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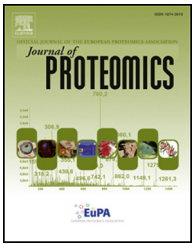

Proteomic analysis of glioblastomas: What is the best brain control sample?

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ABSTRACT

Glioblastoma (GB) is the most frequent and aggressive tumor of the central nervous system. There is currently growing interest in proteomic studies of GB, particularly with the aim of identifying new prognostic or therapeutic response markers. However, comparisons between different proteomic analyses of GB have revealed few common differentiated proteins. The types of control samples used to identify such proteins may in part explain the different results obtained.

We therefore tried to determine which control samples would be most suitable for GB proteomic studies. We used an isotope-coded protein labeling (ICPL) method followed by mass spectrometry to reveal and compare the protein patterns of two commonly used types of control sample: GB peritumoral brain zone samples (PBZ) from six patients and epilepsy surgery brain samples (EB) pooled from three patients. The data obtained were processed using AMEN software for network analysis.

We identified 197 non-redundant proteins and 35 of them were differentially expressed. Among these 35 differentially expressed proteins, six were over-expressed in PBZ and 29 in EB, showing different proteomic patterns between the two samples. Surprisingly, EB appeared to display a tumoral-like expression pattern in comparison to PBZ.

In our opinion, PBZ may be more appropriate control sample for GB proteomic analysis.

Biological significance

This manuscript describes an original study in which we used an isotope-coded protein labeling method followed by mass spectrometry to identify and compare the protein patterns in two types of sample commonly used as control for glioblastoma (GB) proteomic analysis: peritumoral brain zone and brain samples obtained during surgery for epilepsy. The choice of control samples is critical for identifying new prognostic and/or diagnostic markers in GB.

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1. Introduction

Glioblastoma (GB) is the most frequent and aggressive tumor of the central nervous system. Despite the development of new therapies, the prognosis remains poor, with a mean progression-free survival of 7 months and an average survival of 12 to 15 months [1,2]. Even following gross total resection and optimal adjuvant treatment, recurrence is extremely common, mainly from the margin of the resection cavity [3–5].

GB is a very heterogenous groups of tumors [6], involving different zones; both genomic [7,8] and proteomic [9–11] approaches have been used to study these tumors. These analyses led to the identification of different markers, allowing the characterization of different subtypes of GBs and tumoral mechanisms, and may serve as a basis for the development of new therapies focused on the molecular, genetic and proteomic particularities of GB.

In one of our previous proteomic studies, we used an isotope-coded protein label (ICPL) method to compare three areas of GB: the tumor zone (TZ), the interface zone between the tumor and the parenchyma (IZ) and the peritumoral brain zone (PBZ). We successfully identified 35 proteins over-expressed in the core of the tumor by comparison with the periphery and showed that 23 of these belong to a cohesive network of physically interacting proteins linked to several cellular functions [10].

However, few of the 35 proteins that we found to be altered in TZ are the same as those identified by previous studies (Table 1). For example, Khalil [12] used 2DE with MALDI-TOF MS and LC-MS/MS to analyze 30 GB samples with seven control samples obtained from epilepsy surgery for reference. Forty-six differentially expressed proteins were identified of which only ten proteins were in common with our study (β-actin, CKB, GDI1, ALDOA, 14-3-3γ, ATP5A1, ALB, GFAP, NEFL, ENO1). Except for β-actin, most of these proteins showed a different pattern of expression to that described in this previous study. Indeed, we found these proteins to be over-expressed in TZ versus PBZ whereas Khalil [12] observed under-expression in GB samples versus control epilepsy samples. To understand the apparent differences in the protein expression patterns between the two studies, we conducted a bibliographical search for proteomic analyses of fresh brain tumor samples (reported in Table 1). This analysis revealed substantial heterogeneity in results associated with the different proteomic analysis techniques employed and the control tissues used. Indeed, differences in both the analytical methodologies and the control tissues used may explain the only weak similarities between proteomic patterns reported by the various studies.

Obviously, normal live brain samples are not available to be used as control samples under all circumstances, and consequently the control samples commonly used in GB proteomic studies include brain tissue obtained during surgery for epilepsy (EB) or from the walls of the resection cavity during GB surgery (PBZ), with the informed consent of the patient.

However, it is unclear whether PBZ or EB brain samples, commonly used as controls, can be considered to be "normal" brain tissue, and therefore whether they are appropriate for proteomic comparisons and describing the differential proteomic expression pattern of brain tumors.

The aim of this study was to analyze and compare the protein expression patterns of these two control tissues (PBZ versus EB) using the ICPL proteomic method, and to determine which is the most suitable for use as control tissue for proteomic analyses of brain tumors.

2. Materials and methods

2.1. Clinical materials

Six patients whose diagnosis of primary GB (WHO 2007 classification) was confirmed by a central committee of neuropathologists and three patients undergoing epilepsy surgery were included in the study. This study was approved by the relevant ethics committee (CPP Ouest II, Angers, France) and all patients signed an informed consent form for participation in this study.

The tumoral zone and PBZ from GB were defined on preoperative T1 gadolinium-enhanced 3D MRI. Stereotaxic biopsies were performed in the operating theater, by computer-assisted neurosurgery (BrainLab®, La Défense, France). EB was obtained from cortical resection during surgery for epilepsy after identification of the epileptic cradle using per-operative electroencephalograms and electrostimulation.

Histological analysis and protein extraction were performed for each biopsy specimen. For histological analysis, formalin-fixed, paraffin-embedded sections of the biopsy specimens were stained with hematoxylin–phloxin–saffron.

2.2. Tissue protein extraction

Protein extracts of tissue samples were prepared as previously described [10]. Briefly, cell pellets from PBZ and EB samples were resuspended in cold lysis buffer (6 M guanidine HCl, pH 8.5, cells/buffer: 1/2.5(v/v)) and sonicated on dry ice with an ultrasonic processor (Bioblock Scientific, Illkirch, France) six times for 10 s with 30 s pauses between using a microtip setting power level at 40% pulse duration. The homogenates were centrifuged (15,000 *g*, 30 min, 4 °C) and the resulting supernatants were then ultracentrifuged (105,000 *g*, 1 h, 4 °C). Protein concentrations in the resulting supernatants were measured with a BioRad Protein Assay Kit (BioRad, Marnes-la-Coquette, France) according to the manufacturer's instructions. The samples from the three patients undergoing epilepsy surgery were pooled.

2.3. ICPL labeling and protein digestion

The experimental design and the ICPL method are described in Table 2. ICPL labeling was performed on 50 μg of PBZ or pooled EB samples as previously described [10], according to the experimental design described in Table 2. Labeled proteins (50 μg) were separated by SDS-PAGE in 12% precast gels (GeBeGel, Gene Bio Application), which was then stained with Coomasie blue R-350 using the EZBlue gel staining reagent (Sigma-Aldrich, Saint Quentin Fallavier, France).

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