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Construction of mass spectra database and diagnosis algorithm for head and neck squamous cell carcinoma

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ABSTRACT

Objectives: Intraoperative identification of tumor margins is essential to achieving complete tumor resection. However, the process of intraoperative pathological diagnosis involves cumbersome procedures, such as preparation of cryosections and microscopic examination, thus requiring more than 30 min. Moreover, intraoperative diagnoses made by examining cryosections are occasionally inconsistent with postoperative diagnoses made by examining paraffin-embedded sections because the former are of poorer quality. We sought to establish a more rapid accurate method of intraoperative assessment.

Materials and methods: A diagnostic algorithm of head and neck squamous cell carcinoma (HNSCC) using machine learning was constructed by mass spectra obtained from 15 non-cancerous and 19 HNSCC specimens by probe electrospray ionization mass spectrometry (PESI-MS). The clinical validity of this system was evaluated using intraoperative specimens of HNSCC and normal mucosa.

Results: A total of 114 and 141 mass spectra were acquired from non-cancerous and cancerous specimens, respectively, using both positive- and negative-ion modes of PESI-MS. These data were fed into partial least squares-logistic regression (PLS-LR) to discriminate tumor-specific spectral patterns. Leave-one-patient-out cross validation of this algorithm in positive- and negative-ion modes showed accuracies in HNSCC diagnosis of 90.48% and 95.35%, respectively. In intraoperative specimens of HNSCC, this algorithm precisely defined the borders of the cancerous regions; these corresponded with those determined by examining histologic sections. The procedure took approximately 5 min.

Conclusion: This diagnostic system, based on machine learning, enables accurate discrimination of cancerous regions and has the potential to provide rapid intraoperative assessment of HNSCC margins.

Introduction

Although complete tumor resection leads to improved prognosis in patients with head and neck squamous cell carcinoma (HNSCC), functional disabilities such as dysphagia and dysphonia are sometimes unavoidable. The risk of these complications can be minimized by careful dissection [1–4]. Intraoperative rapid diagnosis using frozen sections is the standard means of determining appropriate tumor margins [5,6]. This process requires several steps, including tissue freezing, cryosectioning, hematoxylin and eosin (H&E) staining, and microscopic examination, the whole process usually taking > 30 min. The quality of frozen sections is occasionally inferior to that of paraffin-embedded sections [6,7]. It is especially difficult to delineate malignant cells in irradiated tissues because of the presence of atypical epithelial variations [7,8]. A more rapid and accurate form of intraoperative assessment would be of great value in improving surgical outcomes.

Abbreviations: DESI, desorption electrospray ionization; ESI, electrospray ionization; H&E, hematoxylin and eosin; HNSCC, head and neck squamous cell carcinoma; LR, logistic regression; LOPOCV, leave-one-patient-out cross validation; MMP9, matrix metalloproteinase 9; MS, mass spectrometry; PESI-MS, probe electrospray ionization-mass spectrometry; PLS-LR, partial least squares-logistic regression; REIMS, rapid evaporative ionization mass spectrometry

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Table 1
Patient characteristics.

Patients	Locas of cancer	Gender	Age	Degree of differentiation	TNM classification of malignant tumors			
					T	N	M	Stage
1	Oral (Gingival)	M	81	Well	2	0	0	II
2	Laryngeal	M	70	Well	4a	0	0	IVA
3	Oral	M	81	Moderately	1	0	0	I
4	Oral (Lingual)	M	74	Poorly	4a	2	0	IVA
5	Laryngeal	M	82	Moderately	2	0	0	II
6	Laryngeal	M	66	Poorly	3	0	0	III
7	Laryngeal	M	60	Poorly	2	2	0	IVA
8	Laryngeal	M	61	Moderately	4a	1	0	IVA
9	Oral	M	61	Moderately	4a	2	1	IVC
10	Oral	M	81	Well	2	0	0	II
11	Laryngeal	M	73	Poorly-moderately	4a	2	0	IVA
12	Laryngeal	M	74	Well	4a	0	0	IVA
13	Hypopharyngeal (PS)	M	69	Moderately	2	2	0	IVA
14	Oral (Lingual)	M	65	NA	4a	2	0	IVA
15	Hypopharyngeal (PS)	M	64	Moderately	2	2	0	IVA
16	Pharyngeal (PW)	M	82	Poorly	2	2	0	IVA
17	Oral (Gingival)	M	47	Moderately	4a	0	0	IVA
18	Oral (Gingival)	M	78	Well	4a	0	0	IVA
19	Hypopharyngeal (PC)	M	68	Moderately	3	0	0	III
20*	Hypopharyngeal (PS)	M	67	Moderately	4a	2	0	IVA

PS, piriform sinus; PW, posterior pharyngeal wall; PC, post-cricoid area.
* Sample was used in the simulated situation of intraoperative rapid margin assessment (Fig. 3).

