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Gene Expression Profiling in Parkinson's Disease

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders. Developing therapies for neurodegenerative diseases such as PD should be preceded by research on its exact pathophysiological mechanisms. As a research method to elucidate disease mechanism, gene expression profiling has recently been paid attention. The technique consists of comparing the gene expression levels between healthy populations and PD patients as the basis for inferring the pathological mechanism of PD. Although many studies have been undertaken using this technique, controlling related variables, such as gender, disease stage, and anatomical area, remains a challenge. Additionally, it is necessary to establish strategies for acquiring access to obtain more diversified samples in peripheral tissues such as blood and fibroblast. This review will investigate the hitherto achieved results and current state of research, as well as its limitations, and serve as a signpost for future research directions.
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Key Words Parkinson's disease, Gene expression profiling, Peripheral tissue.

Introduction

Parkinson's disease (PD), the second most common neurodegenerative disorder after Alzheimer's disease, has an average age of onset of 60 years old and affects approximately 1 million people in the United States and more than 4 million people worldwide. The prevalence of PD in industrialized countries is generally estimated at about 1-2% of people over 60 years of age. This prevalence increases to 3-5% in people above 85 years of age.[1,2]

PD is characterized by resting tremor, bradykinesia, muscular rigidity, and postural instability. Pathologically, PD patients show a loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and frequently present with Lewy bodies, eosinophilic intracellular inclusions composed of amyloid-like fibers and α-synuclein.[2,3]

Development of symptomatic treatments for motor and nonmotor symptoms, as well as any potential disease-modifying and neuroprotective therapies, is dependent on an accurate and comprehensive understanding of the pathogenesis and pathophysiology of PD. The majority of studies addressing such issues have been hypothesis-driven 'candidate-mechanism' approaches. Scientifically, this approach is the only way to test and delineate specific mechanisms of disease and therapeutic intervention. The search for unique and unexpected factors impacting the pathophysiology of PD and many other diseases has led to the development of systems-approaches that attempt to assess function on a broader level in the hopes of gaining greater knowledge concerning how individual components fit together as a whole.[4]

One of such methods is gene expression profiling, which has been touted as a way of generating new hypo-theses concerning the pathogenesis of PD, enhancing diagnostic accuracy, improving predictions about progression and prognosis, and predicting disease in asymptomatic individuals.[4-6] For example, one recent experiment used SN dopaminergic neurons expression profiling to search for, and find, novel genetic loci for vulnerability to PD, although two subsequent studies failed to replicate the finding.[4,7]

So far, the majority of studies have been performed on the midbrain and striatum in postmortem samples from PD patients and animal models of parkinsonism.[4,8,9] More recently, studies have targeted enriched populations of dopaminergic neurons, as opposed to tissue pieces, and have begun to explore extra-nigral neurons and peripheral tissues.[4,10,11] This review will investigate the current state of research on gene expression profiling in parkinsonism as well as its limitations, and serve as a signpost for future research.

Current State of Research on Gene Expression Profiling in PD

A critical question is what is actually being evaluated in microdissected pieces of SN or other regions from PD patients. Even under control circumstances, SN samples are extremely heterogeneous, with multiple cell types such as dopaminergic neurons, non-dopaminergic neurons, astrocytes, microglia, oligodendrocytes, and peripheral blood cells represented in different proportions depending on the dissection technique used. In PD, there are dramatic pathological changes that make the situation even more confusing. A loss of SN dopaminergic neurons occurs to varying degrees. Astrocytes and microglia are not only activated, but also recruited to the site. A breakdown of the blood-brain barrier may also occur. These changes make the interpretation of gene expression results very problematic.[4]

Several studies have attempted to minimize the impact of neuron loss by examining more homogeneous populations of SN dopaminergic neurons collected using laser-capture microdissection.[4,12,13] This technique allows for analysis of a highly-enriched sample of SN dopaminergic neurons and has proven successful to a certain degree in both human and animal models of PD. The assumption is that enriched neuronal populations instead of regional microdissections greatly increases the sensitivity with which changes can be detected, and thus the likelihood of generating interpretable results.[4]

Studies in central nervous system

Since 2005, ten groups have conducted gene expression profiling studies in postmortem mixed-cell samples from various brain areas, including parts of the basal ganglia, in patients with PD.[8,14-23] Seven of the studies identified PD-associated differential gene expression in brain areas including the SN, and showed good consensus relating to key gene expression changes, particularly with regard to dysregulation of protein processing and mitochondrial pathways.[8,14-17,21-23] Gene expression profiling analysis of 21 brain areas related to PD revealed that gene expression changes related to mitochondrial function occur throughout the brain, and to varying degrees among the different regions.[14,23]

