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Review Article

Alpha-Synuclein aggregation in Parkinson's Disease

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Introduction

The ability of polypeptides to fold into a three-dimensional, functional structure forms the basis of normal cellular and organ function, however, when this fundamental process goes awry it forms the basis of neurodegenerative diseases. Nearly 1 in 6 people have a neurological condition and these diseases can be debilitating and incurable. Parkinson's Disease (PD) is the second most common neurodegenerative disease. PD is known as a movement disorder, but is also characterized by additional non-motor associated symptoms. The pathophysiological hallmark of PD is the misfolding and aggregation of the protein a-synuclein (a-Syn) and the accompanying loss of neurons that produce dopamine in the brain. There are currently no effective therapies for PD and management options consist mainly of symptom relief. Through the development of multi-disciplinary approaches (including nuclear magnetic resonance, high-resolution imaging and animal models of disease), scientists have made great strides in our understanding of the chemical, genetic and molecular basis of PD. Although it is commonly accepted that aggregation of a-Syn is key in the pathogenesis of PD, whether this aggregation plays a causal role in neurodegeneration is still a matter of intense investigation. This review will provide a critical assessment of the importance of a-Syn aggregation in PD and discuss the experimental approaches and current and future therapies for this neurodegenerative disease. Expanding our knowledge of the role of protein aggregation in the pathophysiology of PD is critical for the identification of biomarkers for early disease detection, as well as for the development of novel and effective therapeutic approaches.

Neurodegenerative diseases

Incorrectly folded (or misfolded) proteins escape the cell's surveillance mechanisms and have a tendency to aggregate and form amyloids; neurodegenerative diseases are thus referred to as 'conformational diseases'. According to the Medical Research Council, 1 in 6 people has a neurological condition [1]. Diseases such as Alzheimer's, Parkinson's, Lewy body dementia and Motor neuron disease, are debilitating and incurable and are characterised by progressive degeneration of neurons. Neurodegenerative diseases place a considerable burden on the NHS (estimated at 17 billion pounds a year) which becomes more problematic given the increased ageing population in the UK [1]. Although treatments improve symptoms associated with these diseases there are no cures or therapeutics that slow disease progression. There is therefore a critical, unmet need to increase our understanding of the molecular mechanisms that cause neurodegenerative diseases.

A hallmark feature of neurodegenerative diseases is the misfolding of proteins such as a-Syn, Tau, Amyloid-β and Prions [1]. Although functionally unrelated, these misfolded proteins adopt a structure that is conducive to their aggregation and polymerisation. These oligomers (several repeating monomers) eventually form insoluble amyloid fibrils that are associated with pathological processes in neurons, such as oxidative stress, disruption of calcium homeostasis and other toxic signalling pathways that ultimately promote neuronal damage and death [2].

Both genetic and environmental factors have been proposed to be responsible for the initiation of these diseases. Familial occurrence of some neurodegenerative diseases suggests a clear genetic basis. However, the vast majority of patients display sporadic disease, thus emphasising the contribution of environmental factors. Although evolution has ensured complex systems that perform quality control to correct protein folding (e.g. chaperones), even small changes in these control mechanisms may lead to protein misfolding.

Parkinson's Disease

PD is the second most prevanlent degenerative disorder which affects approximately 2% of people over 60 and over 4% of people over 80 [3]. PD is a progressive neurodegenerative disease characterised by two hallmark features: loss of dopamine-producing neurons in the brain and accumulation of insoluble a-Syn protein as Lewy bodies (LBs) and Lewy neurites (LNs) [4]. Dopamine is a neurotransmitter that is critical for the control of motor activities. LBs and LNs are protein-rich structures that are localised in the cell neurites and cell body of neurons and are the characteristic hallmarks of degenerating neurons of PD brains. In neurons of PD patients, endogenous a-Syn misfolds, oligomerises and aggregates to ultimately form LBs and LNs [5] (this process will be described in greater molecular detail in the section below).

