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Closing the gap between brain banks and proteomics to advance the study of neurodegenerative diseases

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Abstract

Neurodegenerative diseases (NDs), such as Alzheimer's disease and Parkinson's disease, are among the most debilitating neurological disorders, and as life expectancy rises quickly around the world, the scientific and clinical challenges of dealing with them will also increase dramatically, putting increased pressure on the biomedical community to come up with innovative solutions for the understanding, diagnosis and treatment of these conditions. Despite several decades of intensive research, there is still little that can be done to prevent, cure or even slow down the progression of NDs in most patients. There is an urgent need to develop new lines of basic and applied research, that can be quickly translated into clinical application. One way to do this is to apply the tools of proteomics to well-characterized samples of human brain tissue, but a closer partnership must still be forged between proteomic scientists, brain banks and clinicians to explore the maximum potential of this approach. Here we analyze the challenges and potential benefits of using human brain tissue for proteomics research toward NDs.

Keywords

Brain bank; Human brain; Neurodegenerative diseases; Proteomics; Neuroproteomics

Neurodegenerative diseases (NDs) are extremely debilitating neurological disorders that can be strongly associated with aging, such as in the case Alzheimer's disease (AD) and Parkinson's disease (PD). As life expectancy rises quickly around the world [1], the scientific and clinical challenges of dealing with neurodegenerative diseases will also increase dramatically, along with the economical and psychological burden they place on society. It is estimated that 4.7 million people were affected by AD in 2010, in the United States alone, for example, and this prevalence is expected to triple by 2050 [2].

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Although clinical symptoms associated with NDs, such as cognitive impairment and movement disorders, have been fairly well characterized [3, 4], the understanding of risk factors, mechanisms and etiology of these diseases remains incomplete. It has been well established, for example, that all NDs feature two main neuropathological hallmarks of opposite nature: neuronal cell loss (negative lesions) and deposition of abnormal proteins (positive lesions). The correlation between these two types of lesion, however, is yet to be established. For instance, it is not known if protein misfolding is a phenomenon that precedes or follows neuronal death, if it's a collateral event, or even if it occurs independently of cell death [5]. Furthermore, the same misfolded proteins are found in individuals without any neurological symptoms [6], making the understanding of the neurological basis of NDs even more challenging. In addition, all NDs show selective vulnerability of specific cell populations, and a non-random anatomical progression [7–11]. Nonetheless, what causes this selective neuronal vulnerability is still unknown.

As a result of these knowledge gaps, treatment for NDs remains elusive and our current capacity to curb the growing "dementia epidemic" is limited, despite decades of intensive research. Drug development has been focused primarily on a small number of reductionist mechanistic hypotheses, such as the amyloid cascade in AD, while other hypotheses, such as those related to tau pathology, have been neglected. Thus, it is not surprising that therapeutic options that showed efficacy in animal models that mimic isolated aspects of the disease have failed in human clinical trials [12]. To make this situation even worse, the rate of success in advancing clinical trials from one phase to the next is low, due to regulatory and financial constraints, and the number of compounds that have been tested is very small [13]. Efforts in testing alternative hypotheses are urgently needed.

The potential of neuroproteomics in NDs

Protein misfolding is a key element in NDs, and therefore proteomics has the potential to provide important insights into disease mechanisms, biomarker identification and drug development. For this potential to be fully explored, however, studies must be carefully designed to include appropriate methods. With advances in instrumentation, several proteomic methods may be employed, including gel-based proteomics combined to mass spectrometry or gel-free mass spectrometry-based proteomics, depending on the objectives of the research project (see [14–22] for details on proteomics methods). For instance, it is evident that deposits of misfolded proteins spread via defined transneuronal topographical pathways [23–25]. In this scenario, proteomic research strategies taking advantage of topographical information using for instance MALDI imaging, that allows the analysis of proteins in-situ or proteomic studies that encompass the analysis of single cell types or organelles isolated via laser microdissection or subfractioning [26], rather than homogenates of larger structures, are likely to provide more reliable results, making data interpretation more straightforward and less prone to artifacts induced by area selection [27].

