evaluation of surgical margins to assess whether all malignant tissue has been resected because positive surgical margins lead to a greater likelihood of recurrence. Secondary treatments are utilized to minimize the negative effects of positive surgical margins. Recently, in Science Translational Medicine, Zhang et al describe a new mass spectroscopic technique that could potentially decrease the likelihood of positive surgical margins. Their nondestructive in vivo tissue sampling leads to a highly accurate and rapid cancer diagnosis with great precision between healthy and malignant tissue. This new tool has the potential to improve surgical margins and accelerate cancer diagnostics by analyzing biomolecular signatures of various tissues and diseases.

ARTICLE HISTORY
Received 15 March 2018
Accepted 19 March 2018

KEYWORDS
biochemical recurrence; biomarker; cancer; diagnostics; mass spectrometry; surgical margin

One of the cornerstones in treatment of localized cancer is surgical intervention to remove cancerous tissue with emphasis on maximum preservation of healthy tissue– e.g. for organ functionality in lung cancer or for minimal aesthetic effect in breast cancer.[1] Other cancer types, including thyroid, ovarian, colon, and prostate cancer, also regularly require surgical intervention. Imperfect surgical techniques and difficulties intraoperatively differentiating between cancerous and healthy tissue often result in positive surgical margins (PSM), which indicate a potential for biochemical recurrence (BCR) because cancerous cells may remain at the tumor site. Usually, therapies such as secondary surgery, radiation, chemotherapy, or a combination thereof are utilized to prevent BCR.[1] For example, after mastectomy, 39.2% of patients without post-operative radiation had recurrence at a 20-year follow-up.[2] Similarly, prostate cancer patients had a 20.7% biochemical recurrence post-prostatectomy.[3] Lower rates of recurrence by elimination of PSMs can reduce patients' future morbidity and mortality rates, thereby improving patient care.

To improve the rate of achieving a negative surgical margin, intraoperative tests to distinguish between healthy and tumor tissue are necessary. Post-operative, *ex vivo* techniques, such as immunohistochemistry and gene sequencing,[1] are currently utilized to identify and characterize cancer cells, but these methods are too time consuming to be useful during tumor excision. Traditionally, these biopsies need to be fixed (often with paraffin) and stored until subsequent microscopic analysis by a trained pathologist. This process is time- and labor-intensive as well as subject to human interpretation, and it can delay in making critical decisions for the patient. More surgically relevant techniques using fluorescent probes, Raman spectroscopy, and mass spectrometry have been developed for *in vivo* tissue analysis.[1] Surgical margins are improved with these techniques due to rapid diagnosis of malignant tissue. Mass spectral analysis is particularly useful in differentiating tissue types because healthy and cancerous tissue each produce unique biomolecular spectra.

Mass spectrometry's traditional applications in medicine are widespread, but further development of sensitivity and sampling techniques are allowing this technology to move into the realm of clinical diagnostics by analyzing biomolecular spectra. Drug development has long relied on mass spectrometry (MS) to measure drug concentrations in biological matrices by monitoring molecule-specific fragmentation patterns. Analysis of the obtained concentrations provides insight into the pharmacokinetics of the drug. A similar concept can be applied to perform biomolecular spectra analysis of a sample. Rather than selecting for a specific mass corresponding to a target analyte, a sample is injected directly into the mass spectrometer to monitor the mass of all species present in the sample. By comparing spectra obtained using this technique, typical biomolecular spectra of various tissue types can be established and used to identify unknown samples. Clinical use of MS to distinguish between cancerous and healthy tissue has the potential to impact and expand diagnostics in oncology.

Until recently, destructive sampling techniques such as electrocauterization or ultraviolet/infrared lasers were employed.[1] These procedures inevitably kill portions of the healthy, viable tissue they are used to preserve, which limits their intraoperative applicability.[1] Even nondestructive techniques, such as desorption electrospray ionization (DESI)-MS, are not ideal for obtaining a biomolecular spectra in real-time due to use of

CONTACT William D. Figg figgw@mail.nih.gov 9000 Rockville Pike, Building 10, Room 5A01, Bethesda, Maryland 20892.

organic solvents that could alter the *in situ* profile or harm normal tissue.