PD patients display classic motor associated symptoms, including tremors, slow movements, postural instability and muscle rigidity. However, several non-motor associated symptoms (e.g. cognitive impairment, mood disorders, olfactory impairment) appear even earlier and have a detrimental effect on the quality of life of these patients [6]. Since motor symptoms appear when dopamine levels have already decreased by 60-80%, it is now increasingly appreciated that we need to study biomarkers and early disease mechanisms to slow down neurodegeneration.

Although the pathological mechanisms of PD are still poorly understood, increasing evidence suggests that disruption of dopaminergic systems is a culprit [7]. The death of dopaminergic neurons in the basal ganglia of the brain, a region responsible for coordinating movement via sending signals down the spinal cord, can lead to the physical symptoms of PD. Loss of dopamine-producing neurons occurs in parallel to the appearance of insoluble aggregates of the protein a-Syn in Lewy bodies. Indeed, several neurodegenerative diseases are characterised by the pathological aggregation of a-Syn. a-Syn is a protein that can adopt a number of different conformations and will be discussed in detail in the next section. The appearance of Lewy bodies in several regions in the brain has been linked to alterations in several neurotransmission pathways that may form the basis of the non-motor symptoms observed in PD patients. a-Syn aggregates can lead to neuronal cell toxicity and, ultimately, neuronal death, thus providing a link between Lewy body pathology and PD [7]. However, there are examples of PD patients (examined post mortem) that lack Lewy bodies but instead have mutations in other genes (e.g. *PARKIN* gene) [8].

a-Synuclein and its role in PD

a-Syn is a 14kDa, 140 amino acid-long protein (Figure 1) encoded by the *SNCA* gene and is expressed in the nervous system presynaptic terminals. The protein is composed of an N-terminal region which is responsible for binding to phospholipid membranes by adopting an alpha-helical conformation [5]. This amphipathic domain comprises two a-helical structures separated by a break; this region contains the mutations that are associated with familial

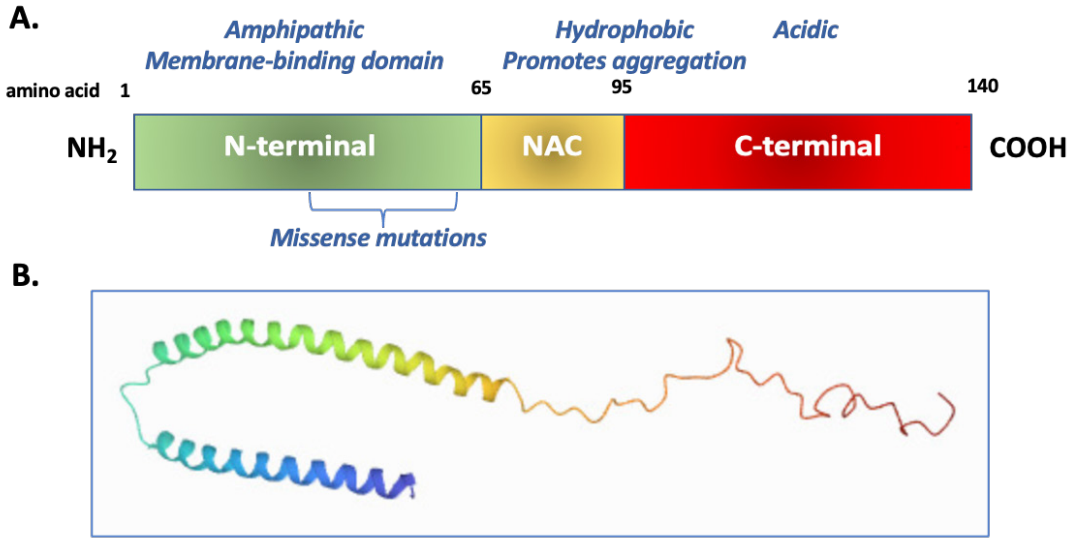

Figure 1. a-Syn structures. *A. Primary structure of a-Syn (140 amino acids long) showing the N-terminal region, the NAC region and the acidic C-terminal region. B. Structure of the human micelle-bound a-Syn, showing the N-terminal alpha helical structures and the rest of the protein's natively unfolded structure. Obtained from the Protein Data Bank (PBD).*