Selection of ideal materials is another crucial part of the equation. Animal models represent an excellent platform for mechanistic and drug development studies. However, they only mimic certain aspects of the human disease and fail to reproduce the full spectrum of NDs' features [28, 29].. For instance, many genetically modified animal models for AD have a

mutation that causes beta-amyloid accumulation, but most NDs are sporadic, and there is no guarantee that the genetic modifications introduced in the animals will replicate what happens in humans [30]. Therefore, it is necessary to further explore the use of well-characterized human samples, that seem to be the ideal material for overcoming limitations related to animal models in basic and clinical studies [31].

Neuroproteomics in human specimens

Body fluids are excellent candidates for biomarker discovery. However, studies in body fluids of patients without a validated diagnosis can lead to false positive and false negative results. In NDs, a definitive diagnosis requires postmortem examination. Not only it allows researchers to look directly at the tissue where the pathology builds-up, but it allows for the identification of overlapping diseases that might influence the proteomic profile of the patient, as well as a more precise staging of the disease. Differently from what happens in animal and cell models, NDs tend to occur in humans in mixed form (with individuals manifesting two or more NDs simultaneously) or in association with cerebrovascular diseases [32], making specific diagnosis a challenge. Even in highly specialized centers, the accuracy of clinical diagnosis of NDs varies from 44.3% to 70.8% [33–36]. Some centers conduct longitudinal studies, in which the patients have body fluids collected annually and receive a postmortem exam, but the number of patients in these centers is limited, and they often lack control subjects for comparison [37].

We propose that the best way to overcome these limitations, for research purposes, is to make use of postmortem human brain tissue. There are several technical and methodological aspects, however, that must be taken into account in order to draw accurate conclusions from this precious material.

Challenges in using postmortem human brain tissue for proteomics

Despite being an excellent platform for research, well-characterized human brain tissue samples are a difficult-to-obtain research commodity [38]. Brain biopsies are usually restricted to the minimal amount of tissue needed for diagnosis, and therefore excess tissue is rarely available. Also, in the case of NDs, biopsies are used more often for ruling out certain dementia diseases, such as Creutzfeldt-Jakob, rather than to confirm the diagnosis of NDs.

Most of the brain tissue available for research, therefore, is obtained via autopsies and deposited in brain banks, which specialize in the structured collection and processing of well-characterized tissue and are regulated by standardized ethical and legal mandates [38]. A striking decrease in the numbers of autopsies worldwide [39] makes this material ever more precious, requiring brain banks and researchers to work in synchrony to make the best possible use of every available sample.

When applied to molecular studies, such as in the case of proteomics, it must be taken into account that, differently from animal models, which live in a completely controlled environment, human samples carry unpredictable confounders. Demographic, clinical, environmental, behavioral and agonal factors (such as education, medication, substance

abuse and health status prior to death) have direct impacts on proteomic results [40]. For example, even a few years of formal education is enough to build a cognitive reserve that protects individuals from cognitive impairment [41]. Although only a small fraction of individuals with NDs have a known genetic basis, genetic factors can influence the risk of manifesting the disease [42]. We have shown recently that African ancestry protects against the formation of amyloid plaques [43]. Therefore, matching cases and controls by as many variables as possible is necessary to minimize false results.

Longitudinal clinical data sets are the most informative about patient clinical status and comorbidities. However, very few brain banks receive cases from longitudinal cohort studies and most only collect limited clinical data from their donors [37]. Moreover, banks that have brains of individuals who participated in longitudinal studies tend to focus more in rare cases and convenience samples, making it difficult to extrapolate results to the general population [44].

In addition to pre-mortem and agonal variables, proteomics studies are very sensitive to tissue state and processing. Therefore, it is best to utilize specimens processed by the same standard procedures. Despite efforts to standardize good practice and routine protocols in biobanks [45], there are still many procedural differences in the way brain banks operate. Samples should be preferentially obtained from a single source to avoid biases created by tissue processing (dissection and homogenization). Tissue processing is an important issue in proteomics, because it can be responsible for a large portion of technical variability [46]. The postmortem interval (PMI) – time between death and storage of the brain – can also vary greatly between banks, and there is data to suggest that PMI, storage temperature and freeze-thaw cycles can influence the proteomic profile of the tissue [47–50]. So, even if samples are collected from the exact same brain region of two matched donors, results may be unreliable if the samples were frozen using different protocols, or if the PMI varied considerably between the two cases.

