Neuroproteomics and Parkinson's disease: don't forget human samples

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Neuroproteomics and Parkinson’s disease: don’t forget human samples

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Parkinson’s disease (PD) is an enigmatic condition, whose clinical manifestations and pathological lesions extend far beyond the classical tremor, rigidity and akinesia developed as a result of the degeneration of the dopamine-producing neurons in the substantia nigra pars compacta (SNpc) [1]. Years, if not decades, before motor abnormalities start, patients may complain of a variety of nonmotor symptoms including loss of sense of smell, sleep disturbances, depression, pain and weight loss among many others [2], coalescing variably in individual patients to produce a highly heterogeneous phenotype. In addition, as the disease progresses, other features may develop, such as gait disorders, autonomic dysfunction and cognitive decline, further adding to the complexity of the clinical picture [2]. As a result, the clinical diagnosis of PD, mainly based on physical examination and some neuroimaging tools, has remained uncertain for a long time, as the distinction from other forms of degenerative parkinsonism is difficult, if not impossible, at an early stage. Moreover, patients are necessarily diagnosed at an advanced pathological stage, as the first detectable motor symptoms manifest when approximately 70% of nigral neurons are already lost (i.e., long after degenerative processes have been initiated) [3]. Underlying these motor and nonmotor manifestations, restricted portions of the peripheral autonomous nervous system and CNS selectively undergo neurodegeneration. Vulnerable neuronal populations exhibit Lewy pathology, the histological hallmark of PD resulting from the abnormal aggregation of α-synuclein (α-SYN) and other cellular proteins. The pathology gains access to the CNS via the olfactory bulbs and the enteric nervous system, to sequentially and selectively target structures of the brainstem, the limbic system and eventually the cerebral cortex, in a cell-to-cell mode of propagation that has been viewed by some as prion-like [4]. Furthermore, the dopaminergic neurons in the SNpc exhibit massive degeneration, whereas adjacent cell populations are relatively spared. Their specific vulnerability may rely on their intrinsic properties as these cells are high-energy consumers, contain neuromelanin and elevated amounts of reactive species arising from oxygen, dopamine, iron or Ca\(^{2+}\) metabolism [5]. Finally, PD pathology is not limited to dopaminergic neurons and affects other nondopaminergic brain structures well before and long after the nigrostriatal system [6,7]. Thus, involvement of the SNpc is just one step in the middle of a much larger and complex multisystem disorder.

The etiology and pathogenesis of PD remain equally mysterious. The general scenario suggests that PD is likely to result from a subtle interplay involving both a predisposing genetic background and some form of environmental toxicity. Indeed, since the late 1990s, a growing list of mutations in various genes including the α-SYN gene, SNCA [8,9], have been associated with PD. These mutations mainly account for...
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early-onset familial forms of PD [10] and those also found in sporadic cases are very rare. For example, the most common point mutation LRRK2 Gly2019Ser is carried by only 1% of typical, sporadic PD cases and 4% of the hereditary counterpart [11], with incomplete penetrance [12] and low concordance in relatives [13]. The processes by which these mutations lead to Lewy body (LB) formation and neurodegeneration is unknown [14]. Moreover, a combination of putative environmental risk factors, such as exposure to various toxins including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, rotenone or paraquat, and protective factors, such as caffeine consumption, cigarette smoking or anti-inflammatory drug intake, are thought to play a role in PD development [15]. The pathogenesis of sporadic PD is even more elusive despite the numerous mechanisms that have been suggested to account for neurodegeneration of PD-related structures, including mitochondrial dysfunction, oxidative stress, abnormal protein degradation through the ubiquitin–proteasome system and/or chaperone-mediated autophagy, glutamate excitotoxicity, neuroinflammation, increased iron deposition and apoptosis. These mechanisms are probably not mutually exclusive and may act at distinct stages of PD pathology, with each triggering the next. However, in this view, it is unclear which pathomechanism is a core element initiating the whole cascade of events and which one is a consequence of it.