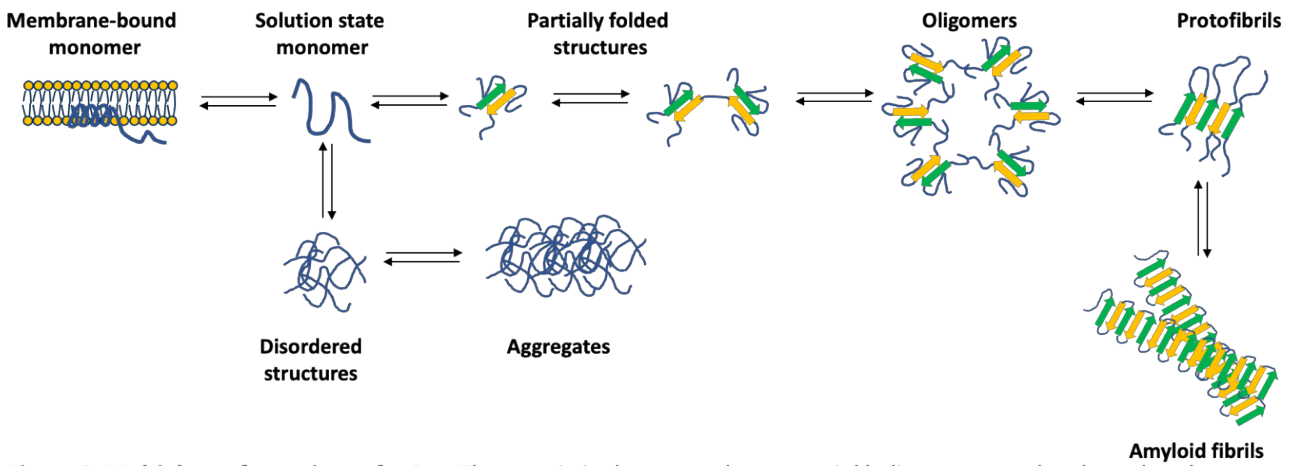

***Figure 2. Multiple conformations of a-Syn**. The protein is shown to adopt a partial helix structure when bound to the phospholipid membrane. It also shows the on-pathway and off-pathway oligomers can form which are rich in beta sheets. These oligomers can be arranged in a very specific way that they form protofibrils and eventually amyloid fibrils. Adapted from Mehra, et al 2019.*

PD (discussed in sections below). The central region, referred to as the NAC region, contains a stretch of 12 amino acids that are crucial for filament assembly. This central region is also referred to as the hydrophobic region and is the domain that promotes aggregation. The C-terminal region is highly acidic and has no secondary structure (Figure 1). The exact function of a-Syn is still unknown, but it has been implicated in neurotransmitter release and plasticity of neurons [5,9].

Aggregated a-Syn has been shown to play a major role in PD, as this protein aggregates and forms toxic oligomers and fibrils [10]. Genetics studies have shown that duplication or triplication of the number of copies of the *SNCA* gene is a risk factor for developing PD. Furthermore, single point mutations (i.e. single amino acid substitutions) can cause autosomal dominant forms of PD [9]. a-Syn is a natively unstructured protein, also referred to as Intrinsically Disordered Protein (IDP). A characteristic feature of IDPs is that, under physiological conditions, these proteins fail to form well-defined three-dimensional structures [11]. Instead, IDPs adopt a range of dynamic structures that can change depending on the environmental conditions (e.g. solvent exposure, pH changes, thermal fluctuations) [12]. When bound to a phospholipid membrane, the a-Syn monomer adopts a partial a-helix structure, while during aggregation it adopts a β-sheet conformation which is highly organised and polymerised into amyloid fibrils (Figure 2) [13]. The fascinating aspect of this protein is that the secondary structure can change entirely without having to change the arrangement of amino acids. The factors that affect this remain unknown and the mechanism by which a-Syn can change into its β-sheet structure is being studied using a variety of methods, including high resolution nuclear magnetic resonance (NMR), Cryo-electron microscopy (EM) and Modelling Dynamic Simulations (which will be discussed below).

