

The Universal Non-Neuronal Nature of Parkinson's Disease: A Theory

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ABSTRACT

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, yet the etiology of the majority of its cases remains unknown. In here, relevant published evidence is interpreted and integrated into a comprehensive hypothesis on the nature, origin and inter-cellular mode of propagation of sporadic PD. We propose to characterize sporadic PD as a pathological deviation in the global gene expression program of a cell: the PD expression-state, or PD-state for short. A universal cell-generic state, the PD-state deviation would be particularly damaging in a neuronal context, ultimately leading to neuron death and the ensuing observed clinical signs. We review why age accumulated damage caused by oxidative stress in mitochondria could be the trigger for a primordial cell to shift to the PD-state. We put forward hematopoietic cells could be the first to acquire the PD-state, at hematopoiesis, from the disruption in reactive oxygen species (ROS) homeostasis that arises with age in the hematopoietic stem-cell niche. We argue why, nonetheless, a cell ageing process is unlikely to explain the shift to the PD-state of all the subsequently affected cells in a patient, thus indicating the existence of a distinct mechanism of cellular propagation of the PD-state. We highlight recent findings on the intercellular exchange of mitochondrial DNA and the ability of mitochondrial DNA to modulate the cellular global gene expression state and propose this could form the basis for the intercellular propagation of the PD-state.

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder of old age. The number of worldwide affected individuals is estimated at 7 to 10 million ¹. With population ageing, particularly in developing countries ², this number is certain to increase steeply. Unfortunately, there is currently no cure for PD, with available medications only providing symptomatic relief ³.

PD is a movement disorder clinically characterized by tremor, bradykinesia, rigidity and postural instability ⁴. The motor dysfunctions are a direct consequence of the death of dopamine-producing neurons in the substantia nigra pars compacta region of the midbrain. Histologically, the most noticeable feature of PD are abnormal aggregates of proteins, called Lewy bodies and Lewy neurites, that appear in the cell body and neurites of PD patient neurons. Their major constituent is the protein alpha-synuclein.

Familial genetic linkage studies have unequivocally associated six genes with Mendelian inheritable forms of PD ⁴. Still, these individual gene mutations account for fewer than 10% of PD cases. They lead generally to juvenile or early onset PD (before 50 years of age). Naturally, genetics still impacts the chance of an individual acquiring non-monogenic sporadic PD later in life. Over a dozen of single nucleotide polymorphisms (SNPs) have been statistically linked with sporadic PD through genome-wide association studies (GWASs) ^{5,6}. However, the differential risks associated with carrying these SNPs, although statistically significant, are mostly very small in absolute terms. Similarly, although some environmental factors, such as exposure to metals or pesticides, have been statistically linked with PD, the associations do not appear to be sufficiently

widespread to explain beyond a minority of PD cases⁷. Thus, the etiology of the over 90% of cases classified as sporadic PD remains undetermined.

We briefly highlight some of the major theories being pursued regarding the nature of sporadic PD. Different aspects of these hypotheses will be presented in more detail as relevant, throughout the article. An overarching hypothesis on the etiology of sporadic PD is that an external agent causes it. Pesticides and metals would be two candidate environmental agents, given their statistical association with PD⁷. Another proposed factor suggested in the literature is a neurotropic pathogen, such as a virus⁸ or a prion-like protein⁹. Entry into the organism could be via the peripheral olfactory system¹⁰ or via the gastrointestinal tract¹¹, two sites associated with early prodromal clinical symptoms of PD. These two sites have also been combined into a dual-hit hypothesis, centered on an external agent simultaneously entering the organism via the two routes¹². Whether with an initially external origin or endogenously generated, the theory that a misfolded, prion-like self-propagating form of alpha-synuclein is responsible for the disease is another major hypothesis currently under investigation^{9,13}. More endogenous, aging-related perspectives of sporadic PD focus on the role of oxidative stress and mitochondrial damage, for which there is significant evidence in PD patients^{14,15}. Finally, although the view of sporadic PD as an autoimmune disease is not typical, the aggravating contribution of the neuro-inflammatory response to the disease is commonly acknowledged^{16,17}.

Starting from an analysis of sporadic PD blood gene-expression data, we have previously argued that sporadic PD could have an hematopoietic origin¹⁸. In the present article, we review and expand on this original hypothesis, placing it in the context of recent developments in both PD and the broader biological research. The article first addresses why sporadic PD may be a systemic, rather than solely neuronal, condition. Then, it discusses why intercellular propagation of the pathology may be involved, rather than the pathology being purely a cell ageing phenomenon. In particular, we present the possibility that the disease transmission may be enabled by the intercellular exchange of mitochondrial DNA. Finally, the article revisits our previously published hypothesis that the pathology may initially begin at hematopoiesis.