Diagnostic systems based on mass spectrometry (MS) have recently been developed [9–13]. Rapid evaporative ionization MS (REIMS) [14] and electrospray ionization (ESI) [15] are two representative ambient ionization techniques that have commonly been used for MS of biological samples. However, these techniques are invasive and require laborious pretreatment. We have developed probe electrospray ionization-MS (PESI-MS) using a very fine needle to achieve direct MS with less invasiveness [16]. The probe needle directly collects a small amount of biological components without any pretreatment [15,17,18]. High voltage is applied to the needle to generate the electrospray. PESI-MS is suitable for direct analysis with minimal invasiveness.

PESI-MS captures metabolic profiles in real time. The composition of low-molecular-weight metabolites in cells or tissues provides important indicators of the presence of malignancy. In the clinical setting of cancer, PESI-MS has been used to discriminate renal cell carcinoma [9] and hepatocellular carcinoma from surrounding normal tissue [10]. These results suggest that PESI-MS can be used to diagnose various cancers and also has the potential to be an additional method of assessing tumor margins during surgery.

Machine learning is a method of creating and evaluating algorithms that facilitate pattern recognition, classification, and prediction based on statistical models using existing data [19]. Automatic construction of an algorithm by a suitably programmed computing machine potentially minimizes the possibility of human biases affecting selection and performance of the algorithm [19]. Machine learning has been broadly utilized in the field of biology and can be applied to analysis of data from MS to select biomarkers and diagnose various diseases, including breast cancer [20]. Although we have recently demonstrated the potential of PESI-MS combined with PLS-LR as a diagnostic system in an animal study [21], it remained unclear whether this approach is applicable to HNSCC diagnosis.

In this study, a total of 255 mass spectra were acquired from non-cancerous and cancerous tissues of 19 HNSCC patients by using both positive- and negative-ion modes of PESI-MS and used to learn partial least squares-logistic regression (PLS-LR). By comparing whole spectral patterns between non-cancerous and cancerous regions, learned PLS-LR discriminated cancerous and non-cancerous areas in HNSCC tissues with 90.48% and 95.35% accuracy in positive- and negative-ion mode, respectively. The time needed to obtain a mass spectrum from a tissue of interest was ≤5 min. This system precisely discriminated the borders of cancerous regions as assessed on permanent sections by pathologists. This combination of PESI-MS and machine learning has the potential to provide rapid intraoperative assessment of HNSCC margins.

Materials and methods

Biological samples

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and the protocol was approved by the ethics committee of the University of Yamanashi (No. 645). Each patient provided written informed consent before participating in this study and all clinical data were anonymized. To construct a database of mass spectra of human mucosal tissues, non-cancerous and cancerous tissues excised from 19 patients with HNSCC were divided into mucosal and connective tissue layers, the latter including muscle. These specimens were immediately stored at −80 °C until PESI-MS analysis.

Additionally, excised tissues that included non-cancerous and cancerous regions were cut in half to assess tumor margins. One half (*i*) was fixed in 20% neutral buffered formalin solution for histological staining, whereas the other half (*ii*) was further divided into 10 smaller specimens, which were then divided into a mucosal and connective tissue layers. These mucosal layer specimens were stored at −80 °C.

Histological staining

Fixed tissue specimens were processed for paraffin sectioning by being cut into 3-μm-thick sections, stained with H&E, and examined under an Olympus BX-53 microscope (Olympus, Tokyo, Japan).

Mass spectrometry

PESI, an ambient ionization method, was installed on a single quadrupole mass spectrometer LCMS-2020 (PESI-MS; Shimadzu, Kyoto, Japan). Frozen specimens were added to 200 μL of 50% ethanol in a 0.5-mL tube and homogenized with a disposable pestle (Argos Technologies, Elgin, IL, USA) for 10 s. The homogenates were centrifuged at 500*g* for 5 min and the supernatants analyzed by PESI-MS (original analysis), as described previously [22]. In the direct analysis, dissected mucosal tissue specimens were analyzed without