There is now growing evidence to support the "prion-like" behaviour of a-Syn; in this model, misfolded a-Syn can transfer between neurons and induce further aggregation of a-Syn [14]. Prions are defined as infectious protein particles which can also adopt different conformations that self-aggregate and are ultimately 'infectious proteins. They can develop 'nuclei' or 'seeds' which are smaller aggregates that multiply to make large amyloids. These types of proteins are seen as a major cause of neurodegeneration as they lead to the rapid progression of diseases such as bovine spongiform. a-Syn has been shown to have the same prion-like propagation of amyloid structures which ultimately result in the formation of toxic species that eventually cause cell death [15]. This is because their natively unfolded state is what allows the -rich conformations and oligomers to form and eventually grow and form amyloids.

There are two proposed mechanisms by which abnormal protein structures may cause disease: 1) loss of homeostatic functions/ mechanisms; 2) acquisition of a toxic function. Under normal conditions, neurons have elaborate quality control mechanisms that involve proteins (chaperones) that aid in correct folding. In addition, cells are capable of degrading misfolded proteins (including a-Syn) via chaperone-mediated autophagy (a process by which cellular material is broken down in the lysosome). Even the smallest changes in the cell's control and protective mechanisms can be detrimental and disturbances in the cell's degradation pathways may lead to the formation and propagation of misfolded a-Syn [14,16]. Furthermore, misfolded proteins may adopt new functions that cause inappropriate cellular changes that ultimately lead to aberrant cellular function. For a-Syn, the mechanism by which it causes neurodegeneration is still unknown, but it has been shown that the oligomers are the toxic species instead of the fibrils themselves.

Role of heat shock proteins

Heat shock proteins are proteins that play a crucial role in protein folding. As polypeptides emerge out of the ribosome during the process of protein synthesis, chaperones aid in their folding into

functional protein structures based on their primary amino acid sequence [10]. As mentioned earlier, although protein misfolding is a common event, the production of harmful proteins is prevented by the cell's quality control mechanisms that target these abnormal or misfolded proteins to the lysosome or proteasome for degradation. However, when quality control mechanisms are compromised, the misfolded proteins may oligomerise and become pathogenic [5]. One of the best-studied members of the heat shock family is Hsp70. A binding site at the C-terminus of Hsp70 recognises denatured proteins via their exposed hydrophobic regions while the N-terminal ATPase domain of Hsp70 hydrolyses ATP to ADP which causes a conformational change at the C-terminus, thus stabilising the denatured protein and allowing it to be refolded. The unique ability of heat shock proteins to either refold denatured proteins or target them for degradation has stimulated interest in their targeting for regulating a-Syn misfolding and associated toxicity [5]. For instance, increasing expression levels of Hsp70 might be a favourable approach to overcome misfolded a-Syn and thus may be beneficial in the context of PD. Indeed, Hsp70 gene therapy using adeno-associated virus in a mouse model of PD was found to be protective against neuronal and dopamine loss [5]. Current efforts are focused on pharmacological approaches to achieve increased expression of Hsp70 as well as on the improvement of gene delivery methods.

Experimental approaches to study PD

There are several research laboratories that are working to increase our understanding of the chemical, genetic and molecular factors that underly the pathophysiology of PD. These investigations not only enhance our understanding of this disease but also provide new avenues for early diagnosis and development of novel therapeutics. The section below will outline some of the experimental approaches that have been instrumental in our understanding of PD; these approaches range from the study of the molecular structures of isolated proteins *in vitro* to the development of animal models that mimic human disease.