Proteomics as a key tool to study PD

At present, PD treatments provide symptomatic benefit at the expense of invalidating side effects (i.e., dyskinesia) and decreasing long-term efficacy. PD research is focused on the development of neuroprotective or neurorestorative strategies that can slow or halt disease progression. The establishment of such disease-modifying strategies mainly depends on our ability to gain a deep understanding of the specific mechanisms underlying neurodegeneration in order to identify therapeutic targets and diagnose PD accurately and at an early stage with new specific and sensitive tools, such as biomarkers.

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In view of the currently limited knowledge surrounding PD pathogenesis and despite the enormous amount of data already available on this topic, we believe it is time to revisit the pivotal issues previously described using different, unbiased, non-hypothesis-driven approaches. An example of such new lines of thinking involves the recent achievement of several genome-wide association studies performed in very large populations of PD patients and controls, which have been able to link a number of loci to increased risk for PD, including MAPT, SNCA, HLA-DRB5, BST1, GAK, LRRK2, ACMOD, STK39, MCCCIILAMP3, SYT11 and CCDC62/HIP1R [16]. While the significance of most of these genes in PD pathogenesis remains to be established, findings from genome-wide association studies somehow validate a discovery, hypothesis-free approach, a strategy that is highly typical of most proteomic studies. In the context of PD, we propose proteomics as an essential exploratory tool that can be used to identify new proteins of interest and thus decipher new mechanisms underlying degeneration, to independently validate existing candidate gene products or hypotheses, and to discover disease biomarkers, another unmet need in PD clinical research. In fact, during the past few years, proteomics has already allowed us to unravel a number of pathways associated with PD pathogenesis as well as potential PD biomarkers (for a review see [17]) and we believe that this trend will further increase in the near future.

Nonhuman models or human samples?

A combined PubMed query for ‘Parkinson disease’ and ‘proteome’ or ‘proteomics’ terms (up to February 2011) identified 119 papers, including 44 dedicated reviews. Of the 75 original articles, 25% were based on the analysis of human samples (cerebrospinal fluid [CSF], blood or brain tissue) whereas the majority (75%) were related to cellular and mostly animal models of PD. Various nonhuman cell lines, invertebrates and rodents were most frequently used, whereas one single proteomic study examined a nonhuman primate model of PD. Overall, modeling human neurodegenerative diseases has proved to be instructive in understanding their pathogenesis and has offered platforms to test novel therapeutic interventions. However, disease models have also demonstrated serious limitations and, in the field of neurodegeneration, it is possible that this traditional approach has reached its limits. PD has been modeled through the administration of neurotoxins in vitro or in vivo or, more recently, genetic manipulations. Such models are far from ideal as they do not, or only partly, exhibit the progressive, age-dependent pattern of neurodegeneration involving selected nigral and extra-nigral brain structures, the full spectrum of motor and nonmotor symptoms of the condition, or the characteristic LB pathology (for a review see [18,19]). Studies on animal models should therefore be interpreted with caution and potential pathological modifiers and biomarkers must be ultimately validated in human samples. In fact, it is our view that human samples from PD patients – either biological fluids from living patients (CSF, blood or urine) or neuropathologically assessed brain tissues – remain inescapable sources of information and may be an avenue of choice to detect PD-specific abnormalities through proteomic strategies.