THE UNIVERSAL NATURE OF THE PD-STATE

In this section we argue for a universal cell-generic nature of sporadic PD. Our case is centered on the appropriate interpretation of recently published evidence obtained by applying induced pluripotent stem cell (iPSC) technology to PD research^{19,20}. Using iPSC techniques, conveniently collected cells from PD patients, such as skin fibroblasts, can be reverted to a pluripotent state and subsequently differentiated into dopaminergic neurons. This allows investigation of the disease *in vitro*, using dopaminergic cells with the full genetic background of a PD affected individual. Such *in vitro* models may prove invaluable in the pursuit of disease-modifying therapies. However, just as important, we believe that these early PD iPSC experimental results already permit a fundamental re-evaluation of the nature of the sporadic non-monogenic form of the disease.

We start by recalling the key observations from two published PD iPSC studies^{19,20}. In both cases, skin fibroblasts from sporadic PD patients (presuppose sporadic henceforth) were reprogrammed back into pluripotent stem cells, which were then differentiated into dopaminergic neurons. Sánchez-Danés et al.¹⁹ reported that, in comparison with neurons derived from fibroblasts from disease-free controls, neurons originating in fibroblasts from PD patients consistently showed PD phenotype associated alterations. These included reduced numbers of neurites, more limited neurite arborizations and increases in caspase-3 activity, a marker for cell apoptosis. Woodard et al.²⁰ utilized neurons derived from fibroblasts of two monozygotic twins discordant for PD. Multiple alterations that can be linked with a PD phenotype were present exclusively in the fibroblasts derived from the PD twin. These included lower dopamine levels, an

elevated presence of alpha-synuclein in neurites, a delay in the emergence of spontaneous action potentials and an absence of synchronous neuronal activity.

An epidemiological study by Tanner et al.²¹ reported a mere 15.5% concordance of monozygotic twins in developing PD. An analogous study in Sweden published by Wirdefeldt et al.²² corroborates this result, placing the concordance rate at 11%. Thus, excluding the monogenic cases, heredity does not ensure the emergence of PD. Therefore, the consistent, regular emergence of a PD phenotype in neurons derived from fibroblasts from PD patients cannot be attributed to a PD favorable germline genetic background in the patients. The conclusion is that the disease must have been present in the skin fibroblasts from the patients. PD is thus a cell-generic state, not confined to neuronal cells.

We propose to characterize PD as a pathological deviation in the expression program of a cell: the PD expression-state, or PD-state for short^{23,18}. Reports of a characteristic PD gene-expression signature across multiple tissues support this view^{18,24,25,26}. The recent observation of a unique, concordant pattern of methylation in post-mortem frontal cortex samples and peripheral blood leukocytes from PD patients²⁷ reinforces this standpoint, further suggesting that the PD-state may be stabilized by DNA epigenetic modifications.

THE ROLE OF AGEING IN PD

In spite of possessing a multitude of self-repair mechanisms, all cells undergo an ageing process²⁸. They gradually accumulate dysfunctional molecules, as well as random mutations and other assorted alterations in their genetic code. Ultimately, this leads to the organism ageing phenotype. As a source of free radicals, mitochondria and the mitochondrial DNA are particularly vulnerable to oxidative stress damage²⁹. This observation has led to the theory that the mitochondrial dysfunction caused by oxidative stress plays a central role in ageing^{30,31}. With PD arising at old age and with mitochondrial function specifically known to be compromised in a variety of cell types in PD patients^{32,33,34,35}, the mitochondrial theory of aging broadly views PD as yet another manifestation of this phenomenon¹⁴.

It is conceivable that accumulated random damage, possibly in mitochondrial DNA and due to oxidative stress, eventually triggers the gene expression program of a cell to shift to the PD-state. The shift to the PD-state in a cell could thus be viewed as a probabilistic event, its likelihood being a (nonlinearly) increasing function of that built up damage. However, it is not tenable that all PD-state cells in a PD affected individual acquired the PD-state in this fashion.

To see the above, consider the following valid scenario. Imagine two monozygotic twins, one diagnosed with PD in the past, the other not so up to the present. Let the unaffected twin presently exhibit a greater amount of age accumulated damage than the affected twin did at the time of his PD diagnosis - this would be the case given enough years had elapsed since then. Now, assume that accumulated random damage is the only possible trigger of the PD-state in a cell. Then, the observed greater age accumulated damage in the undiagnosed twin at present would guarantee a currently greater probability for its individual cells to acquire the PD-state than that probability was for the cells of the affected twin at the time of his PD-diagnosis. Yet, the unaffected twin continues to show no clinical signs of PD at present, in contrast with the affected twin at the time of his PD diagnosis. Thus, assuming that accumulated random damage is the only possible trigger of the PD-state in a cell leads to a probabilistic paradox.