Nuclear Magnetic Resonance (NMR)

Although a-Syn is difficult to study as a monomeric protein as it is unfolded, NMR can be used to study the various conformational states of a-Syn. Small aggregates of a-Syn can be studied by solution NMR and the data can provide useful information about the intermolecular and dynamic properties of the protein. An advantage of NMR is that, unlike X-ray crystallography, crystals are not required to study a-Syn fibrils (it can be difficult to crystallise proteins that aggregate in aqueous solutions). However, NMR spectroscopy is a less sensitive method for structure determination and requires high concentrations of protein to obtain a sufficient NMR signal. Another challenge is that larger aggregates of a-Syn have too large a molecular weight for solution NMR studies but are more amenable to structural characterisation using solid-state NMR and cryo-EM (to be discussed below).

Despite the challenges, researchers have used NMR successfully to study membrane-bound a-Syn; several laboratories have used various models to mimic phospholipid membranes, including SDS micelles, artificial lipid vesicles and nanodiscs [17]. Trifluoroethanol can also be used in NMR spectroscopy as it provides a different environment for the polypeptide with its secondary structure. These types of alcohols are useful in gaining a better understanding of the dynamics of protein folding. Changing the temperature or lowering the pH of the solutions also changes the conformation of proteins and is used to study thermodynamic changes. The environment that is produced *in vitro* is often similar to the environment that the protein is exposed to inside the cell. This is useful in exploring possible therapeutic strategies and also understanding the reasons why such proteins adopt new conformations.

Researchers have used quenched hydrogen/deuterium (H/D) exchange measured by solution-state NMR to identify secondary structural elements in a-Syn [18]. This technique allows identification of amide protons that are solvent-protected and are thus indicative of hydrogen bond formation. It has been shown that the a-Syn fibrils have a solvent-protected core (NAC region) which may potentially have a role in the secondary structure of the protein. More recent work has used NMR to examine the effect of a-Syn on synthetic vesicles that model the synaptic vesicles in the brain. Neurotransmitters (such as dopamine) carried inside synaptic vesicles are being passed across synapses (the junctions between neuronal cells). These synaptic vesicles move to the surface of the synapse and fuse with the membrane while releasing the neurotransmitter in a matter of milliseconds. Using NMR, researchers have shown that a-Syn plays a critical role in controlling the synaptic vesicles during the fusing process as a-Syn can attach itself to vesicles and hold some of them while releasing others in what has been described as a 'marshalling' function [19].

Size Exclusion Chromatography (SEC)

SEC is a widely used and most powerful chromatography technique to obtain information about the size of macromolecules and proteins under different conditions. SEC allows size profiling of protein samples using a resin column that is packed with spherical beads containing pores of a specific size; the pore size of these beads is used to estimate the size of proteins. Small molecules/proteins enter the pores and their flow through the column is slowed down compared to large molecules/proteins that do not enter the pores and are thus eluted faster. Therefore, proteins are separated based on their size as they pass through the column.

Modelling Dynamic Simulations can be used to predict how the protein will fold from a-helices to β-sheets by using our knowledge of the most energetically favourable pathways. This theoretical data can then be compared with experimental data from NMR, Cryo-EM etc. When specifically studying amyloid fibrils, Paramagnetic Relaxation Enhancement (PRE) is used frequently. This is when an unpaired electron is added to the protein and the magnetic dipolar interactions are measured which describes how the protons in the polypeptide adapt to their environment. Overall, most of the techniques mentioned are used in combination with each other

to gain as much information about the protein and its dynamic properties.

