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Pc12 Cell Line as a In-Vitro Model For Parkinsons Disease

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ABSTRACT: Parkinson's disease (PD) is a common serious neurological disease that affects millions of people worldwide, there is only symptomatic treatment is availableto develop a effective disease-modifying medicine, we must improve our current understanding of the molecular and cellular mechanisms underlying PD development and progression. For the Standardization of research techniques and disease, cell line-based models are essential. The current PD research is mostly carried out with permanently established cell models that are in the PC12 line. However, there is no consensus on many essential features of its application, such as the impacts of different culture media compositions and differentiation techniques. We give a survey of scientific papers that have employed PC12 cells to investigate Parkinson's disease. This study focuses on cell source, culture conditions, differentiation protocols, methods/approaches used to imitate PD, and preclinical validation of PC12 findings using alternative cellular models.As a result, this paper may be beneficial in standardising the use of the PC12 cell line in PD research in the future, as well as serving as a user's guide.

KEYWORDS: Cellular differentiation, Cellular model, Dopaminergic neuron, Neuroblastoma, Cell culture conditions, Neural growth factor.

I. INTRODUCTION

Parkinson's disease (PD) is a neurological condition characterized by tremor, muscle rigidity, balance problems, and bradykinesia. Selective loss of dopaminergic neurons and build-ups of α-synuclein and other proteins are two pathological markers of Parkinson's disease. PD has a significant impact on the ability of middle-aged and older people to care for them and decreases the quality of their lives. The pathogenesis of PD is multifactorial. The proteins that are accumulated causes degeneration of dopaminergic neurons, especially in the substantia nigra (SN) area, which reduces the concentration of dopamine and weakens the dopaminergic transmission, leading to the motor symptoms characteristic of PD. In addition to the SN area, in the basal ganglia, hypothalamus, and olfactory bulb, loss of neurons is observed. It subsequently triggers the transmission disorders in other systems, namely the glutaminergic, cholinergic, adenosine, gabanergic, noradrenergic, serotonergic, and histaminergic systems. (1)

Due to oxidative stress, neuron degeneration occurs, caused by disturbances in calcium regulation and dysfunction of mitochondria, lysosomes, and proteasomes. Oxidative stress is one of the major factors which leads to mitochondrial dysfunction which causes neuronal death in PD.(2) During oxidative stress, ROS induces the release of cytochrome c from the mitochondria and the consequent activation of the caspase family, which plays a role in cell apoptosis. Once caspase-3 is activated, it induces nuclear DNA condensation and fragmentation and ultimately, apoptosis. Therefore, drugs that can prevent ROS-induced cell injury might be an appropriate choice for the treatment of PD.(3) The ideal PD model should have a high degree of construct validity (e.g. Underlying oxidative stress, inflammation, complex I inhibition, or proteasomes inhibition), face validity that is, similar symptoms (e.g. Akinesia, rigidity), and biochemistry (e.g. Reduced striatal dopamine and changed downstream neurochemistry, nigrostriatal tract degeneration and Lewy body deposition) and predictive validity — the ability to positively identify therapeutically useful medicines.Different PD models available are MPTP treated mouse model,6 OHDA treated rat model, pesticide-induced animal models, different cell lines like SHSY5Y cell line, PC12 cell line.

Main applications of cell cultures in scientific studies include; before in vivo testing using cell cultures, pharmacological studies of newly made medicines and found natural substances are screened for toxicity. Investigating

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how medications affect tissues, cells, and their organelles, Discovering disease-causing pathways and pathophysiological mechanisms and development, Manufacturing of some proteins or biological components that have not yet been discovered in nature, Virology and microbiology (mainly multiplication of intracellular pathogens).(4)