We make two remarks regarding the presented argument. Firstly, accumulated random damage causing a cell to shift to the PD-state does not pose a paradox. Only the presence of every PD-state cell in the organism cannot be explained by such a process. Therefore, ageing-associated damage, caused by oxidative stress in mitochondria, could still be the trigger for a primordial cell in the organism to shift to the PD-state. Secondly, ageing-associated cellular damage may not be

required for the subsequent dissemination of the PD-state across the organism since, as argued, this dissemination would occur via a distinct mechanism.

There are two direct pieces of evidence supporting a propagation dynamic in PD. First, analyses of post-mortem neuronal tissue from patients that passed away at different stages of the disease appear to support a chronological, physical spread of Lewy-bodies across the nervous system³⁶. Second, there is the published evidence on the surgical transplantations of fetal ventral mesencephalic dopaminergic neurons as a treatment of PD. A number of post-mortem analyses, performed over 10 years after the transplant, detected Lewy-bodies and Lewy neurites in grafted neurons, in spite of the still young age of the transplanted tissue^{37,38}. This latter fact is in addition evidence of the non-essentiality of cellular ageing to the propagation of the PD-state.

In this section we argued that ageing-associated damage caused by oxidative stress in mitochondria could be the trigger for a primordial individual cell to shift its gene expression program to the PD-state. The shift to the PD-state would be a probabilistic event, its likelihood increasing nonlinearly with that accumulated damage in the cell. On the other hand, the subsequent appearance of the PD-state in numerous other cells could no longer be explained by cell ageing. Rather, it likely involves a separate mechanism of propagation of the PD-state.

THE PROPAGATION OF THE PD-STATE

The spread of the PD-state is a slow process, as evidenced by the approximate decade that it takes an implanted fetal neuron in the brain of a PD patient to develop Lewy bodies^{37,38}. The mechanism of propagation of the PD-state remains undetermined. However, new modes of inter-cellular communication continue to be discovered³⁹. In this section, we discuss how propagation of the PD-state may occur.

One hypothesis posits that alpha-synuclein can behave as a prion and that PD is a prion disorder^{9,13}. Under this theory, there exists a misfolded form of alpha-synuclein that is self-propagating, having the ability to induce similar misfolding in well-conformed alpha-synuclein. The transfer of the misfolded form of alpha-synuclein from cell to cell would thus result in the intercellular spread of PD. However, a western blot analysis did not detect any alpha-synuclein in the fibroblasts utilized in the PD iPSC experiments discussed earlier²⁰. Therefore, a PD phenotype in fibroblast-derived neurons cannot be explained by the lingering presence of a hypothetical infectious form of alpha-synuclein.

Nevertheless, the possibility that a different molecule (or higher-level biological entity) is responsible for transmitting the PD-state remains open to investigation. Recently, the intercellular exchange of mitochondrial DNA (mtDNA) has been demonstrated^{40,41}. We suggest that intercellular mtDNA transfer could be the vehicle for the propagation of the PD-state.

Comprehensive research with neuron-platelet cytoplasmic hybrids supports that anomalous mtDNA may suffice to set off the PD-state in a cell. A PD cybrid cell is created in vitro by the fusion of a neuronal cell depleted of endogenous mtDNA with an enucleated platelet from a PD donor. Thus, the mtDNA of the cybrid cell is that of the platelet from the PD patient, while its nuclear DNA is that of the disease-free neuronal cell. Various PD characteristic alterations have been observed in PD cybrids, most prominently, inclusions that replicate the essential biochemical and structural features found in Lewy-bodies in the brain of PD patients^{15,42,43}.

The sufficiency of mtDNA to trigger the PD-state in a cell is supported by its ability to induce epigenetic modifications and to modulate gene-expression in nuclear DNA. In the context of tumorigenesis, work by Smiraglia et al.⁴⁴ and by Xie et al.⁴⁵ shows that alterations to mtDNA affect the methylation pattern of various nuclear genes. Bellizzi et al.⁴⁶ report that methylation and gene expression patterns of nuclear genes in cybrids depend on the mtDNA donor haplogroup. Kelly et al.⁴⁷ proposed that mtDNA haplotypes play a pivotal role in the process of differentiation and

mediate the fate of the cell. In mouse undifferentiated and differentiating embryonic stem cells, with the same nuclear DNA haplotype but distinct mtDNA haplotypes, they observed mtDNA haplotype-specific expression of genes involved in pluripotency, differentiation, mitochondrial energy metabolism, and DNA methylation.