Cryo-EM

Technological advances in recent years have allowed a revolution in the field of cryo-EM and the determination of the structure of protein fibrils at near-atomic resolution. While techniques such as NMR, X-ray crystallography and transmission electron microscopy have been used to study fibril structure, cryo-EM can address several disadvantages of these techniques (e.g. requirement for large amounts of protein or drying/dehydrating proteins) [20]. Researchers have used cryo-EM to capture images of a-Syn fibrils that readily aggregate. Processing these high-resolution images with computer software revealed a 3D model of the a-Syn fibril structure in which proteins cluster to form a helix. Each turn of the helix is formed by two a-Syn molecules that are facing each other but are rotated by approximately 180 degrees from each other [21]. Having this 3D structure is particularly useful in the design of molecules that would make the fibrils detectable via medical imaging and thus allow doctors to diagnose PD at an earlier stage. Ongoing research is focussed on possible differences in fibril structure between patients, how fibrils interact with each other and how they damage neuronal cells.

Animal models of PD

Several animal models of PD have been generated in the last few years and have been extremely useful in modelling this disease in mice. These models fall into two categories: a) inoculation of pre-formed fibrils of a-Syn and b) overexpression of a-Syn by adeno-associated viral vectors.

Inoculation of a-Syn pre-formed fibrils

One approach to model PD in animals involves the injection of either a-Syn pre-formed fibrils or Lewy body-containing brain extracts from patients. Preformed fibrils are formed in the test tube *in vitro* from recombinant a-Syn monomers which have been induced to aggregate into fibrils. The fibrils have been successfully injected into mice, rats and non-human primates. Similarly, brain extracts from patients have been injected into mice, rats and non-human primates. These approaches have been successful in producing aggregation of a-Syn in neurons, progressive neuronal degeneration and an accompanying reduction in dopamine levels that is characteristic of PD. Importantly, a-Syn fibrils can spread from the injection site (ipsilateral) to other regions (contralateral) of the brain within 12 weeks, as shown in [9].

Adeno-associated virus vectors model

An additional model involves the use of AAV vectors to deliver the gene of interest (a-Syn) in the brain. The advantage of AAV is that it transduces efficiently and allows expression of the gene for long periods of time. Another important feature of AAV is that can transduce non-dividing cells, which is particularly critical since neurons are post-mitotic cells and thus do not divide. Finally, AAV is small and this allows the injection of many AAV viral particles in the same volume thus providing a greater chance of transducing the tissue of interest. Overexpression of a-Syn and its mutant forms using AAV mimics the human pathology of PD, with a progressive loss in dopaminergic neurons and reduction in dopamine levels.

In summary, animal models of PD can reproduce the human disease up to a certain extent. They are useful for studying the prion-like behaviour of a-Syn and the mechanisms of its propagation in the brain. Most importantly, animal models are valuable tools for discovering biomarkers of PD as well as testing novel therapeutics.

Therapeutic efforts for PD

Therapeutic strategies for protein aggregation/misfolding disorders include inhibition of proteins prone to misfolding, targeting the aggregation of misfolded proteins, removal of aggregates and reducing the toxic effects of misfolded proteins. Several of these strategies have yielded promising candidates that have been successfully moved from pre-clinical into human clinical testing [22]. For PD, strategies for reducing a-Syn and its propagation include: reducing a-Syn synthesis using single interference RNAs (siRNAs), modulating the levels of heat shock and chaperone proteins, and inhibiting the fibrillation and oligomerisation of a-Syn.

Approved Therapies

PD is a progressive neurodegenerative disease that leads to both loss of motor control and non-motor symptoms, including cognitive impairment, speech and swallowing problems and mood disorders. Symptoms of PD are primarily caused by reduced levels of the brain chemical dopamine.

