

National Biobank Program workpackage 5.4 Brain Proteomics Biobanking Final Report, January 2006

Christer Ericsson and Monica Nistér

Karolinska Institutet

Department of Oncology Pathology
Cancer Center Karolinska, R8:05
Karolinska University Hospital, Solna
171 76 Stockholm
Sweden
christer.ericsson@ki.se, monica.nister@ki.se

Background

Recent developments in protein analysis, particularly highly parallel detection and identification, through progress in the fields of protein mass spectrometry instrumentation, together with previous and ongoing developments in protein chromatography and protein immuno-detection have increased the utility of clinical specimens for translational research. The prospects are to understand pathogenic mechanisms at the protein level. Based on this information rational suggestions for new drug-targets can be made. Analysis of blood samples holds the prospect to detect new characteristic signatures of protein leakage from tumors into the blood stream, and of reactive proteins, opening the prospect of an increased applicability of this fairly non-invasive protein-based molecular diagnosis (Ludwig and Weinstein, 2005).

To realize these prospects the tissue and blood samples need to be handled in ways that preserve their essential character, and are compatible with downstream analytical techniques of the appropriate sensitivity and throughput (Ericsson, 2006c). The current handling of pathological specimens will have to be modified to incorporate these new demands.

This workpackage aimed at finding relevant criteria for optimal tissue and blood sample handling, applying those criteria to prospective brain tumor tissue collection and to compare the sensitivity and throughput of current protein analysis technology.

The mission for this workpackage, initiated in late 2002, was the following:

1. Development of procedures for handling and quality assessment of samples for maximal reproducibility and utility for proteomics
2. Building of a fresh tissue brain biobank
3. Test of the minimal amount of protein required for different proteomic techniques
4. Definition of Standard Operating Procedures for handling of plasma and serum for proteomics
5. Implementation of a Quality Manual for the solid tissue sample collection (initiated by Bertil Hamberger in 1986) within the biobank at the Karolinska University Hospital

The results pertaining to each item are reported and discussed below.

1. Development of procedures for handling and quality assessment of samples for maximal reproducibility and utility for proteomics

The effects of elapsed time and of temperature on brain tissue protein quality were studied. The study was designed to evaluate if the demands of histopathological diagnosis and those of proteomic studies (other than by immunohistochemistry) were compatible. The parameters measured were: microscopical morphology, immunoreactivity, protein integrity, and integrity of phosphate groups on proteins. The study (Ericsson, 2006a) showed considerable benefit from cooling tissue, under sterile conditions, in wet ice, and that the requirements for diagnosis and for proteomics were indeed compatible.

A Standard Operating Procedure for handling of brain tumor tissue was developed, as well as a validation assay (please see www.biobanks.se for details),

We state that similar studies of other tissues are warranted to determine optimal handling conditions and to suggest validation assays, such as Western blots of specific proteins determined to be especially sensitive to degradation.

A study of optimal total tissue protein extraction and solubilization with SDS was performed, resulting in an optimized procedure regarding disintegration method, buffer, time, temperature and volume (Ericsson, 2006b).

2. Building of a fresh tissue brain biobank

Fresh brain tumor collection, and collection of blood samples, has been initiated in a collaboration between the department of Pathology and the department of Clinical Neuroscience. The samples are cryopreserved and stored within the infrastructure of the Solid Tissue Collection (executive, professor Bertil Hamberger), within Biobank Karolinska of the Karolinska University Hospital, Huddinge and Solna, Sweden (executive, senior consultant Tommy Söderström). Fresh tumor material as well as blood samples are now collected routinely from consenting brain tumor patients, at Solna. Currently (2006-01-12) samples from 75 individuals have been collected. It is the goal of the members of this workpackage to contribute to the continuing optimization of the Solid Tissue Collection and to Biobank Karolinska.

3. Test of the minimal amount of protein required for different proteomic techniques

No proteomic techniques currently exist that can analyze the entire concentration range of proteins in solid tissue (estimated to be about 10^6) or of blood plasma (estimated to be about 10^{10} to 10^{11}). A survey of the literature (Ericsson, 2006c) shows that the two most commonly used massively parallel proteomic techniques, 2-D PAGE/MALDI MS and LC/ESI MS, have a sensitivity of at best 10^4 . The targeted technique of Western blot has a concentration range of about 10^5 , but requires that specific antibodies exist. Targeted ELISA assays currently have the highest sensitivity, and seem to be adequately sensitive and specific for the currently developed blood analytes in clinical practice. ELISA does require preexisting antibodies of sufficient quality. Conceivably fractionation, especially multidimensional fractionation, would increase the sensitivity of each technique. Currently no example of comprehensive protein analysis exists for any eukaryotic tissue or cell, and so the ultimate potential of this approach remains to be determined. It has been suggested that immunoaffinity, or other affinity-, purification may be required to analyze

entire proteomes. The proteome-wide antibody project, the Human Proteome Resource (Uhlen and Ponten, 2005), is important progress towards that goal. Recent improvements in antibody arrays (Wingren and Borrebaeck, 2004), improvements in assay sensitivity, without loss of specificity, such as proximity ligation (Gustafsdottir et al., 2005) likewise represent important progress towards that goal.

4. Definition of Standard Operating Procedures for handling of plasma and serum for proteomics

The Plasma Proteome Project (PPP) within the HUman Proteome Organization (HUPO) has recently recommended use of plasma with EDTA or citrate as inhibitors of coagulation, rather than serum, due to the greater ease of reproducibility, less protease activation and compatibility with downstream analysis (Omenn et al., 2005). Currently two different rationally based protocols exist for isolating the plasma, one cold to prevent proteolysis (please see appendix) and one room temperature (please see appendix), to prevent platelet activation (and subsequent protein release) (Tammen et al., 2005). It has presently not been determined if the room temperature protocol provides adequate protein quality. We therefore currently recommend cold preparation of plasma, pending evaluation of the room temperature, platelet activation reduced protocol.

There exists a need to develop one or more validation markers for successfully handled plasma.

5. Implementation of a Quality Manual for the solid tissue collection (initiated by Bertil Hamberger in 1986) within the biobank at the Karolinska University Hospital

A quality manual is currently being written, that is compatible with the current legislation and with the guidelines provided by the Association of County Councils (“Landstingsförbundet”). The quality manual describes the purposes of the collection, the type of samples (human solid tissue and blood derivatives), ethical considerations, patient informed consent, sample transport, handling, storage, sample quality validation, data management, access privileges, policies, policy update mechanisms, responsibilities, training and executive powers, equipment, buildings, maintenance and oversight.

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