Samples from living patients are likely to offer immediately testable diagnosis, prognosis and therapeutic biomarkers. Although such samples are rarely neuropathologically confirmed, idiopathic PD can be accurately diagnosed during patient’s lifetime with a positive predictive value of over 95% when assessed by movement disorder specialists [20]. The levels of various pathogenic proteins can be easily and repeatedly measured in the CSF, which might be a particularly suitable source of biomarkers. Indeed, CSF may contain pathogenically relevant molecules released by brain structures and allow the study of protein profile changes at any time during the entire disease course. As an example, α-SYN, a major component of LB, has been repeatedly found to be decreased in the CSF of PD patients compared with age-matched controls [21–23] and Alzheimer’s disease (AD) patients [22,23]. Hong and colleagues recently demonstrated that both total DJ-1 and α-SYN levels in
human CSF may represent useful PD diagnostic markers, when blood contamination and age factors are controlled. It is also possible that a combination of several biomarkers may prove superior to individual ones. For example, a large-scale study reported the performance of a panel of eight CSF proteins (tau, amyloid β1-42, β2-microglobulin, vitamin D-binding protein, apolipoprotein A-II, apolipoprotein E, brain-derived neutrophic factor and IL-8) to distinguish PD from AD patients and controls. At present, however, most potential biomarkers, have not achieved a level of robustness high enough for routine clinical use and preliminary results require further validation. This leaves the door open for the design of innovative, sensitive and more targeted (i.e., post-translational modification detection) proteomic studies to identify additional biomarkers of interest in CSF or other biological fluids.

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Direct analyses of autopsy samples from PD-affected structures may provide a comprehensive, unbiased and unique view of the degenerative mechanisms taking place in PD. Thus far, proteomic studies have not only confirmed existing theories but also identified novel potential pathogenic molecules (for a review see), which, in turn, could also lead to the identification of PD biomarkers. A firm advantage of post-mortem samples is that the diagnosis is definitely confirmed by neuropathological examination. Moreover, regions of interest such as the SNpc can be precisely dissected out, cell populations as well as subcellular fractions isolated, and LBs purified for analysis by sensitive proteomic tools to unravel their proteomic specificities. Selecting tissue samples might therefore be a way to address the difficult question of pathology selectivity and cellular vulnerability. Some limitations that have been implicated against the use of such tissues can now be overcome. Indeed, although the type, number, quantity and quality of samples might be limited, access to them may be made easier through scientific collaborations with PD-experienced medical centers running brain donation programs and brain banks. Furthermore, recent studies have shown that only a minority of proteins undergo massive degradation after a prolonged post-mortem delay of 72 h at room temperature. The problem of artifactual post-mortem changes can thus be circumvented within the range of a relatively long post-mortem delay. Finally, the constantly evolving variety of proteomic techniques available, either gel-based (i.e., 2DE) or gel-free analyses involving shotgun approaches combined with mass spectrometry techniques, may allow us to dig increasingly deeper into the proteome of PD relevant structures.

Irrespective of the samples tested, patient selection and sample quality are of enormous importance for proteomic approaches. Patients from the ‘Parkinson’ and ‘control’ groups should be carefully selected and PD diagnosis of samples from living patients should be formally assessed by an expert using standardized criteria, whereas neuropathological confirmation is mandatory for autopsy tissues. Patients’ medications and demographic data, such as age, gender, ethnicity, genetic background, environmental and occupational specificities, should also be taken into account and groups of samples under study should be stratified accordingly. For example, as PD is a heterogeneous disease, it might be relevant to select patients with a similar phenotype to identify markers that may vary according to clinical subtypes. Furthermore, from a clinical point of view, it might be more relevant to differentiate PD from other mimicking synucleinopathies than from AD or other phenotypically distinct neurodegenerative conditions. In addition to patient selection, sample quality is equally pivotal and many pre-analytical parameters have to be considered and monitored, including blood contamination, storage conditions and post-mortem delay, among others.

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In conclusion, we believe that neuroproteomics has an underestimated potential to offer new, unbiased and highly sensitive strategies to study the biological and molecular mechanisms underlying PD pathology and to identify diagnostic, prognostic or therapeutic biomarkers. As PD is an exceedingly complex condition that can only be partly modeled in the laboratory, we propose to tenaciously explore human samples from PD patients that, in the end, may represent the ideal material for basic and clinical research.

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