To date, no potential drugs have been identified for the treatment of PD and no treatment has been identified that slows down disease progression. Given that PD is associated with loss of dopamine in the brain, treatment options have focussed on providing additional dopamine to patients. This is accomplished by using the natural chemical levodopa (which is converted to dopamine in the brain). Dopamine agonists can also be used, as they mimic dopamine. Since its discovery, L-Dopa has proven to be the most efficacious therapy for symptomatic treatment of PD, even after 30 years of extensive research into drugs for PD. L-Dopa is highly potent during the first few years of treatment so much so that the term "L-Dopa honeymoon" has been coined. However, long-term treatment of PD patients with L-Dopa has limitations and the drug stops being as effective. In addition, there are several limitations of L-Dopa treatment, including limited improvement of motor dysfunction; limited effects on disease progression; and association with various side-effects (e.g. toxicity, nausea, loss of appetite, hypotension) [23].

In addition to supplying extra dopamine, PD treatments have also focussed on inhibiting pathways that eliminate dopamine in the body, such as inhibitors of monoamine oxidase- and catechol-O-methyltransferase [24,25]. There are also treatments for the non-motor symptoms associated with PD; these include drugs

to treat hypotension and psychosis in PD patients. Finally, some patients may be suitable for surgical treatments, such as deep brain stimulation, which can treat tremors, stiffness and dyskinesia. All of the treatments described above are complemented by supportive therapies (such as speech therapy, physiotherapy and occupational therapy) to alleviate and manage symptoms of PD.

An important consideration for therapeutics for PD is the difficulty associated with crossing the blood-brain barrier. The blood-brain barrier prevents access of most administered drugs to the central nervous system and the identification of structurally compatible analogues of drugs is an intense area of investigation.

Experimental Therapies and Future Directions for PD research

Scientific breakthroughs in our understanding of the aetiology and disease pathogenesis open up new avenues for therapeutic intervention and research into this debilitating disease is ongoing. Non-profit foundations and charities, such as Parkinson's UK, the Michael J Fox Foundation and The Parkinson's Foundation, have dedicated funding towards research into therapies for PD. A number of investigational treatments and drugs are currently being tested in clinical trials [26].

Drugs that target alpha-synuclein

As mentioned earlier, intracellular accumulation of insoluble a-Syn protein as Lewy bodies is the major pathological hallmark of PD. As such, a-Syn has become the focus of therapeutic developments in PD and several therapeutics are under investigation:

a. Reduction of extracellular a-Syn via immunisation: A number of immunotherapies for Parkinson's are under clinical development, most are based on passive immunisation (i.e. administration of antibodies) while a couple are based on active immunisation (i.e. administration of peptides to mimic epitopes on a-Syn). Most of these studies have completed Phase 1 trials and are recruiting patients for Phase 2. Many questions require answers in these studies: will immunogenicity promote clearance of a-Syn and will a decrease in circulating a-Syn translate into less disease? What about impacts on motor- and non-motor symptoms?

b) blocking a-Syn aggregation: A small-molecule inhibitor of a-Syn dimerisation, which blocks its propagation to oligomers and fibrils, has been developed (NPT200-11) and a Phase 1 trial has shown that it is well-tolerated. A Phase 1b trial is underway.

Embryonic stem cell-based therapies

Since loss of dopaminergic neurons is a critical feature of PD, a number of approaches to provide a source of dopamine-producing cells are currently under investigation. Clinical trials using stem cell-derived dopaminergic neuron progenitor cells are underway [27]. Embryonic stem cells are isolated from surplus human embryos derived during *in vitro* fertilisation and, using specialised protocols in the laboratory, it is now possible to reprogramme these cells to become dopaminergic neuronal progenitors. Apart from the ethical considerations, there are specific drawbacks associated with this approach, as it would require immunosuppression because these stem cells would originate from a different host. In contrast, induced pluripotent stem cells (iPSCs) is a very promising new approach that involves reprogramming of somatic cells (differentiated cells) into pluripotent cells (i.e. cells that can differentiate into any lineage) that then be differentiated into dopaminergic neurons. This approach has been used successfully in non-human primates [27]. An advantage of this approach that iPSCs are derived from the patient and therefore there is no need for immunosuppression, however, the same genetic susceptibility factors that caused PD in the first place will also be present in the iPSCs. A new Phase 1 clinical trial received clearance earlier this year (2021); this is the first trial in the USA that investigates pluripotent stem cell-derived dopaminergic neurons in patients with advanced PD. The primary objective of the trial is to replace the dopaminergic neurons that are lost due to PD and thus allow the neural circuit to rebuild and restore motor control of patients.