Continuously replicating cell lines with differentiated features has long been recognized as an effective model system for research. Such models are very appropriate for chromaffin cells and neurons, which are similar cell types. The development of neuronal cell lines is very difficult. As mature neurons do not divide, a neural model that is most useful should be able to change between a condition in which it can replicate and a state in which it is nondividing and neuronally differentiated. Furthermore, neurons exhibit a variety of specialized and distinguishing characteristics, such as electrical excitability and neurite development, as well as several discrete phenotypic classes. Therefore, an ideal model neuronal line should have the distinct characteristics of a distinct subclass within the total neuronal phenotype. In the same way, an ideal chromaffin cell model should not only capture the overall characteristic behaviour of chromaffin cells, but also the phenotypic heterogeneity that exists among them. The key point in selecting the appropriate cell model is to consider the aim of the experiment and the limitations of the model systems. The choice of cell line also depends on the aspect of the disease and the type of therapy we want to develop. Cell cultures are a very good model for research because they develop pathology faster, pharmacological and genetic manipulations are relatively easy and reliable. (5)

Advantages of using cell cultures in scientific research are:Cells are easy to maintain and provide information quickly than in vivo experiments, Cells can be easily genetically modified and allow multiple replicates of an experiment, Cellular cross-talk is enabled via co-cultures, which have a 3D structure and a culture environment that can be modified.

There are also disadvantages of using cell culture: The human organism is difficult to link to the obtained data, adequate handling and preparation to become a viable cell model, features, and phenotype. Cell research is crucial for evaluating the outcome.

It's important to remember that PC12 cells, Models, like all others, have limitations. Any findings made with this tumour cell line are not guaranteed to be replicated in vivo models or in human disease. As a result, any theories derived from PC12 cell research should be tested in primary neuronal cell cultures, in vivo models, and, if possible, in patients with Parkinson's disease.

The PC 12 line of rat pheochromocytoma cells is one system that meets several of the above requirements. This line is being employed in a growing number of laboratories since it has the potential to be incredibly useful for studying both chromaffin cells and neurons. PC12 cells are popular due to their exceptional adaptability in terms of pharmacological manipulation, their simplicity of culture, and a wealth of understanding about their proliferation and differentiation. Furthermore, they resemble neurons more closely than chromaffin cells, with smaller vesicles and quantal sizes.

The major purpose of the present article is to review the currently known properties of the PC12 line and to discuss how it has been and could be exploited to increase our knowledge of neurosecretory and neuronal cells. Practical suggestions for the growth and experimental use of PC 12 cells will also be included. This review is meant both to acquaint new workers with the PC 12 system and to provide an overview for those already familiar with it. Also, it will discuss the advantages and disadvantages of using PC12 cell lines that will be needed in the future and their characteristics, which will lead to the development of a new generation of molecules that will treat both the symptoms and the disease process.

II. PC12 CELL LINE

PC12 iscell line derived from a pheochromocytoma of the rat adrenalmedulla. It has embryonic origin from the neuralcrest that has a mixture of eosinophiliccellsandneuroblasticcells. They are kept in the growth medium, multiply, and, like chromaffin cells, produce, store, and release catecholamines in response to stimulation. Dopamine is the most abundant monoamine in PC12 cells, and the cells also have dopamine beta-hydroxylase. Catecholamines are stored in chromaffin granules and synaptic vesicles, and the cells have active transporters for catecholamine uptake across the plasma membrane and into granules/vesicles. The ability of the PC12 cell line to respond to nerve growth factor (NGF) by terminating the cell cycle and undergoing neuronal differentiation is one of its most notable characteristics.(6)The differentiation of

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pheochromocytoma is accompanied by the action of the neural growth factor (NGF). NGF-mediated stimulation leads to changes in their shape, an increase in the volume of cells, and the generation of neurotic processes also by promoting the growth of axons. In response to the nerve factor, cells exhibit the properties of sympathetic neurons, and their differentiation occurs through the Tyrosine kinase receptor. (4) PC12 cells are an excellent model for studying neurotoxicity in PD due to their remarkable properties that help to compare the pathogenic mechanisms. The characteristics of such proliferating cells i.e. homogeneous, abundant as well as easy to culture, transfect or infect with viral particles, make them ideal for drug screening, gene manipulation, and signalling pathway investigation. (7)

III. CELLSOURCE AND CULTURE CONDITIONS

The American Type Culture Collection (ATCC) is the most frequently mentioned source for the PC12 cell line. (JL Biedler deposited CRL-2266). Additional resources retrieval of cells from other cell banks, such as the Authenticated Cell Cultures in Europe is a collection of authenticated cell cultures from across Europe. (ECACC, 94030304, deposited by PFT, Catalogue number: 94030304, Vaughan) or National Centre For Cell Science, Pune