In *Science Translational Medicine*, a recently published article by Zhang et al. describes the MasSpec Pen – a new *in vivo* and *ex vivo* cancer diagnostic tool using a mass spectrometer with a nondestructive tissue sampling technique.[1] A single, pre-determined volume of water from a handheld sampling probe is automatically dispensed and exposed to the tissue of interest for a pre-determined length of time (1sec, 3sec or 5 sec, typically). A smaller droplet results in more precise margins, but less intense signal. Upon exposure, the water extracts biomolecules from the target tissue to which it is exposed without harming or otherwise altering the nature of the tissue. The water droplet is pulled under vacuum through the injector and into the MS, where masses of the extracted biomolecules are detected and scanned, providing a spectrum of the sampling area's water-soluble biomolecules.[1] Surprisingly, many bio-relevant lipids are able to be monitored as well.

Diagnostic pathology using MS can be performed based on cancer-specific biomarkers in a tissue sample. To be considered a strong cancer biomarker, the signal at a specific mass to charge ratio (*m/z*) must have a consistent, significant difference in intensity between the healthy and cancerous tissues. Previous studies using destructive sampling techniques coupled to mass spectrometric analysis have shown this to be effective in cancers of the brain, lungs, stomach, intestine, prostate, testicles, bladder, kidneys, liver, breast, and lymph nodes.[4] In general, the abundance of specific lipid molecules allow for accurate diagnoses, but there are other tissue-specific molecules (especially metabolites and fatty acids) that differentiate healthy and cancerous tissue, albeit they do not present with the same level of regularity, potentially limiting their applicability as biomarkers.[4]

Initial tests of the MasSpec Pen were conducted on normal thyroid tissue and papillary thyroid carcinoma tissue thin sections. A much higher abundance of various lipid species in the range of *m/z* 650–900 were found in papillary thyroid carcinoma versus normal thyroid tissue. These species, including cardiolipin and glycerophospholipids, have been previously described as mass spectroscopic biomarkers of the disease.[5] Cardiolipin is a glycerophospholipid typically localized within the mitochondrial membrane.[5] This is of particular interest because cancer cells almost universally adjust their metabolism either towards or away from oxidative phosphorylation given the environment, energy needs, and mutations of the cancer cell.[6] As such, mitochondrial abundance, and therefore cardiolipin prevalence, will be changed from normal tissue to cancerous tissue, making it important to monitor as a potential biomarker using MS.

Another potential biomarker with clinical relevance is phosphatidylinositol (PI) (38:4). Prior studies show PI (38:4) abundance increase in glioblastomas, prostate, pancreatic and colon cancers; the present study shows increased PI (38:4) abundance occurs in lung, ovarian, breast, and thyroid cancers.[1,4,5,7] PI is a membrane phospholipid with 3 sites of phosphorylation.[8] Its intracellular effects depend upon the site and number of phosphorylations.[8] In cancer biology, $PI(3,4)P_2$ and $PI(3,4,5)P_3$ are major signaling lipids, controlling cell growth, survival, and proliferation.[8] $PI(3,4,5)P_3$ controls intracellular signaling upon binding to Akt, PDK1, and BTK.[8] Increased $PI(3,4,5)P_3$ expression leads to more rapid cell proliferation.[8] This can occur from mutations in PI3K, which generates $PI(3,4,5)P_3$. When these mutations happen, the tumor suppressor PTEN dephosphorylates $PI(3,4,5)P_3$ to its precursor $PI(4,5)P_2$[8,9]. However, somatic mutations of PTEN are among the most common mutations found in cancer cells, and as such $PI(3,4,5)P_3$ often avoids having its signal repressed by this mechanism.[8,9] $PI(3,4)P_2$ exhibits a similar effect on cell proliferation through the Akt pathway after it is generated by PI-5-phosphatase dephosphorylating $PI(3,4,5)P_3$[8]. Because of the importance PI (38:4) holds in cancer biology, it could serve as a clinically-relevant predictive biomarker of healthy versus cancerous tissue. Further analysis is necessary before determining if these species are biomarkers, which will likely depend on the cancer's tissue type as well as disease subtype.

