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

André X. C. N. Valente[1,2,3,*], Altynai Adilbayeva[4], Tursonjan Tokay[4], Albert A. Rizvanov[3]
[1]Center for Neuroscience and Cell Biology, University of Coimbra, Cantanhede, Portugal
[2]Biocant - Biotechnology Innovation Center, Cantanhede, Portugal
[3]Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia
[4]National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan

[*]Corresponding author, email: axcnvalente@gmail.com

ABSTRACT

Various recent developments of relevance to Parkinson's disease (PD) are discussed and integrated into a comprehensive hypothesis on the nature, origin and inter-cellular mode of propagation of late-onset sporadic PD. We propose to define sporadic PD as a characteristic pathological deviation in the global gene expression program of a cell: the PD expression-state, or PD-state for short. Although 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, such a 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 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 as a movement disorder clinically characterized by tremor, bradykinesia, rigidity and postural instability [1]. 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 [1]. Still, these individual gene mutations account for fewer than 10% of PD cases. They lead generally to juvenile or early onset PD (before age 50).

Naturally, genetics still impacts the chance of an individual acquiring non-monogenic sporadic PD later in life. Over a dozen single nucleotide polymorphisms (SNPs) have been statistically linked with sporadic PD through genome-wide association studies (GWASs) [2,3]. 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 cases [4]. Thus, the etiology of the over 90% of cases classified as sporadic late-onset 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 called upon and presented in more detail whenever relevant, throughout the article. An overarching hypothesis on sporadic PD is that it is

caused by an external agent. Pesticides and metals would be two candidate environmental agents, given their statistical association with PD alluded to above [4]. Another possibility would be a neurothropic pathogen, such as a virus [5] or a prion-like protein [6]. Entry into the organism could be via the peripheral olfactory system [7] or via the gastrointestinal tract [8], two sites associated with early prodromal clinical symptoms of PD. These two sites have also been combined into a dual-hit hypothesis, that proposes that the external agent simultaneously enters the organism via the two routes [9]. 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 [6,10]. More endogenous, aging-related perspectives of sporadic PD are very centered on the role of oxidative stress and mitochondrial damage, for which there is significant evidence in PD patients [11,12]. 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 [13,14].

In this article, we combine some recent developments of relevance to sporadic PD with a number of more established PD findings in a comprehensive hypothesis on the nature, origin and inter-cellular mode of propagation of sporadic PD.

THE UNIVERSAL NATURE OF THE PD-STATE

The application of induced pluripotent stem cell (iPSC) technology to PD research is a recent development in the field. Using iPSC techniques, conveniently collected cells from PD patients, such as skin fibroblasts, can be first reverted to a pluripotent state and then further differentiated into dopaminergic neurons. This allows investigation of the disease *in vitro*, in dopaminergic cells with the full genetic background of a PD individual. Such *in vitro* models may prove invaluable in the pursuit of disease-modifying therapies. However, future developments aside, we believe that the existing elementary observations from PD iPSC experiments already permit a deep, fundamental re-evaluation of the nature of the sporadic late-onset form of the disease.

We start by recalling the key observations in two of these PD iPSC studies. 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. [15] reported that, by comparison with neurons identically derived from fibroblasts from healthy controls, neurons originating in fibroblasts from PD patients consistently showed alterations that could be associated with a PD phenotype. These included reduced numbers of neurites, more limited neurite arborizations and increases in caspase-3 activity, a marker for cell apoptosis. Woodard et al. [16] specifically utilized neurons derived from fibroblasts of two monozygotic twins discordant for PD. Vis-à-vis the neurons derived from the healthy twin fibroblasts, the neurons derived from the PD twin fibroblasts again showed multiple alterations that can be linked with a PD phenotype. 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.

Now, a well-established study by Tanner et al. [17] reported a mere 15.5% concordance of monozygotic twins in developing PD. An analogous study in Sweden by Wirdefeldt et al. [18] essentially confirmed this result, placing the concordance rate at 11%. In other words, 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 fundamentally define PD as a

characteristic pathological deviation in the expression program of a cell: the PD expression-state, or PD-state for short [19,20]. Reports of a characteristic PD gene-expression signature across multiple tissues directly support this view [20,21,22,23]. The recent observation of a unique, concordant pattern of methylation in post-mortem frontal cortex samples and peripheral blood leukocytes from PD patients [24] reinforces this standpoint, further indicating that the PD-state may be stabilized by DNA epigenetic modifications. 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.

THE PD-STATE DOES NOT ARISE INDEPENDENTLY IN CELLS ACROSS THE ORGANISM

In spite of possessing a multitude of self-repair mechanisms, cells age [25]. At a primary level, they gradually accumulate dysfunctional molecules, as well as random mutations and other assorted alterations in their genetic code. Ultimately, at the phenotypic level this produces the external signs of aging that we are all able to recognize. As a source of free radicals, mitochondria and the mitochondrial DNA are particularly vulnerable to oxidative stress damage [26]. This has led to the fundamental theory that mitochondrial dysfunction caused by oxidative stress plays a central role in aging [27,28]. With PD arising at old age and with mitochondrial function specifically known to be compromised in a variety of cell types in PD patients [29,30,31,32], the mitochondrial theory of aging broadly views PD as yet another manifestation of this phenomena [11].