Other therapies

Repurposing of other drugs is a desirable approach for the treatment of any disease since these drugs are already approved by the appropriate regulatory bodies. Produced initially as a cancer therapy, nilotinib was identified by scientists at Georgetown University as a potential therapy for PD. The mechanism of action of nilotinib is via promoting autophagic degradation of a-Syn; this approach has been shown to be neuroprotective and beneficial in animal models of Parkinson's. A small Phase 1 trial was completed and a Phase 2 trial completed last year showed that nilotinib appeared to be relatively safe and supports the development of a Phase 3 study to investigate the effects of this drug in patients [28]. Additional drugs under development include CVT-301 (an inhaled formulation of levodopa and aims to provide quick relief of symptoms in between doses) and MSDC-0160 (an insulin sensitiser that is being developed to treat dyskinesia caused by levodopa).

The protein aggregation theory on trial

It is an undisputed fact that degenerative diseases are intimately connected with the aggregation of proteins into pathological fibrillar structures. For PD, in particular, aggregation of a-Syn is considered a gold standard for definitive diagnosis of the disease [29]. Despite this intimate connection between protein aggregation and pathology, the development of effective therapies to prevent neurodegenerative diseases has proved to be very challenging. This has led to a rethink of the protein aggregation theory and the questioning of the causal role of protein aggregation in disease pathogenesis.

Critics of the protein aggregation theory argue that a-Syn aggregation is not a causal pathway in PD, but simply an epiphenomenon. This view argues that protein aggregates are by-products of several pathological pathways that converge onto aggregation of a-Syn and that aggregation *per se* is therefore not causative for disease [29]. Another prevailing view along a similar line of thought is that protein aggregation serves as a protective mechanism against toxic

insoluble forms of proteins by sequestering them into insoluble structures in Lewy Bodies [29]. Sequestration of proteins could allow neurons to function normally for years, thus delaying the onset of symptoms. According to this model, targeting protein aggregates would have detrimental effects on neuronal function, as it would remove the protective mechanisms associated with protein aggregation.

In contrast, supporters of the causal role of protein aggregation argue that the presence of plaques is a universal characteristic of postmortem Parkinson's patients. As mentioned above, the aggregation of a-Syn is thought to cause a cascade of pathological events, including inflammation, neuronal dysfunction and cell death. These observations strongly implicate a-Syn as the culprit in PD [30]. Further support of this hypothesis comes from both human and animal models of PD. Mutations in the *SNCA* gene cause familial forms of Parkinson's, while animal models of PD based on a-Syn have also been generated, thus providing further support for the role of the aggregation of this protein in pathology [9].

Conclusions

Compelling evidence from biochemical, cellular and animal studies, coupled with postmortem histological examination, has shown that aggregation of a-Syn in the brain constitutes the main event that triggers PD. As discussed already, a-Syn undergoes misfolding from its native state to form oligomers and large fibrillar aggregates during disease; these aggregates display prion-like behaviour and can propagate into larger amyloids in the brain. The most convincing evidence for the causative role of a-Syn aggregation in PD comes from animal studies in which inoculation with brain homogenates from diseased patients or transgenic mouse models that exhibit a-Syn aggregates results in the development of PD. Overall, these observations provide overwhelming evidence for a causative role for a-Syn aggregation in PD.

There are still many unanswered questions regarding the role of protein aggregation in Parkinson's and other neurodegenerative diseases. What is clear though is that there is a pressing need for increasing our understanding of the molecular nature of protein aggregation in neurodegenerative diseases. Recent advances in imaging and resolution of protein structure offer great promise.

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