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Transcriptome profiling in Parkinson's leukocytes: from early diagnostics to neuroimmune therapeutic prospects

Alon Simchovitz[1], Lilach Soreq[2] and Hermona Soreq[1]

Parkinson's disease (PD) involves motor symptoms reflecting the progressive degeneration of dopaminergic neurons in the substantia nigra. However, diagnosis is only enabled late in the disease, limiting treatment to palliative assistance. Here, we review recently generated transcriptional profiling datasets from blood and brain RNA of human PD cohorts and animal models that may offer unprecedented progress in PD research. Specifically, advanced analysis techniques demonstrated functionally inter-related underlying impairments of RNA metabolism and neuroimmune signalling processes. Identifying novel biomarkers in serum and nucleated blood cells, including protein networks and non-coding RNAs can drive discovery of the molecular mechanisms involved and reveal new targets for therapeutic intervention, posing a dual diagnosis/treatment opportunity for limiting the exacerbation of neuroinflammatory events in PD.

Addresses
[1] Department of Biological Chemistry and The Edmond and Lily Safra Center for Brain Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
[2] Department of Molecular Neuroscience, UCL Institute of Neurology (ION), Queen Square, London WC1N 3BG, UK

Corresponding author: Soreq, Hermona (hermona.soreq@mail.huji.ac.il)

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Current PD diagnosis is delayed and only allows palliative treatment

Parkinson's disease (PD) is the second most common neurodegenerative disease, with a current prevalence of up to 2% [1]. PD risk increases with age, and the prolonged life expectancy in Western societies predicts heavier costs in the coming decades [2]. Most cases of the disease are sporadic or non-familial, as hereditary mutations in several genes explain only up to 10% of the cases [3]. Hallmark symptoms are motor, and may include bradykinesia, resting tremor, rigidity and postural reflex impairments [1] due to massive death of dopaminergic neurons in the substantia nigra pars compacta brain region. Current disease diagnosis is based on the appearance of two out of the four aforementioned motor symptoms, which follow the death of approximately 50–60% of the nigro-striatal dopaminergic cells and the depletion of 80–85% of the striatal dopaminergic content [1]. Interestingly, non-motor symptoms whose aetiology is still in debate, such as anosmia, sleep problems and constipation, might precede the appearance of motor symptoms by up to 20 years [4]; however, these symptoms are not considered specific enough to allow early diagnosis and are variable across different populations. Combined with late diagnosis, incomplete understanding of the molecular mechanisms underlying the PD neurodegenerative process, and resultant failure to attenuate its progress pose major problems to PD treatment. Current treatment mainly remains palliative and is based on the primary use of agents increasing dopaminergic signalling, such as the dopamine precursor 3,4-dihydroxyphenyl-L-alanine (L-DOPA) [5]. While involvement and potential targeting of additional neurotransmitters is being investigated [6,7], these studies merely aim to provide a more successful and specific palliative, anti-symptomatic treatment. Likewise, intracranial subthalamic transplantation of deep brain stimulation (DBS) micro-electrodes only improves the motor symptoms, may entail adverse side effects (e.g. cognitive and language problems) and reflects an alternative palliative solution [8]. DBS is offered when dopamine agonists cause motor complications or are no longer effective (which often occurs after 5–10 years of usage), but can only be performed in patients who pass inclusion criteria [9]. Altogether, these suboptimal diagnostic approaches lead to delayed diagnosis and lack of disease-modifying therapy for PD, calling for urgent solutions.

Studies in patients and animal models implicate neuroimmune malfunctioning

The molecular mechanisms that underlie dopaminergic cell death in PD are still incompletely understood. Nevertheless, the relevant body of knowledge has grown substantially in the past decades, thanks to breakthrough developments in neurogenetics and molecular neurochemistry. These breakthroughs implicate several pathways including nuclear, mitochondrial and immune system signalling as shaping the neurodegenerative process [10]. Several mutations were discovered as responsible for the familial forms of the disease, such as duplications, triplications and mis-sense mutations

(mainly the A53T substitution) of the nuclear protein α-synuclein (Park1) in early-onset PD patients [10]; and the most common G2019S mutation in the mitochondrial regulator Leucine-rich repeat kinase (LRRK2) with its more subtle PD phenotype [11]. In parallel, neurotoxic compounds were discovered that cause Parkinsonism, including 1-methyl-1,2,3,6-tetrahydropiridine (MPTP) and 6-hydroxydopamine (6-OHDA) [12]. Chemically induced Parkinsonism provided the basis for creating PD animal models, allowing closer investigations of cell death mechanisms and enabling experimental manipulations aimed to prevent cell death. In comparison, transgenic animals over-expressing disease-related genes, in either mutated or human wild-type form, sometimes show motor phenotypes but mainly lack PD-like neurodegenerative events [12]. These discoveries helped portray a new landscape of PD pathology, with hallmark pathways implicated including oxidative stress involvement in cell death [13], generation of Lewy bodies neuropathology containing disease-related proteins [14] and prominent immune system involvement [10]. Technology-based breakthroughs are thus an essential element in PD research.

