APPLIED SCIENCES AND ENGINEERING

Three-dimensional histological electrophoresis enables fast automatic distinguishment of cancer margins and lymph node metastases

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Tissue diagnosis is important during surgical excision of solid tumors for margin evaluation. Conventional histopathologic methods rely heavily on image-based visual diagnosis by specialized pathologists, which can be time-consuming and subjective. We report a three-dimensional (3D) histological electrophoresis system for rapid labeling and separation of the proteins within tissue sections, providing a more precise assessment of tumor-positive margin in surgically resected tissues. The 3D histological electrophoresis system uses a tumor-seeking dye labeling strategy to visualize the distribution of tumor-specific proteins within sections and a tumor finder that automatically predicts the tumor contour. We successfully demonstrated the system's capability to predict the tumor contours from five murine xenograft models and distinguish the tumor-invaded region of sentinel lymph nodes. Specifically, we used the system to accurately assess tumor-positive margins from 14 patients with cancer. Our 3D histological electrophoresis system serves as an intraoperative tissue assessment technology for more accurate and automatic pathologic diagnosis.

INTRODUCTION

Surgery remains the primary form of treatment for solid tumors, and the key is ensuring that the resected tumors have tumor-free margins. Despite histologic differences, neoplastic tissue is often indistinguishable from healthy tissue in the operating room. Intraoperative techniques such as fluorescence-guided surgery (FGS) have produced promising outcomes in the delineation of tumors and coexisting benign tissues using contrast agents (e.g., fluorophore-labeled antibodies) that target tumor cells. However, the low sensitivity/selectivity of tumor cell targeting caused by overexpression in the coexisting benign tissues limits the routine clinical use of tumor-targeted FGS (*1*–*3*). While mass spectrometry (MS)–based techniques such as rapid evaporative ionization MS (or the iKnife) and MS coupled with ultraviolet (UV) and infrared lasers have also been used intraoperatively to obtain tumor-free margins, they are operationally constrained to a specific surgical modality and rely heavily on specialized instrumentation (*4*–*8*). Thus, conventional histopathologic analysis of frozen sections remains the gold standard of intraoperative assessment for tumor-positive margins.

During intraoperative pathologic analysis, pathologists determine tumors from coexisting benign tissues to achieve the complete resection of tumors. This is based on a variety of tissue features, including disturbed tissue architecture, the presence/absence of specific cell characteristics, and an abundance of inflammatory cells. However, rapid and accurate assessment of tumor margins by pathologic analysis of frozen sections during surgery remains challenging. For some diagnostic tasks, determining the delicate boundary between tumor and normal tissues may exceed the capabilities of human visual inspection of cellular/histic morphology, resulting in the reproducibility among pathologists being less than optimal (*9*, *10*). Therefore, margin specimens are frequently processed postoperatively as permanent specimens. Unfortunately, when positive margins are found during the final pathologic evaluation, the patient is subjected to additional surgical procedures for re-excision of the involved margin, which increases health care costs and places the patient at risk for additional surgical complications, discomfort, and anxiety (*11*–*17*). Although immunohistochemistry (IHC) protocols targeting protein biomarkers and gene sequencing technologies for identifying specific genetic mutations and chromosomal translocations are aimed to aid in the pathological diagnosis of tissue sections, they are often labor and time intensive, limiting their use in intraoperative pathological diagnosis (*18*, *19*). This clinical challenge highlights the need for a complementary strategy for rapidly, accurately, and automatically assessing surgical resection margins.

Here, we describe the development and application of a three-dimensional (3D) histological electrophoresis system that can be flexibly applied to various existing surgical modalities, independent of histomorphologic diagnosis, to rapidly and accurately assess tumor-positive margins. The workflow of the 3D histological electrophoresis is described in Fig. 1A. This system incorporates several components engineered to maximize the differences between malignant and normal tissues. First, we adopted a tumor-seeking dye labeling strategy that efficiently labels tumor-positive regions within tissue sections by selectively binding to proteins with certain pocket conformation and thiol groups (*20*). Second, because unbound dye molecules inserted into tissue cytoskeleton and unlabeled proteins still produced signal interference that obscures the trace protein differences between tissues, we envisioned extending the traditional

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