It is conceivable that accumulated random damage, perhaps in mitochondrial DNA and due to oxidative stress, eventually induces 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 a (nonlinearly) increasing function of the built up damage associated with aging.

However, it is no longer plausible that cells throughout the organism acquire the PD-state *independently*, in the above fashion. To see this, consider two monozygotic twins, one diagnosed with PD long ago, the other so far not so, in spite of having by now a far greater amount of aging-associated damage than his PD twin did at the time of diagnosis. This ordinary scenario [17] poses a paradox, in light of the huge differential in the number of cells that have shifted to the PD-state in each twin. The fact that at the time of clinical diagnosis over 50% of the dopaminergic neurons are believed to have already died [33] does not dispel the paradox. Thus, we must conclude that the PD-state cannot have arisen independently in each affected cell of a PD patient. A mechanism for the propagation of the PD-state must exist.

We make two additional remarks regarding the above argument. Firstly, the described stochastic process shifting one or a few cells to the PD-state in one twin but none in the other does not pose a paradox. Only the collective shifting of many more cells in one twin than the other is not tenable. Therefore, aging-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, aging-associated cellular damage may not be essential to the subsequent dissemination of the PD-state across the organism since, as argued, this dissemination is occurring via a distinct propagation mechanism.

There are two more direct pieces of evidence supporting a propagation dynamic in PD. First, there are the post-mortem histo-pathological analyses of neuronal tissue from patients that passed away at different stages of the disease. These appear to support a chronological physical spread of Lewy-bodies across the nervous system [34]. Second, there are the surgical transplantations of fetal ventral mesencephalic dopaminergic neurons as a treatment of PD. A number of post-mortem analyses over 10 years after the transplant detected Lewy-bodies and Lewy neurites in grafted neurons, in spite of the neurons still young age [35,36]. This latter fact is in addition evidence of the non-essentiality of cellular aging to the propagation of the PD-state.

In sum, in this section we have argued that aging-associated damage caused by oxidative stress in mitochondria could well be the trigger for an 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 large numbers of cells in the PD-state across the organism can no longer be explained by such a cell autonomous process. Rather, it must result from a separate mechanism of propagation of the PD-state.

THE PROPAGATION OF THE PD-STATE

The spread of the PD-state is a rather slow process, as evidenced by the 10 or so years that it takes for an implanted fetal neuron in the brain of a PD patient to produce Lewy bodies [35,36]. The mechanism of propagation of the PD-state remains undetermined. However, new modes of inter-cellular communication continue to be discovered [37]. It is safe to say that we still underestimate the ways in which cells can communicate. Therefore, the fact that a mechanism for the transmission of the PD-state is not known at present, does not detract from the possibility of such said transmission. In this section, we discuss how propagation of the PD-state may take place.

A currently popular hypothesis is that alpha-synuclein can behave as a prion and PD is a prion disorder [6,10]. Under this theory, a misfolded form of alpha-synuclein would be self-propagating, having the ability to induce further misfolding in well-conformed alpha-synuclein. This process would underlie the spread of misfolded alpha-synuclein from cell to cell. However, a western blot analysis did not detect any alpha-synuclein in the fibroblasts utilized in the PD iPSC experiments discussed earlier [16]. 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 particular molecule (or higher-level entity) is responsible for transmitting the PD-state remains open. Recently, the intercellular exchange of mitochondrial DNA (mtDNA) has been demonstrated [38,39]. We suggest that such intercellular mtDNA transfers could enable propagation of the PD-state.

Comprehensive research with neuron-platelet cybrids supports that a particular 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 [12,40,41].

The ability of mtDNA to both induce epigenetic modifications and modulate gene-expression in nuclear DNA supports the sufficiency of mtDNA to trigger the PD-state in a cell. In the context of tumorigenesis, work by Smiraglia et al. [42] and by Xie et al. [43] shows that alterations to mtDNA affect the methylation pattern of various nuclear genes. Bellizzi et al. [44] report that methylation and gene expression patterns of nuclear genes in cybrids depend on the mtDNA donor haplogroup. Kelly et al. [45] 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 PD [46]. However, it will be worth exploring whether the PD-state is stabilized in mitochondria by characteristic mtDNA epigenetic modifications. Methylation of mtDNA was reported decades ago [47,48,49], but it was since then mostly ignored. Fortunately, there has been a recent renewed interest in this possibility

and its implications, that started with the report by Shock et al. [50] that DNA methyltransferase 1 translocates to mitochondria and that 5-hydroxymethylcytosine and 5-methylcytosine are present in mtDNA.

Finally, an mtDNA propagating the PD-state would have to possess a differential intra-cellular replication ability in order for its intra-cellular frequency to reach functionally significant levels. This is certainly possible, as evidenced by cells containing mutated mtDNA being often homoplasmic, which indicates a preferential accumulation of a specific mutated mtDNA variant [51,52].