There are two types of PC12 cell lines available from the ATCC collection: cells growing in suspension and cells with a well-attached adherent phenotype. Undifferentiated PC12 cells have the following characteristics: they are pheochromocytoma cells, they synthesise and produce catecholamines, they express NGF receptors, and they can be used as a model for adrenal chromaffin tumour and differentiated PC12 cells. Secrete and synthesise dopamine, express different neural markers, form connections with primary neurons, and are morphologically comparable to sympathetic neurons. It's a model of a neuronal cell because it's sensitive to neurotransmitters and electrically excitable.

Several cell line distributors have made recommendations for the formulation of growth medium for PC12 cell propagation: ATCC recommends Modified Eagle Medium (MEM) supplemented with 10% foetal bovine serum (FBS); ECCAC recommends MEM/F12 with 2 mM glutamine, 1% non-essential amino acids, and 15% foetal bovine serum; and ECCAC recommends MEM with 15–20 percent foetal bovine serum. The most widely used regimen in PD-related articles was Dulbecco's Modified Eagle Medium (DMEM), followed by DMEM/F12, MEM/F12, DMEM high glucose, RPMI 1640, Cosmedium-001, and MEM. Antibiotics/antimycotics, glutamine, non-essential amino acids, sodium pyruvate, or other components like HEPES, sodium carbonate, uridine, or L-lysine were also added to the media in different combinations. (8)

PC12 cultures should be differentiated for 48 hours to 7 days in the presence of NGF. PC12 cells should be differentiated for at least 72 hours. As a result, the study's normal time for investigating the influence of differentiation on susceptibility to hazardous elements will be 72 hours.

The elongation of the branches, i.e. emerging neurites from cells, polygonal form, and adherence of the culture to the surface of wells, are all characteristics of PC12 cell differentiation that may be examined after 72 hours of incubation with 100 ng/mL NGF.(4)

The type and composition of the medium must be carefully selected; for example, DMEM can modify the metabolomes and differentiation capacity of a number of cell lines. Intracellular levels of glutamine, alpha-ketoglutarate, and pyruvate may be assessed by many metabolic pathways, and the redox ratio and concentration of NAD+/NADH are important factors in gene epigenetic control during differentiation. (9) The addition of sodium pyruvate to media has been shown to protect against oxidative stress, which could have an impact on the outcome of trials looking into the function of oxidative damage in Parkinson's disease. In addition, some researchers utilise other quantities of serum, ranging from 5 to 20%, or serum from other species, such as horses. However, in the majority of the articles, the media were supplemented with 10% foetal bovine serum. (10)

IV. DIFFERENT MECHANISM OF INDUCTION IN PD

PC12 cells are vulnerable to mitochondrial poisons such as 6-hydroxydopamine, 1-methyl-4-phenylpyridinium (MPP +), rotenone, or paraquat, which mimic Parkinson's disease (PD),which can be used to research in neurodegeneration. Within 24–48 hours of exposure, these toxins cause cellular deterioration.(11) They're also employed to assess the pathogenic qualities of -synuclein. They are the most commonly utilized PD cellular model,

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along with the SH-SY5Y cell line, although data acquired with these oncogenic cellular models should be interpreted with caution and confirmed in other cellular and animal models(12) Here, we'll look at the two most commonly utilized neurotoxins in Parkinson's disease research, MPTP and 6-OHDA, and their characteristics and modes of action

MPTP(13)

