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Review Article

Cellular models as tools for the study of the role of alpha-synuclein in Parkinson's disease

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ABSTRACT

Neurodegenerative diseases are highly debilitating conditions characterised primarily by progressive neuronal loss and impairment of the nervous system. Parkinson's disease (PD) is one of the most common of these disorders, affecting 1–2% of the population above the age of 65. Although the underlying mechanisms of PD have been extensively studied, we still lack a full understanding of the molecular underpinnings of the disease. Thus, the *in vitro* and *in vivo* models currently used are able to only partially recapitulate the typical phenotypes of the disease. Here, we review various cell culture models currently used to study the molecular basis of PD, with a focus on alpha-synuclein-associated molecular pathologies. We also discuss how different cell models may constitute powerful tools for high-throughput screening of molecules capable of modulating alpha-synuclein toxicity.

Contents

1. Introduction

1.1. Parkinson's disease

PD is the second most common age-related neurodegenerative disorder affecting 1–2% of individuals above the age of 65 (de Rijk et al., 2000; Van Den Eeden et al., 2003). The progressive loss of nigrostriatal dopaminergic neurons, resulting in a series of motor symptoms such as bradykinesia, resting tremor, rigidity and postural instability, is characteristic of the disease. PD is also accompanied by a wide range of non-motor symptoms like sleep disturbances, constipation, dementia, cognitive decline or olfactory deficits that further impact on the quality of life of patients (Lees et al., 2009; Obeso et al., 2010; Savica et al., 2010).

The typical pathological hallmark of PD is the accumulation of protein inclusions known as Lewy bodies (LBs) and Lewy neurites inside the surviving neurons (Spillantini et al., 1997). LBs are mainly composed of alpha-synuclein (aSyn), a protein that exists not only in the brain but

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also in the peripheral nervous system, in tissues such as the skin (Rodriguez-Leyva et al., 2014), the heart (Iwanaga et al., 1999), and in the gastrointestinal tract (Lebouvier et al., 2008; Lebouvier et al., 2010; Shannon et al., 2012).

Intracellular aSyn inclusions represent the hallmark of a group of progressive neurodegenerative diseases collectively as synucleinopathies. These include not only PD, but also Multiple System Atrophy (MSA) and Dementia with Lewy Bodies (DLB) (McCann et al., 2014). For the purpose of this review, we will focus on PD and the cell models that have been develop throughout the years.

PD, as other complex disorders, is thought to derive from the interplay between environmental and genetic factors. About 10% of all PD cases present classical Mendelian inheritance, with known alterations in various genes (Verstraeten et al., 2015). These include dominant mutations in the genes encoding for the Leucine-rich repeat kinase 2 (LRRK2) or aSyn, as well as recessive mutations in Parkin (*PARK2*), PTEN-induced putative kinase (*PINK1*) (Bonifati, 2014; Hernandez et al., 2016; Trinh and Farrer, 2013), or DJ-1 (*PARK7*) (Bonifati et al., 2003; van Duijn et al., 2001). However, variations within additional genes such as the glucocerebrosidase gene (*GBA*) are associated with familial PD susceptibility (Sidransky et al., 2009). Genome-wide association studies (GWAS) have uncovered a few new genes associated with late-onset PD, and the list is likely to continue to expand as new studies are conducted.

Despite tremendous advances in the field of PD genetics, the exact mechanisms underlying neuronal degeneration remain unclear, limiting current therapeutic strategies to the attenuation of the motor symptom. The use of cellular models provides, despite inherent limitations, unique opportunities for assessing molecular pathologies and for the identification of therapeutic strategies capable of modulating those pathologies.

1.2. Alpha-synuclein

aSyn is a negatively charged protein that belongs to a family with three members: alpha-, beta-, and gama-Syn (Lavedan, 1998; Maroteaux et al., 1988). aSyn comprises 140 amino acids (aa), and is routinely divided in three domains: an N-terminal domain (1–65 aa), a non-amyloid-β component of plaques (NAC) domain (66–95 aa), and a C-terminal acidic domain (96–140 aa) (Amer et al., 2006; Eliezer et al., 2001; Serpell et al., 2000). aSyn is highly abundant in neuronal cells, representing ~1% of the total protein (Mizuno et al., 2012). It is mainly present in presynaptic terminals, and associated with synaptic vesicles (Larsen et al., 2006; Maroteaux et al., 1988; Nemani et al., 2010). Thus, the function of aSyn is thought to be related with the trafficking of synaptic vesicles, and the regulation of vesicle exocytosis (Diao et al., 2013). Additionally, aSyn is capable of promoting the assembly of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-complex, which is associated with neurotransmitter release (Burre et al., 2010).