As an alternative to the above suggested mitochondrial based transmission, propagation of the PD-state could result from a complex interplay between multiple inter-cellular signals and the cell's expression-state [53]. In biology there is traditionally an emphasis on identifying pathways and signaling cascades that permit explaining events in a linear, mechanistic fashion. But in reality, living organisms were fashioned by evolution rather than by intelligent design. Therefore mechanistic descriptions, although reassuring, may not always add much predictive value. With many biological processes, it may be better to aim for a phenomenological understanding that allows prediction and hopefully modulation of the relevant dynamics. The propagation dynamics of the PD-state could be one such case.

A SITE OF ORIGIN FOR THE PD-STATE

The previous section was concerned with the spread of the PD-state. Now, propagation implies the existence of an origin. We have mentioned that aging-associated damage caused by oxidative stress in mitochondria could be the trigger for a primordial cell, or few cells, to shift their gene expression program to the PD-state. A relevant question is where, if anywhere in particular, would a primordial PD-state cell most commonly arise. In this section, we suggest the hematopoietic stem cell niche as a site to consider.

First recall that gene expression [20,22], DNA methylation [24], neuron-platelet cybrid [41,40,12] and bioenergetic [29] analyses, all support the presence of the PD-state in circulating hematopoietic cells. Given the short lifespan of blood cells (days for platelets [54] and granulocytes [55] and weeks for lymphocytes, with the exception of memory cells [56]) vis-à-vis the decade long timescale for the transmission of PD across the neuronal system [34,35,36], 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 aging. Research in the topic relies heavily on work with mice. The evidence alluded to henceforth in this section is all based on mouse models, except where noted otherwise. In terms of global gene expression in hematopoietic stem cells (HSCs), nitric oxide mediated signal transduction, the NF-kB 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 ones [57]. Alterations at the HSC epigenetic level are supported by many chromosomal regions showing a coordinated change in transcriptional activity [57]. Fate-wise a skewing to the myeloid line and a diminished lymphoid potential are observed with aging [58].

It has been known for long that reactive oxygen species (ROS), if not properly checked, have the potential to cause indiscriminate cellular damage [27]. However, today ROS are also appreciated to play a functional signaling role in activating processes such as the inflammatory and stress responses [25,59]. There is additionally indication of a specific ROS role in regulating hematopoiesis [60]. In particular, evidence associates abnormal ROS levels at old age with a dysfunction in both the proliferation and the differentiation dynamics of HSCs. We briefly highlight some of this evidence. In vitro work has shown that exposure to H_2O_2 can lead to chromosomal

translocations in HSCs [61]. Ionizing radiation is also known to affect HSCs, namely it has been found to promote differentiation, short-term apoptosis and long-term senescence of HSCs [62]. 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 progenitors triggers their precocious differentiation [63]. Conversely, scavenging ROS from these hematopoietic progenitors retards their differentiation into mature blood cells [63]. 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 senescence [65]. Finally, the same protective effect was achieved by SIRT3 up-regulation in HSCs [67]. HSCs are highly-enriched in this mammalian sirtuin, except for its suppression at old age [67]. SIRT3 regulates the global acetylation landscape of mitochondrial proteins and reduces oxidative stress [67]. Mechanistically, the FoxO transcription factors [68] and the p53 [69], Akt [70], MAPK [66] and ATM [71] 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 PD [73]. DZIP1 is a component of the Hedgehog signaling pathway [74]. Besides its role in directing embryonic pattern formation, the hedgehog pathway has been implicated in the maintenance of adult stem cell niches, including both neuronal [75] and hematopoietic stem-cells [76]. 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 disease [77]. Gaucher's disease is characterized by low blood platelets levels, anemia, and the accumulation of the glycolipid glucocerebroside in the mononuclear phagocyte system [78].

In summary: i) a large body of evidence points to PD patients consistently having circulating hematopoietic cells in the PD-state; and ii) we have argued why their PD-state may be acquired at hematopoiesis rather than after maturation, from the disruption in ROS homeostasis that arises with age in the hematopoietic stem-cell niche.

CONCLUSION

Hypotheses, even if eventually proved incorrect, can elicit valuable directions for experimental research efforts. 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 late-onset sporadic Parkinson's disease. We now review this 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.

Aging-associated 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 relatively unlikely, mostly probabilistic event, its odds not greatly affected by genetics or lifelong environmental exposures. The observed low statistical 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 mtDNA, with its ability to move across cells and to modulate the cellular global gene expression state, could form the basis for the 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 aging-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 aging phenotype just as humans do [79]. As per our hypothesis, the aging-dependent PD initiation dynamics should occur in aged mice, just as well. The absence of a PD phenotype in mice would thus follow instead from the non-aging dependent mtDNA-based spread dynamics of PD. This propagation dynamics would be too slow relative to the lifetime of a mouse, for the condition to affect the neuronal system.

Two of the early symptoms of PD in humans are an impaired sense of smell [7] and gastrointestinal dysfunction [80]. Both have been reported as much as 10 years before the appearance of symptoms at the motor level. They are typically interpreted as supporting the role of an invading external agent - via the olfactory or gastro-intestinal entry points - in inducting PD [9]. 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 applicable to Alzheimer's disease.

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