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- mediated selective damage to dopaminergic neurons of the nigrostriatal pathway has been widely used as a model of Parkinson's disease (PD)[1–3]. MPTP is a lipophilic molecule that can cross the blood-brain barrier. MPTP has provided important information on the potential mechanisms of PD. It induces toxicity through its conversion of astrocytes to 1-methyl-4-phenylpyridinium (MPP+) in the reaction catalysed by monoamine oxidase type B (MAO-B). MPP+ can be selectively transported into the dopaminergic neurons through high-affinity dopamine (DA) transporter and accumulates in the neuronal mitochondria, where it disrupts cellular respiration inhibiting complex I of the electron transport chain, depletes adenosine triphosphate (ATP) in the mitochondria. It increases sensitivity to oxidative attack and eventually causes apoptotic or necrotic neuronal cell death. It is reported that the toxic effects of MPTP are results of the generation of oxygen free radicals either during oxidation of MPTP to MPP+ or during electron transport chain disruption by MPP+. In addition, MPP+ might induce the release of DA, which in turn could generate free radicals from auto-oxidation. Also, it is found that the accumulation of MPP+ in dopaminergic neurons is a critical step for DA neurotoxicity, the exact molecular mechanisms underlying MPP+ -induced neuronal death have not been clear. Thus, elucidating the molecular basis of the cell death triggered by MPP+ can provide valuable insight into the pathogenesis of PD.(14)

6-OHDA(15)

The neurotoxin 6-OHDA has been proposed for use in animal models of Parkinson's disease. 6-OHDA is administered unilaterally into the nigrostriatal pathway to induce neurodegeneration, and several 6-OHDA animal models have been used in Parkinson's disease research. 6-OHDA is a toxin that primarily affects the peripheral and central nervous systems. T the neurotoxic 6-OHDA is unable to cross the blood-brain barrier, and central nervous system toxicity can only be achieved through direct injection into the brain. The toxic effects of 6-OHDA are caused by toxins accumulating in catecholaminergic neurons, which then alter cell stability and lead to neuronal death. Because it resembles the structure of endogenous catecholamine, dopamine or norepinephrine membrane transport protein absorption hence 6 OHDA is used.

The toxicity of 6-OHDA is related to its ability to the oxidative stress and production of free radicals, which is similar to the hydrogen peroxide effect. The inhibition of brain mitochondrial complexes (I and IV) and oxidation to form semiquinone radicals (which participate in reactive oxygen species production) are the main biochemical properties of 6-OHDA.Then the toxicity of 6-OHDA results in the loss of respiratory activity from oxidative stress by free radicals. the auto oxidation-derived oxidative stress and the inhibition of the mitochondrial respiratory chain, initially microglial stimulation and NADPH oxidase-derived reactive oxygen species functions synthetically with 6-OHDA and establish an appropriate and early constituent of 6-OHDA-convinced cell death.

V. EVALUATION PARAMETERS FOR PC12 CELL LINE

Cell viability assay

Cell viability can be determined using a thiazolyl blue tetrazolium bromide (MTT) assay. PC12 cells can be cultured in 96-well plates. After incubation, 5 mg/mL MTT solution (prepared in 1× PBS) is added to each well and then the cells will be incubated at 37°C in a 95% air/5% CO2 atmosphere for 4 h. After removing the medium with MTT, 100 µL DMSO can be added to each well and shaken at room temperature for 10 min. Then, the optical density at 570 nm (OD570) can be measured by a micro plate reader. Victor2 micro plate reader (PerkinElmer, Waltham, MA, USA) is most commonly used for cell viability assay.

Lactate dehydrogenase (LDH) and total superoxide dismutase (SOD)

LDH release is considered to be an important indicator of cell membrane integrity and is widely used in cytotoxicity assays. The changes in the LDH leakage rate can be measured using an LDH kit according to the manufacturer's protocol. PC12 cells were seeded into a 96-well plate, and the absorbance of the samples will be measured at 490 nm using a micro plate reader. The SOD activity of PC12 cells can be measured using a total SOD assay kit. PC12 cells were seeded into a 6-

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well plate, and the absorbance of the samples should be measured at 450 nm using a micro plate reader. Also, Victor2 micro plate reader (PerkinElmer, Waltham, MA, USA) is used to take absorbances of the sample

Length and Density of Neurites

The On days 2, 3, 5, 7, 14, and 21, following incubation with 100 ng/mL rat NGF, the average length and density of neurites were measured using microscopic images taken 24 hours after seeding (day 0). Cells were seeded at a low density (1000 cells per well) in these tests. The use of NGF to differentiate cells leads in a dense network of neurites, and the use of fewer cells has made the examination of neurites belonging to specific cells easier. The software was used to determine the density and lengths of 50 cells in three distinct wells in five independent replicates.

