Direct-Tissue SELDI-TOF Mass Spectrometry Analysis: A New Application for Clinical Proteomics, *Ali Bouamrani,[1†] Jessica Ternier,[1,2†] David Ratel,[1] Alim-Louis Benabid,[1,2] Jean-Paul Issartel,[1] Elisabeth Brambilla,[3‡] François Berger[1*‡]* ([1] INSERM U318, Université de Grenoble; Departments of [2] Neurosurgery and [3] Pathology, Centre Hospitalier Universitaire Grenoble; † A. Bouamrani, and J. Ternier are cofirst authors; ‡ F. Berger and E. Brambilla are colast authors. * address correspondence to this author at: Neuroscience moléculaire, INSERM U318, Pavillon B, Centre Hospitalier Universitaire BP 217, 38043 Grenoble cedex 9 France; fax 33-4-76-76-56-19; e-mail fberger@ujf-grenoble.fr)

Background: **New molecular profiling technologies can aid in analysis of small pathologic samples obtained by minimally invasive biopsy and may enable the discovery of key biomarkers synergistic with anatomopathologic analysis related to prognosis, therapeutic response, and innovative target validation. Thus proteomic analysis at the histologic level in healthy and pathologic settings is a major issue in the field of clinical proteomics.**

Methods: **We used surface-enhanced laser desorption ionization-time-of-flight mass spectrometry (SELDI-TOF MS) technology with surface chromatographic subproteome enrichment and preservation of the spatial distribution of proteomic patterns to detect discrete modifications of protein expression. We performed in situ proteomic profiling of mouse tissue and samples of human cancer tissue, including brain and lung cancer.**

Results: **This approach permitted the discrimination of glioblastomas from oligodendrogliomas and led to the identification of 3 potential markers.**

Conclusion: **Direct tissue proteomic analysis is an original application of SELDI-TOF MS technology that can expand the use of clinical proteomics as a complement to the anatomopathological diagnosis.**

Proteomic analysis at the histological level in healthy and pathological tissue is an important aspect of clinical proteomics and has been enhanced by the validation of proteomic imaging *(1)*. Many recent important findings are related to serum/plasma biomarkers *(2–5)*, but tissue is also an important target for closer investigation of pathological processes *(6)*. New molecular profiling technologies for direct tissue analysis may reveal new key biomarkers for prognostics, therapeutic responses, and innovative target validation and facilitate analysis of the increasingly small histologic samples obtained by minimally invasive biopsy approaches.

The validation of a direct mass spectrometric analysis was a major advance in area of proteomic tissue analysis *(7–8)*. This in situ proteomic approach has been used for anatomoproteomic classification of diseases such as lung carcinoma *(9)* and brain tumors *(10)*, thus enhancing anatomopathological diagnostic techniques. Recognizing an urgent need for a fast, high-throughput assay that can be used in the anatomopathological laboratory as a proteomic complement to histological analysis, we developed an approach for direct tissue analysis with surface-enhanced laser desorption ionization-time-of-flight mass spectrometry (SELDI-TOF MS) technology *(11)*. One of the main innovations of this MS approach is molecular enrichment on chromatographic surfaces. To validate the impact of this direct-tissue SELDI-TOF MS proteomic method, we tested 3 chromatographic surfaces by use of anionic Q10, cationic CM10, and hydrophobic H50 arrays *(12–13)*. Thin-tissue cryostat sections (8 μm) were deposited directly on the different protein chip surfaces (Ciphergen Biosystems). After a fast drying step, each array was washed with binding buffer. After the arrays were air dried, saturated sinapinic acid matrix was added to each array spot (see Fig. 1a in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue11). For gold arrays, matrix was added directly to tissue sections without washing steps.

We analyzed the arrays with the Ciphergen ProteinChip Reader PCS4000 model and analyzed the data with Ciphergen Express software (Ciphergen Biosystems). We calibrated the peak intensities to the total ion current, starting at 1500 Da after baseline subtraction. All calibration factors were 0.5 to 2.0 times the mean of all included samples. We achieved peak labeling with signal-to-noise ratio set to 10 for the first pass and 5 for the second pass with 0.3% of the mass window and added the estimated peaks. We used a 2-tailed *t*-test for statistical analysis of differences in peak intensity between sample groups. The level of significance was assigned at $P < 0.02$. With Eisen's software *(14)* we applied the agglomerative hierarchical clustering algorithm to investigate the pattern among these statistically significant differential proteins.

We obtained profiling data from frozen tissue samples in ~30 min. Furthermore, except for the manual apposition of the samples, all the other steps, including data acquisition, can be automated and processed in a 96-well format (see Fig. 1b in the online Data Supplement). This protocol is a very fast method for analyzing 96 different samples in <3 h, offering high-throughput analysis of tissue samples. We compared direct proteomic profiles obtained with cryostat sections with profiles obtained with the corresponding lysate. Interestingly, more peaks were detected with direct in situ analysis compared with classic lysate procedure essentially in the low mass range (see Fig. 1c in the online Data Supplement), a finding that is probably attributable to protein loss during the extraction steps before MS analysis.