The brain is the most complex organ of the body, harboring hundreds of morphologically and functionally distinct regions [51]. The hippocampus, for example, contains several different regions, each one participating in distinctive circuits and bearing a wide range of cell types [52]. In addition to these structural differences, the human brain also shows functional asymmetry [53, 54]. It means that even a tiny, millimeter-size brain region can contain areas with very different proteomic profiles. Such differences exist not only among regions, but also among individuals. Therefore, the choice of the best brain area is critical in the planning of the study and can vary greatly depending on the objectives of the research.

In order to overcome all these challenges, proteomics researchers, brain bank personnel, pathologists and clinicians need to work together to identify the best samples and the best areas for the study of NDs, going beyond the traditional approach of just matching disease and control subjects by age and gender. A good study design and a reliable source of material are mandatory to guarantee the precision of the results.

Opportunities for proteomic investigations using human tissue

Many significant efforts have already been made by proteomics to advance the understanding of NDs (see reviews [55–59] for details), but there are still many untapped opportunities and novel strategies that can be pursued in partnership with brain banks to push this research forward. Here are some that we consider to be the most valuable for the near future:

I. Characterization of normal brain proteome across age groups: Much data has been generated by comparative studies between disease and control cases, but results are not conclusive for a series of reasons. One of them is that the proteomic characterization of a “normal brain” and data about the initial stages of disease are still lacking. As long as there is no reference base (i.e. a universally accepted “normal brain proteome”) for comparative studies, and no proper characterization of the variability that exists within NDs, it will be difficult to interpret results extracted from diseased brains, especially in studies with a small number of cases [60].

II. Study of asymptomatic cases: The onset of neurodegenerative diseases normally precedes clinical symptoms by several years [61]. Some groups, including ours, have shown that it is necessary to have a significant loss of neurons to develop clinical dementia [6]. The identification of biomarkers in these individuals holds great potential for developing preventive strategies and early therapies and diagnostic tools, capable of slowing down (or even canceling) the progression of NDs before they cause great harm to patients and therapies are no longer effective.

III. Explore areas of the brain that are neglected at large: Some areas of the brain that are less studied than others, such as the brainstem, hold great promise for new discoveries. Recent studies conducted by our group and others [62, 63] have uncovered unexpected links between the brainstem nuclei and Alzheimer’s disease. Further analysis of this brain structure could potentially unveil important information about the initial stages and the progression of AD, as well as other common neurodegenerative diseases. In the case of PD, it is already known that neuromelanin-harboring neurons in the substantia nigra are vulnerable [64], but the brainstem may play an even bigger role in the progression of the disease.

Proteomics research is expanding quickly, and new technologies are being developed at a fast pace, creating a whole new set of approaches for the study of NDs. It is important to note, however, that just generating new data is not enough, as this new proteomic data must still be validated through direct analysis of brain tissue. This can be done through various techniques, such as arrays, immunohistochemistry, and also MS-based measurements and methods for absolute quantitation of peptides, such as multiple reaction monitoring (MRM) [14, 65].

Final remarks

Multidisciplinary teams, including proteomic researchers, human brain banks and clinicians, must work closely together to build the capacity necessary to meet the demands that are

brought forward by proteomics and to identify priorities in this new field of research. This collaboration must be permanent and not restricted to the immediate needs of specific projects. In doing so, proteomic researchers will become better versed on how biobanks operate (i.e. how samples are collected, processed and stored, and what exactly can be done with them), while biobanks will be able to better understand the needs of proteomic research and adapt their protocols accordingly, at the same time that clinicians will be using their expertise to develop translational research goals. It is important to extract as much information as possible from each sample, using gold-standard tools and working collaboratively. The Brain Proteome Project of the Human Proteome Organization [66], an open international group of researchers from several disciplines, is a good example of how this multidisciplinary approach can be beneficial to advance the understanding of NDs using the tools of proteomics.

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List of abbreviations

ND	Neurodegenerative diseases
AD	Alzheimer’s disease
PD	Parkinson’s disease
PMI	postmortem interval

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