Contrasting the years-long view of the central nervous system (CNS) as immune-privileged [15], recent reports implicate both the adaptive and innate branches of the immune system in PD pathology. Polymorphisms in inflammation-related genes, coding for histocompatibility antigen (HLA) [16], cytokine interleukins (IL)-10 and -1β as well as tumour necrosis factor α, and other immune genes [10] were identified as associated with PD in certain populations, although not in others [17]. This indicated possible interaction of immune-related risk alleles with other population-specific genetic variables or environmental factors, compatible with the variable risks of Parkinsonism in different populations [18]. DNA polymorphisms/RNA expression relationships also showed immune relevance, with the identification of leukocyte expression quantitative trait loci (eQTLs) in PD-related disease variants [19]. Moreover, many inflammatory cytokines and chemokines are up regulated or show higher activation in patients' serum (Including CCL-2, IL-8 and IL-1β). Increased cytokines and chemokines gene expression is correlated to disease severity, as is leukocyte hyperactivity measured by their reaction to pro-inflammatory bacterial lipopolysaccharide (LPS) [20,21]. Brain-to-blood communication processes may hence be important elements in the PD disease process.

The cellular composition of peripheral blood leukocytes is also affected by, and connected to the PD process, albeit with subtle and specific changes. Combining peripheral blood composition with functional brain imaging revealed up regulation of leukocyte apoptosis in PD patients, in a manner correlated to dopaminergic neuron death in their brain [22]. Changes were also witnessed in monocyte subpopulations: while the overall monocyte level remained unchanged in patients, classical monocytes expressing the cellular differentiation antigen CD14 were up-regulated [20], and increased CCL2-receptor levels were identified in this subpopulation [23], possibly potentiating leukocytes crossing the blood-brain barrier, as is schematically displayed in Figure 1. CCL2, excreted by various immune cells including activated microglia [24], is also up regulated in the serum of PD patients [20], suggesting microglia-induced recruitment of a pro-inflammatory monocyte subpopulation to the CNS. Immune activation might also be mediated by PD-related genes, for example the mitochondrial regulatory protein LRRK2, which is increasingly expressed in maturing monocytes, possibly with a function in their maturation and activation [25]. These findings shed a new light on the early treatment of PD with cholinesterase blockers; the cholinergic system's immunomodulatory function [26•] further implicates it in PD progression and potential treatment. This evidence clearly indicates active involvement of the peripheral immune system in PD pathology.

RNA-sequencing and microarray data analyses indicate RNA-mediated neuroimmune impairments

Recent datasets of brain transcripts from human PD cohorts [27] and animal models [28] substantiate the functional relevance of RNA metabolism and neuro-immune signalling in the PD process [29]. These include changes in regulatory RNA elements such as small vault RNAs [30], micro-RNAs (miRNA) [31–33] and miRNA binding sites in their target genes (due to alternative polyadenylation) [34••] (prominent changes are listed in Table 1). Many PD-related genes are involved in neuronal transcription and RNA metabolism processes [35,36], raising the question of causal RNA metabolism involvement in disease pathology in general and in its immune involvement in particular. Similarities between transcriptional profiles of peripheral blood cells and brain tissues from PD patients [32] further support the notion of impaired RNA metabolism in PD [35] and the hope to extract disease 'signatures' from the altered RNA expression patterns of PD patients' blood leukocytes [29,32,34••,37–41]. Leukocyte RNA studies involved PD patients pre-DBS and post-DBS, as well as after a 60 minutes cessation of DBS, and covered transcriptional profiling using both microarrays and RNA-sequencing followed by advanced analysis techniques. This allowed comparison between data obtained by different transcriptional profiling methods and revealed strong evidence of comprehensive RNA dysregulation. Both disease and treatment caused various RNA changes including differential mRNA, long non-coding (lncRNA) and miRNA expression and alternative splicing. Beyond the use of different profiling methods, studying various disease conditions by analysis of different datasets allowed a comparative view of transcriptional changes