To validate this direct tissue analysis method, we used mice obtained from IFFA-CREDO and human tissue samples obtained from our hospital. This study was approved by the institutional Human Research Ethics committee at our center. All patients signed an informed consent form.

We used this method for in situ SELDI-TOF MS proteomic analysis for differentiation of specific tissues in

different mouse organs. We deposited 4 serial cryostat sections of each organ on anionic Q10 protein chips. Small intestine, liver, kidney, heart, muscle, spleen, and lung were clearly differentiated according to their specific profiles (see Fig. 2, a and b, in the online Data Supplement). Reproducibility of the method was tested for each organ by calculating the intensity percentage CV of specific markers. The mean CV was , <15% (see Table 1 in the online Data Supplement).

To document the advantage of the chromatographic surface, we compared the pathologic tissue sample differentiation results obtained with gold, anionic, cationic, and hydrophobic surfaces. The gold surface enabled differentiation of healthy brain from glial tumor formations including glioblastomas and oligodendrogliomas (Fig. 1A) but not differentiation of glioblastomas from oligodendrogliomas (Fig. 1A). With chromatographic surfaces, differentiation of tumor types revealed 3 biomarkers

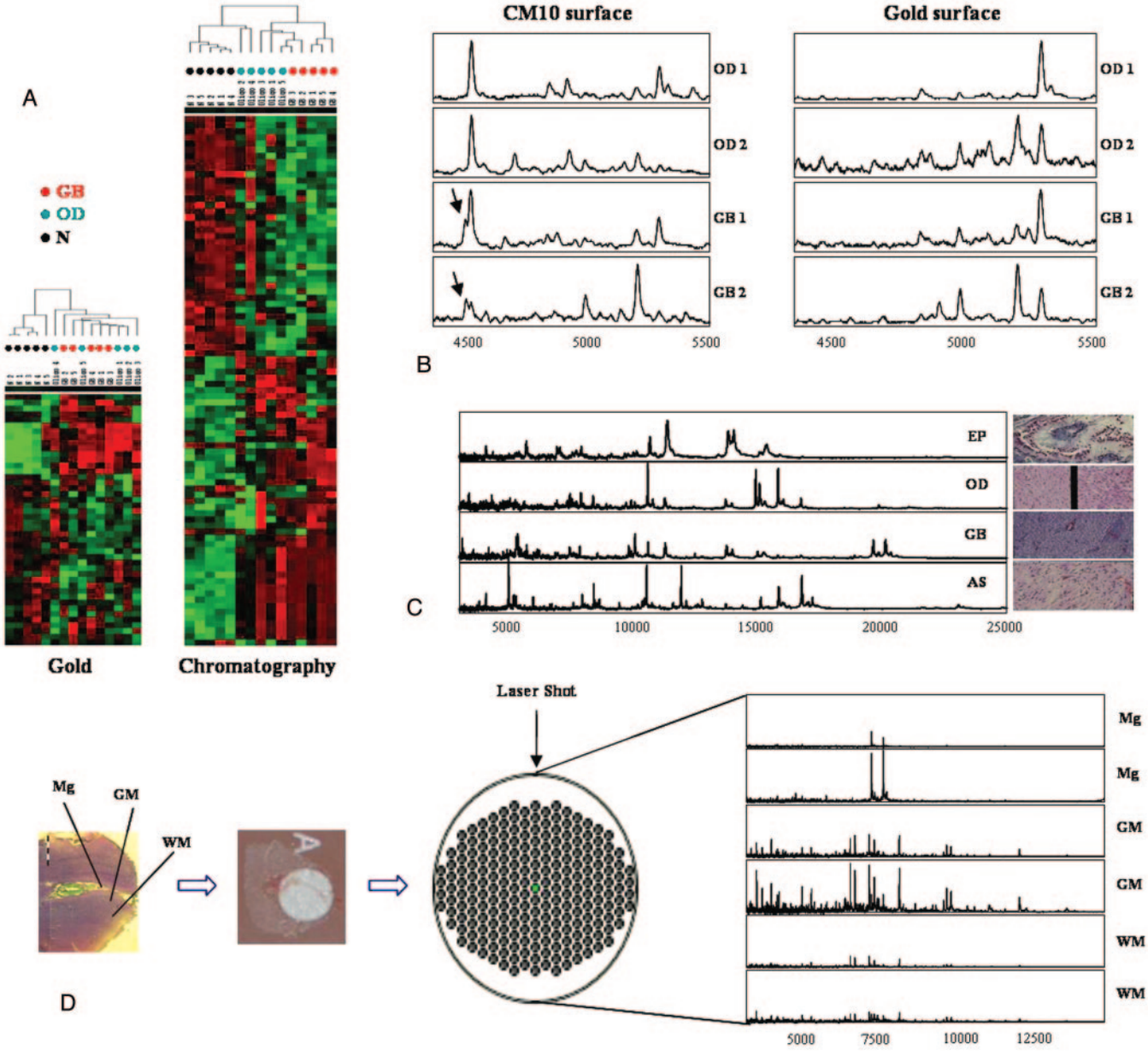

Fig. 1. Validation and clinical application of direct-tissue SELDI-TOF MS proteomics.