Neurite Outgrowth (Spectrofluorimetric Evaluation)

Neurite outgrowth is one of the most essential characteristics for determining the morphological phenotype of neuronal cells, which is linked to their effective mechanism and condition. The "Neurite Outgrowth Staining Kit" can be used to measure neurite outgrowth spectrofluorimetrically. Neurite outgrowth was assessed on collagen and non-coated surfaces, with or without NGF addition, on day 3 in this test to examine the effect of surface modification and NGF administration. The cells should be rinsed with PBS after the supernatant has been removed, and the staining solution should be applied to each well. At 37°C, the cells should be cultured for 20 minutes. After incubation, cell cultures were washed in PBS, a background suppression dye was added, and a spectrofluorimetric reading was taken with a microplate reader at 485/535 nm (excitation/emission wavelength).

VI. IN VIVO AND IN VITRO MODELS USED IN PARALLEL WITH PC12 CELLS

The loss of DAergic neurons in the SN is a feature of Parkinson's disease. As a result, primary cultures of neurons from patients' brain areas are thought to be the most reliable models for studying the disease's molecular causes. The inaccessibility and lack of multiplication of such neurons, on the other hand, limit their utility. Furthermore, using a proliferative and more homogeneous model, such as the PC12 cell model, has drawbacks. In addition to the PC12 cell line, cell lines with a neuronal phenotype, such as mouse mesencephalic primary cultures and mostly cortical primary neurons, stem cells, SHSY5Y cell line, Neuro-2a cell line, or MN9D cell line research, and other cell types have been used. (18)

The identification of the indicated primary pathways underlying PD was made possible by analysing the functions of the associated (mutated) proteins and, as a result, the cellular abnormalities that resulted. Mitochondrial, proteasomal, autophagy failure, protein aggregation, dopamine metabolism, and oxidative stress are some of the processes that lead to DAergic cell death.

Rodent mesencephalic primary cultures are enriched for the cell population of interest, the SN DAergic neurons, because the SN is found within the mesencephalon or midbrain. Primary cultures from other brain regions, primarily the cortex, are also commonly utilised as complementing in vitro models, however these cultures are made up of mixed populations of different neuronal subtypes.

It has been employed primary cultures of DAergic neurons from different species, such as the worm C. elegans. Because cell lines are generally immortal and can sometimes be differentiated into terminal neuronal populations, physiologically relevant permanently formed cell lines are easier to maintain than cells in original cultures.

PC12 cells treated with nerve protein differentiate into a catecholaminergic-like phenotype. The Neuro-2a cell line was produced from a neuroblastoma in a mouse brain and can be differentiated into neuronal-like cells and, more particularly, DAergic neurons. (19)

SH-SY5Y cells are a subtype of the SK-N-SH line derived from a neuroblastoma bone marrow biopsy.SK-N-SH was sub cloned to SH-SY, then to SH-SY5, and finally, to SH-SY5Y. Neuroblastoma cells can be differentiated into mature human neurons with the help of neurotrophins for e.g (brain-derived factor—BDNF) or retinoic acid. Moreover, literature data show that differentiated SH-SY5Y cells are characterized by the presence of markers typical of mature neurons. The neuron biomarkers present SH-SY5Y include NeuN protein, which is a neuronal nuclear antigen, synaptophysin protein (SYN), neuron-specific enolase (NSE) or a protein related to the growth and plasticity of neurons (GAP-43). The use of various methods of cell differentiation also allows the selection of specific subtypes of neurons (adrenergic, cholinergic,

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dopaminergic neurons). SH-SY5Y cells also express dopamine β-hydroxylase or secretion nor epinephrine, and therefore they can increase catecholaminergic activity. Moreover, the SH-SY5Y cell line is a good model for the study of disturbed dopamine homeostasis, which is an important aspect of the development of PD. The production of free radicals caused by the spontaneous oxidation of dopamine causes the malfunction of the mitochondria, and consequently increases oxidative stress, which in turn plays a key role in the development of neurodegenerative disorders. The presented line, due to its neoplastic origin, is also characterized by many genetic aberrations, however, most of the genes and pathways involved in the pathogenesis of PD remain unchanged, and therefore it is one of the most frequently chosen research models. (21)