aSyn was reported to exist in equilibrium between different conformational states and, possibly, in a tetrameric form (Bartels et al., 2011; Wang et al., 2011). Nevertheless, it still not clear how aSyn converts to amyloid aggregates, and whether these are a cause or consequence of disease processes in PD. One of the current hypotheses is that prefibrillar oligomeric species may represent the toxic forms of aSyn, but this is still unclear (Conway et al., 2000a, 2000b; Diogenes et al., 2012; Outeiro et al., 2008; Villar-Pique et al., 2016), aSyn oligomers may also lead to synaptic dysfunction by interfering with the axonal transport proteins like synapsin-1 (Scott et al., 2010).

Recent studies have shown that aSyn can spread from diseased to healthy cells, possibly in a prion-like manner (Recasens et al., 2014). This idea comes from the Braak staging hypothesis (Braak et al., 2003) and from the examination of post-mortem brains from patients who had received transplants of embryonic neural tissue in the striatum to replace the degenerated dopaminergic neurons. In these studies it was observed that the transplanted young embryonic tissue contained pathological aSyn inclusions (Kordower et al., 2008; Kurowska et al., 2011; Li et al., 2008, 2010), suggesting a trans-synaptic spreading of aSyn pathology (Braak et al., 2003; Del Tredici and Braak, 2016). In cell culture and in animal models, exogenous preformed aSyn fibrils (PFFs) can act as seeds and recruit aSyn to aggregate and transmit from cell-to-cell, similar to prion protein propagation (Luk et al., 2009, 2012; Volpicelli-Daley et al., 2011).

The occurrence of aSyn strains has been described, and is currently a hot topic of investigation, as it is thought to explain the basis of different pathologies (Bousset et al., 2013; Guo et al., 2013; Peelaerts et al., 2015). Thus, it is also possible that the fibrils themselves, rather than the process of fibril formation, may be key players in the process of aSyn toxicity.

Due to our limited understanding of the mechanisms associated with aSyn-mediated cytotoxicity, it is essential to explore cell-based models that mimic important aspects of aSyn biology, aggregation, and toxicity. This knowledge will be instrumental for the development of future strategies for therapeutic intervention and, possibly, for early detection of synucleinopathies.

1.2.1. Cellular models for studying PD

The molecular intricacies of PD have been difficult to address, as stated above. This is due to the fact that none of the currently existing models is able to fully recapitulate all disease features. In multifactorial disorders such as PD it, is particularly important to dissect the complex pathological processes into simpler molecular events. The purpose of any cellular model is to simplify experimental variables present in the whole organism, and to enable more precise manipulation of specific genes and environmental factors while, at the same time, avoiding complex ethical issues associated with the use of mammalian models or studies in human subjects.

Specific advantages can be pinpointed in the various culture systems already available (Table 1). First, it is possible to generate homogenous cell cultures that can be expanded, virtually indefinitely, to generate the numbers of cells required for any experiment, such as the assessment of aSyn aggregation, to assess the metabolic stage of cells, or to investigate biochemical pathways such as mitophagy, oxidative stress, apoptosis and others. The possibility of manipulating cells in order to direct them to distinct fates enables, for example, the production of cells with dopaminergic phenotype in a synchronized manner. Nevertheless, it is important to keep in mind that cell-based assays have limitations, and are obviously not able to recapitulate *in vivo* physiology (Astashkina et al., 2012) (Table 1). A critical point that should be taken into consideration when working with post-mitotic cells is that they do not "possess" the age of the subject they were derived from. For example, it has been difficult to identify pathological differences in patient-derived iPSCs considering their naïve neuronal stage. In an attempt to model the late-onset characteristics in these cells, iPCS-derived neurons are usually extensively differentiated (up to 75 days) (Sanchez-Danes et al., 2012), in order to mimic aging (Miller et al., 2013).

Cell models can be viewed as "living test tubes" in which to study the contribution of specific cellular processes towards disease. This minimalistic approach facilitates the interpretation of the effect of single events/pathways, but fails to recapitulate non-cell autonomous features that are essential for disease onset and progression (Alberio et al., 2012). Obviously, the crosstalk between different cell types, and with the vasculature, is lost when individual cell types are cultured *in vitro*. Although afferent and efferent connections are made between differentiated neurons, this only occurs in two-dimensional (2D) culture. In addition, the intact brain tissue is substituted by a culture medium lacking several factors that might be required to trigger the progression of PD pathology. The development of microfluidic and brain-on-a-chip devices, enabling the culture of cells in three dimensional (3D) surroundings, holds promise in order to more accurately mimic the *in vivo*