(*A*), comparison of protein expression in human brain and glial tumors, including glioblastoma (GB) and oligodendroglioma (OD). On gold surfaces tumors can be differentiated from healthy samples. Proteomic profiling on chromatographic surfaces differentiates healthy brain and tumoral subtypes of gliomas, as illustrated on the hierarchical clustering analysis. (*B*), spectra of tissue sections on CM10 and gold surfaces. An *arrow* indicates a marker detected at *m/z* of 4535. (*C*), direct-tissue SELDI-TOF MS proteomics in neurooncology. Anatomopathological analysis was performed in parallel with SELDI-TOF MS profiling on different neuropathological samples. Specific patterns are obtained on a CM10 array for each histologic type, including ependymoma (EP), oligodendroglioma (OD), glioblastoma (GB), and astrocytoma (AS). (*D*), 2 serial cryostat sections of human brain were used for tissue staining and protein chip apposition, respectively. Three histologic entities corresponding to meninges (Mg), gray matter (GM), and white matter (WM) are differentiated.

(Table 1); for example, a potential marker for glioblastoma at 4535 Da was detected exclusively on the CM10 surface (Fig. 1B). The use of chromatographic surfaces also facilitated differentiation of discrete histological differences.

To assess the potential use of this approach for clinical applications, we analyzed different human cancer samples, including brain and lung cancer. Immediately after surgery, tissue samples were frozen and cryostat sections were deposited on the protein chip for rapid MS analysis. The same samples were analyzed in parallel in the anathomopathological laboratory. We applied this new proteomic method to differentiate neuropathological entities (Fig. 1C). Typical profiles were obtained for the different histological entities, including glioblastomas, astrocytomas, oligodendrogliomas, and ependymomas. Similarly, the proteomic profiles obtained by direct apposition of tissue sections from different histological types of lung cancers were clearly specific (see Fig. 2c in the online Data Supplement). The proteomic fingerprints of lung squamous cell carcinoma and adenocarcinoma were easily differentiated, as shown on hierarchical clustering analysis.

To validate the in situ proteomic analysis, we checked the conservation of the spatial proteomic representation of the tissue after the washing step (Fig. 1D). Laser resolution of the Ciphergen ProteinChip Reader PCS 4000 system (Ciphergen Biosystems) is ~ 50 μm, and each spot of 2 mm is divided into 210 different laser shot positions. Two serial cryostat sections of human brain were used for histological staining and proteomic profiling, respectively. Specific profiles associated with white matter, gray matter, and meninges as visualized on the stained tissue section are shown in Fig. 1D. These results demonstrated the ability to differentiate different areas of the tissue section at the proteomic level.

These data demonstrate that the addition of a chromatographic surface in our method leads to the binding of more proteomic biomarkers, thus providing better differentiation between pathological entities. This effect has been demonstrated for protein lysate and is a main focus of SELDI-TOF MS technology *(15–17)*. Whole tumor biopsy lysate as starting material is too heterogeneous for marker detection, however, and is not feasible for clinical samples. Laser microdissection coupled with SELDI-TOF MS can overcome these limitations *(18)*, but it is a time-consuming and labor-intensive procedure. Direct-tissue SELDI-TOF MS analysis is an alternative method, compatible with clinical applications, and it provides more detected proteins than tissue lysates analysis, which may generate some protein losses during protein extraction procedures *(19–20)*. The apposition of 8-μm cryostat sections combined with specific washing conditions eliminates the tissue outside the affinity chromatographic surface, retaining only a specific subproteome. Furthermore, we have demonstrated that this tissue fingerprint maintains the spatial location of the proteins. Each chromatographic surface provides the opportunity to investigate specific subproteome enrichment. Addition of a chromatographic surface may enhance the differentiation of different but closely related pathological subtypes, as illustrated by oligodendrogliomas compared with glioblastomas (Fig. 1). In addition, changing planar chromatography to small-column chromatography makes purification of potential biomarkers easier *(21)*.

In conclusion, we have demonstrated that direct-tissue proteomic analysis is a fast, highly sensitive, and reproducible application of SELDI-TOF that opens the door to new perspectives in clinical proteomics. This method offers unique high-throughput characteristics that can be used for biomarker discovery in large cohorts of patients. In addition, this application allows proteome analysis of tissue samples as a complement to other anatomopathological diagnostic methods.

Table 1. Benefit of chromatographic surfaces for in situ proteomics.

	Detected peaks in mass range, 2.5 kDa–100 kDa		
		Significant markers	Significant markers
	All peaks	Normal/Tumor	OD/GB
Gold surface	77	15	0
Chromatographic surfaces	156	8 H50	20 246 Da H50
		5 CM10	4535 Da CM10
			9896 Da CM10
		15 Q10	0

Peak labeling was achieved with signal-to-noise ratio set at 10 for the first pass and 5 for the second pass with 0.3% of the mass window, and estimated peaks were added. Student 2-tailed *t* test was used for statistical analysis of differences in peak intensity between sample groups. The degree of significance was assigned at $P < 0.02$.

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