The implication of dysregulation of protein processing and mitochondrial pathways is consistent with other researches on the pathophysiology of PD.[23-25] Several disease-causing mutations in PD impair mitochondrial complex I function[23,26] or the ubiquitin-proteasome system.[23,27] Moreover, the susceptibility gene *DJ1* encodes a chaperone protein that is also involved in proteolytic stress.[23,28] In fact, many genes and associated pathways implicated in familial PD are differentially expressed in the SN of sporadic PD cases compared with controls.[23,29]

Previous studies used a clustering technique to show that downregulation of mitochondrial and ubiquitin-proteasomal gene clusters correlate with each other and with clinical phenotype, suggesting a close relationship between impairments of these two systems in PD.[23,30] Studies comparing gene expression in the putamen of PD patients with a mutation in leucine-rich repeat kinase 2 (*LRRK2*) also showed evidence that *LRRK2* is involved in mitochondrial function.[23,31] These pathways could, therefore, contain a common therapeutic target.

Studies in peripheral tissue

Expression profiling from extra-nigral tissues is attractive for several reasons. First, many symptoms of PD are not referable to the SN or the rest of the basal ganglia.[4] Second, PD pathology such as either α-synuclein pathology or neuronal loss, is prominent in many areas outside the SN.[4,32,33] Third, peripheral tissues are more accessible for biopsy in living patients.[4]

A gene expression profiling study in peripheral blood from a large number of patients with sporadic PD aimed to generate a gene signature for this disease.[23,34] Blood samples were predominantly taken from patients with early-stage disease and compared with control samples from healthy individuals. The patient groups were chosen to facilitate the development of a biomarker for diagnosis in early PD. A molecular marker consisting of eight genes such as *VDR, HIP2, CLTB, FPRL2, CA12, CEACAM4, ACRV1*, and *UTX* was then validated.[23,34] Another gene expression profiling study in peripheral blood used exon-level probes,[23,35] and showed altered transcript splicing in venous blood from patients with PD. The researchers suggested this result could be related to altered expression of SRRM2, a splicing factor that was found to be differentially expressed in previous studies.[23,34]

Gene expression profiling analysis of peripheral blood mononuclear cells from LRRK2-PD cases found dysregulation of similar pathways to those identified in central nervous system studies of idiopathic PD, including mitochondrial function and the ubiquitin-proteasome system.[23,36] This result conflicts with that of another study suggesting that a direct comparison is difficult because the peripheral blood work did not include direct samples from the brain in patients with idiopathic PD.[23]

In addition with peripheral blood, other groups have investigated the expression profile of primary skin fibroblasts with PINK1 (PARK6) and Parkin (PARK2) mutations at the global transcriptome and proteome levels,[37-40] and found that mRNAs of several PARK genes such as alpha-synuclein and Parkin were dysregulated.[37,38,41]

Hurdles of Gene Expression Profiling Interpretation

The promise of gene expression as an unbiased method of determining potential causes of PD is unfulfilled at present.[4] There is an important point to be made concerning the impact of basal gene expression levels on changes caused by PD or PD-related insults. In particular, individual or even systematic expres-

sion changes in PD are potentially seriously misleading to the point of irrelevance if considered in the absence of baseline expression differences between cell types.[4]

To date, gene expression profiling experiment performed on neural tissue from PD patients has employed postmortem tissue samples. A large majority are obtained from PD patients in advanced stages of the disease. This raises significant issues concerning interpretation, especially with regard to disease pathogenesis. Pathological changes in the composition of the midbrain in advanced PD patients make it difficult to know what expression differences signify. RNA changes seen in advanced cases may not be at all indicative of what occurs in early PD due to the differing and variably abundant cell types in the SN of PD. Laser-capture microdissection of homogenous populations of SN dopaminergic neurons can correct, to a certain degree, for concerns about sample composition, but interpretive hurdles remain. For instance, surviving SN dopaminergic neurons captured from advanced PD patients may be a unique population of cells that are less vulnerable. They may also have engaged in effective compensatory behaviors resulting in survival. As such, the expression profile in these cells may actually be one indicating survival, not impending damage or not susceptible to damage.[4-12]

Conclusion

In light of these facts, gene expression profiling in PD will have to be investigated, taking account of related variables. In other words, by controlling variables such as cell type, gender, age, and pathological stage, in order to find a PD-specific gene expression. To enable this, large sample sizes should be ensured, which can become possible by conducting studies on peripheral tissue and thus increasing the accessibility to samples.

Although many studies have been undertaken using this technique, controlling related variables, such as gender, disease stage, and anatomical area, remains a challenge. Additionally, it is necessary to establish strategies for acquiring access to obtain more diversified samples in peripheral tissues such as blood and fibroblast. The gene expression profiling technique can serve as the basis for inferring the pathological mechanism of PD and as a signpost for future research directions.

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