No specific mutations in mtDNA have been consistently associated with PD⁴⁸. However, heteroplasmy of mtDNA, i.e., the presence of multiple mtDNA variants within a cell, is now widely appreciated⁴¹. Thus, undetected lower frequency mtDNA variants could potentially be involved in the transmission of the PD-state. Further, the number of PD-state triggering variants could be too large for effective statistical detection. Alternatively, it will be interesting to investigate if the PD-state is stabilized in mitochondria by characteristic mtDNA epigenetic modifications. Whether mtDNA can be methylated is currently a matter of active debate^{49,50}. Hong et al.⁵¹ make a strong case for the absence of mtDNA methylation, at least under most biological conditions. On the other hand, recently Bacarelli et al.⁵², via bisulfite-PCR pyrosequencing, found the presence of significant mtDNA methylation in platelets from blood samples of cardiovascular disease patients. For instance, in sequenced sites in the MT-CO1 gene region, average percentage site methylation was found to be on the order of 25%⁵².

A SITE OF ORIGIN FOR THE PD-STATE

We have mentioned that ageing-associated damage caused by oxidative stress in mitochondria could be the trigger for a primordial cell to shift its gene expression program to the PD-state. A relevant question is where would an initial PD-state cell most commonly arise. In this section, we suggest the hematopoietic stem cell niche as a site to consider.

Research based on gene expression^{18,25}, DNA methylation²⁷, neuron-platelet cybrid^{43,42,15} and bioenergetic³² analyses supports the presence of the PD-state in circulating hematopoietic cells. Given the short lifespan of blood cells (days for platelets⁵⁴ and granulocytes⁵⁵ and weeks for lymphocytes, with the exception of memory cells⁵⁶) by comparison with the decade long timescale for the transmission of PD across the neuronal system^{36,37,38}, the above signs of PD in blood point to circulating hematopoietic cells acquiring the PD-state at hematopoiesis, rather than after maturation.

Hematopoiesis is altered with ageing, with most published evidence coming from mouse models as described in this section. In terms of global gene expression in hematopoietic stem cells (HSCs), nitric oxide mediated signal transduction, the NF- κ B cascade and the pro-inflammatory response are the most age up-regulated processes, while chromatin silencing, single-strand break repair, SMAD protein nuclear translocation and chromatin remodeling are the most down-regulated ones⁵⁷. Alterations at the HSC epigenetic level are supported by many chromosomal regions showing a coordinated change in transcriptional activity⁵⁷. Fate-wise, a skewing to the myeloid line and a diminished lymphoid potential are observed with ageing⁵⁸.

It has been known for a long time that reactive oxygen species (ROS), if not properly checked, have the potential to cause indiscriminate cellular damage³⁰. Today, it is recognized that ROS may also play a functional signaling role in activating processes such as the inflammatory and stress responses^{28,59}. Additionally, there is indication that ROS play a role in regulating hematopoiesis⁶⁰. In particular, evidence associates abnormal ROS levels at old age with a dysfunction in both the proliferation and the differentiation dynamics of HSCs. In vitro research has shown that exposure to H₂O₂ can lead to chromosomal translocations in HSCs⁶¹. Ionizing radiation is also known to affect HSCs, as it has been found to promote differentiation, short-term apoptosis and long-term senescence of HSCs⁶². In vivo, work with *Drosophila* supports the role of ROS in the regulation of hematopoietic cell fate. Increasing ROS beyond its basal level in *Drosophila* multipotent hematopoietic progenitor cells triggers their precocious differentiation⁶³. Conversely, scavenging

ROS from these hematopoietic progenitors retards their differentiation into mature blood cells⁶³. It is well-established that serial transplantation of human HSCs into immunodeficient mice leads to both elevated intracellular ROS levels and to premature HSC senescence^{64,65,66}. Yahata et al.⁶⁴ and Ito et al.⁶⁶ independently reported that antioxidant pharmacological inhibition of ROS can mitigate this deteriorating HSC phenotype. Caloric restriction in BalbC mice was similarly shown to postpone HSC senescence⁶⁵. Finally, the same protective effect was achieved by SIRT3 up-regulation in HSCs⁶⁷. HSCs are highly-enriched in this mammalian sirtuin, except for its suppression at old age⁶⁷. SIRT3 regulates the global acetylation landscape of mitochondrial proteins and reduces oxidative stress⁶⁷. Mechanistically, the FoxO transcription factors⁶⁸ and the p53⁶⁹, Akt⁷⁰, MAPK⁶⁶ and ATM⁷¹ pathways have all been implicated in the ROS modulation of hematopoiesis.