To assess the accuracy of diagnosis achieved by the MasSpec Pen, the researchers analyzed 253 human tissue samples, encompassing multiple tissue types (lung, n = 95; ovary, n = 57; thyroid, n = 56; and breast, n = 45), multiple cancer types, and healthy tissue.[1] The accuracy was very high and comparable to other, established techniques, such as MALDI and DESI-MS.[1,10,11] Breast cancer was diagnosed with an accuracy of 95.6%, ovarian was 94.7%, lung was 96.8%, and thyroid was 94.7% and 97.8% for follicular thyroid adenocarcinoma and papillary thyroid carcinoma, respectively.[1] Overall predictive accuracy was 96.3% for the 253 ex vivo human tissue samples.[1]

In vivo biomarker profiling during a surgical procedure were necessary for proof-of-concept to further elucidate the potential intraoperative impact of the MasSpec Pen. Using a human breast cancer cell line (BT474), researchers subcutaneously engrafted nude mice.[1] After allowing the tumors to grow over 4 weeks, tumors and surgical margins were analyzed utilizing the MasSpec Pen. Two distinct biomolecular spectra were obtained from cancerous tissue and healthy tissue, with cardiolipin and PI (38:4) being among the most distinct differences between sample types.[1] Previously observed spectra in breast cancer using prior techniques compared well to the biomolecular spectra reported using the MasSpec Pen.[1] Importantly, no macroscopic or microscopic tissue damage was observed.[1]

With the high level of accuracy obtained, the tissue preserving sampling technique, and the potential for improvements in surgical treatment of many cancer types, the MasSpec Pen has the ability to improve both cancer diagnostics and treatment. Diagnostic tests on biopsy samples could be performed without damage to the tissue; thereby, preserving the sample for other tests such as immunohistochemistry staining for confirmation of the MasSpec Pen diagnosis. Combination of the two could improve accuracy of cancer diagnoses and give faster initial feedback to physicians and patients because of the rapid output from the MasSpec Pen.

PSMs are a major contributor to recurrence in patients after undergoing surgery for tumor excision. For surgical breast cancer treatment, re-excision and/or mastectomy was performed in 18–29% of patients.[12] Patients 5 years after radical prostatectomy with positive surgical margins had a 47% rate of BCR while patients with a negative surgical margin had less than 20% rate of recurrence.[13] In pancreatic cancer, surgical

margin analysis of frozen sections matched MS analysis in 24 of 32 samples (75%) post-operatively. Patients, whose tests were both negative, had a median survival of 26 months while patients with a negative frozen section analysis and a positive MS analysis had a median survival of 10 months.[7] MS analysis proved more sensitive at detecting PSMs than frozen section analysis. Intraoperative utilization of MS to detect surgical margins could have eliminated PSMs and potentially increased survival in this subset of patients.

Elimination of PSMs through MasSpec Pen implementation in both *in vivo* and *ex vivo* cancer diagnosis could drastically decrease the number of secondary operations and/or treatment courses patients must endure. While current MS methods to analyze tissue intraoperatively exist, they require destructive sampling techniques that prevent extensive clinical utilization. The MasSpec Pen would remove the negative side effects and rapidly provide clinicians with the same necessary diagnostic information as has now been proved in a murine xenograft model.[1]

Further advances are necessary for the MasSpec Pen to be applied in the operating room. The technique needs to be tested in a human study to ensure safety and efficacy. Simultaneously, a database of cancer type mass spectral signatures is needed to keep diagnosis time relevant to surgical applications. Establishing these databases and integrating them to form an automated diagnostic tool will take time, but the promise of immediate feedback about surgical margins with a non-destructive sampling technique, and its *ex vivo* applicability gives this technology great promise for future clinical applications.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the National Institutes of Health.

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