Dopaminergic neurons may often be successfully separated from fibroblasts from Parkinson's disease patients. According to available somatic cell research, fibroblasts are frequently converted to a pluripotent state before being used to make induced pluripotent stem cells (iPSC). iPSC-based cell models are extremely effective for researching the molecular underpinnings of Parkinson's disease [22]. The LUHMES line is another advanced in vitro model for Parkinson's disease research. It's a human foetal mesencephalon cell line that has been immortalised. The MESC2 line is a subclone of these cells. The v-myc tetracycline-responsive (TET-off) gene is used to keep LUHMES cells at an undifferentiated stage of proliferation. Tetracycline, neurogliacyte line-derived neurotrophic factor (GDNF), and cyclic AMP can be used to differentiate this cell culture (cAMP). (22)

Cells exit the cell cycle as a result of the administration of such substances, causing them to develop into post-mitotic neurons. Neurons that have been differentiated have phenotypically similar properties to human dopaminergic neurons, including biochemical expression of mature neuron markers.

Because of the parent MESC2 cells, LUHMES have similar neuronal and dopaminergic characteristics. Furthermore, LUHMES cells exhibit significant levels of L1CAM and -synuclein, which are helpful markers for researching Parkinson's disease. Furthermore, within the proposed cellular model, the expression of the microtubule-associated protein tau (MAPT) and synapsin-1 (SYN1) proteins is a critical element accountable for the danger of PD and thus the disease's primary pathomechanism.

Because of its human origin, the provided cell model is ideal for PD research. The LUHMES line, in comparison to the models described above, has no limits due to its origin and should be properly referenced inside the in vivo testing system. Three-dimensional (3D) cell cultures have become one of the most popular and cutting-edge in vitro models for the research of neurodegenerative disorders in recent years. Scientists have a wide range of possibilities when it comes to developing new therapeutic compounds and understanding illness pathomechanism because to the diversity of cell models available. (19)

Cell hybrids, such as the MN9D cell line, and variants of the neuroblastoma SK cell line, are other PD cellular models. It's worth noting that the majority of the chosen lineages come from animals other than humans. Finally, patient-derived induced pluripotent stem cells (iPSCs), which will be developed into dopaminergic neurons and maybe other important cell types such as glial cells, are an emerging new category of cell models for Parkinson's disease (23).

The mouse, rat, worms, and fruit fly are used as animal models, and the PD features of these in vivo models are reproduced using MPTP, 6-OHDA, rotenone, or genetic alterations. (12)

CONCLUSION

This review highlights the PC12 cell line's "popularity" and widespread use in Parkinson's disease research, as well as some of its shortcomings. PC12 cells are being used to investigate the cellular and molecular mechanisms behind the effects of a number of PD-related toxins, as well as to do functional investigations on family PD genes and to test protective drugs for PD treatment. As a result, this cell line has proved valuable in understanding the molecular intricacy of Parkinson's disease. PC12 cells have physiological characteristics that are very different from those of typical DAergic neurons. Differences in cell source and culture maintenance, possibly of epigenetic character, could explain these variations, but drawing firm conclusions is impacted by a lack of accurate reporting of experimental protocol parameters and inaccurate listing of individual characteristics of cell lines kept at different laboratories. To match findings and promote reproducibility and advancement with this PD model, the cell source must be precisely mentioned, and more investigations on the effects of medium

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composition on the cell population are required. In addition, using other neuronal cell lines, such as those discussed here, and animal models alongside PC12 cells could help to validate the findings.

Aspects such as species differences, tumorigenic characteristics, time and resource needs should all be considered when selecting additional models. Because the onset of this complex disease involves both hereditary and environmental variables, choosing the best way to mimic PD is difficult. In functional investigations on PC12 cells, chemical and genetic techniques are used to focus on one or more pathways connected to PD. In any case, current developments in the fields of chemical biology and reverse-genetics (i.e. CRISPR/Cas9 applications), which will allow a much broader application of chemical libraries for cell signalling inhibition and genome editing, respectively, suggest and are expected to facilitate the use of multiple approaches in parallel. These novel opportunities alongside the right exploitation of the already well-established procedures for cell culturing will allow the standardization of the utilization of the PC12 cell line as a model for PD. (20)

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