At the genetic level, some emerging evidence may also turn out to connect PD and the hematopoietic system, although its interpretation is not yet completely clear. A new study by Xiao et al.⁷² found hematologic abnormalities in alpha-synuclein knock-out mice indicative of an heretofore unknown role of this molecule in late-stage hematopoiesis. A genome-wide association study found a rare non-synonymous mutation in DZIP1 as a risk factor for PD⁷³. DZIP1 is a component of the Hedgehog signaling pathway⁷⁴. Besides its role in directing embryonic pattern formation, the hedgehog pathway has been implicated in the maintenance of adult stem cell niches, including both neuronal⁷⁵ and hematopoietic stem-cells⁷⁶. Finally, PD patients are over five times more likely to be carriers of the mutated form of GBA responsible for the Gaucher's autosomal recessive disease⁷⁷. Gaucher's disease is characterized by low blood platelet levels, anemia, and the accumulation of the glycolipid glucocerebroside in the mononuclear phagocyte system⁷⁸.

In summary, we argued for the hematopoietic stem cell niche as a possible site for the appearance of a primordial PD-state cell based on evidence that supports: i) PD patients consistently having circulating hematopoietic cells in the PD-state; ii) Those hematopoietic cells more plausibly having acquired the PD-state at hematopoiesis, rather than after maturation; and iii) the critical role of ROS in regulating the hematopoietic niche and the disruption of this homeostasis with ageing.

CONCLUSION

Calling upon some recent findings that we considered relevant to the PD field, as well as on various established lines of PD research, we presented a comprehensive theory on the nature, origin and inter-cellular mode of propagation of sporadic Parkinson's disease. We now review our hypothesis and interpret a few additional observations in its light.

We propose to define PD as a characteristic pathological deviation in the global gene expression program of a cell: the PD expression-state, or PD-state for short. Most significantly, any cell could be in the PD-state, it would be a universal cell state. However, due to the cell processes it affects the most, the PD-state deviation would be particularly damaging in a neuronal context, ultimately leading to neuron death and the ensuing observed clinical signs of PD.

Ageing-associated accumulated damage caused by oxidative stress in mitochondria could be the trigger for a primordial cell to shift to the PD-state. In particular, hematopoietic cells could be the first to acquire the PD-state, at hematopoiesis, as a result of the disruption in ROS homeostasis that arises with age in the hematopoietic stem-cell niche. The appearance of the PD-state deviation at hematopoiesis would be a lifetime statistically relatively unlikely event, its odds not greatly affected by genetics or lifelong environmental exposures. Low correlation of PD incidence with genetic and environmental factors would follow from this PD-state initiation dynamics.

Propagation of the PD-state across the organism would occur in a second phase and via a distinct mechanism. We suggested that the ability of mtDNA to move across cells and to modulate

the cellular global gene expression state could form the basis for this intercellular propagation of the PD-state. The mtDNA-based PD propagation dynamics would occur on a time-scale of years, as observed in patients, and not be ageing-dependent, in contrast with the PD-state initiation dynamics.

Under physiological conditions, mice are not susceptible to PD late in life, in spite of clearly showing an ageing phenotype just as humans do ⁷⁹. This absence of a PD phenotype in mice could thus more likely follow from the ageing-independent PD spread dynamics, than from the ageing-associated PD initiation dynamics. Namely, going from human to mouse, the mtDNA propagation dynamics would not scale appropriately time-wise to permit the condition to reach the neuronal system in the lifetime of a mouse.

Early symptoms of PD in humans include impaired sense of smell ¹⁰ and gastrointestinal dysfunction ⁸⁰. Both have been reported as much as a decade before the appearance of symptoms at the motor level. They are typically interpreted as supporting the role of an external agent - entering via the olfactory or gastro-intestinal entry points - in inducing PD ¹². However, another characteristic shared by the olfactory bulb and the gastro-intestinal tract is that they are both sites of very active stem-cell based tissue regeneration ^{81,82}. The rapid cell renewal and the plasticity of immature cells could facilitate both the cellular uptake of carriers of external mtDNA and the global cellular reprogramming to the PD-state, explaining the olfactory bulb and the gastro-intestinal tract being some of the earlier sites to which the PD-state would spread.

Finally, we note that although our hypothesis was presented in the context of PD, it is apparent that, if correct, an analogous, parallel etiology may be at the core of Alzheimer's disease.

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