Mol Neurobiol (2012) 46:495–512
DOI 10.1007/s12035-012-8291-8

Rodent Models and Contemporary Molecular Techniques: Notable Feats yet Incomplete Explanations of Parkinson's Disease Pathogenesis

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Received: 14 May 2012 / Accepted: 13 June 2012 / Published online: 27 June 2012

Abstract Rodent models and molecular tools, mainly omics and RNA interference, have been rigorously used to decode the intangible etiology and pathogenesis of Parkinson's disease (PD). Although convention of contemporary molecular techniques and multiple rodent models paved imperative leads in deciphering the role of putative causative factors and sequential events leading to PD, complete and clear-cut mechanisms of pathogenesis are still hard to pin down. The current article reviews the implications and pros and cons of rodent models and molecular tools in understanding the molecular and cellular bases of PD pathogenesis based on the existing literature. Probable rationales for short of comprehensive leads and future possibilities in spite of the extensive applications of molecular tools and rodent models have also been discussed.

Keywords Parkinson's disease · Rodent models · Genomics · Transcriptomics · Proteomics · RNA interference

Introduction

James Parkinson offered the first landmark portrayal on shaking palsy; however, the name Parkinson's disease (PD) was given by Jean-Martin Charcot [1, 2]. PD is recognized as the most common progressive, baffling, and devastating neurodegenerative disorder in the elderly after the Alzheimer disease [3, 4]. This movement disorder is distinguished by the selective degeneration of the nigrostriatal dopaminergic neurons, accumulation of cytoplasmic protein aggregates and onset of phenotypic features, such as resting tremor, rigidity and bradykinesia, etc., leading to loss of control over the movement [3–6]. The degeneration of selective neurons is accountable for the decreased dopamine level in the striatum that ultimately results in the clinical manifestations [6]. Although an early diagnosis is dreadfully difficult, physical and clinical examinations and symptomatic features are used to diagnose the patients after considerable dopaminergic neurodegeneration and manifestation of noticeable complications [7]. Moreover, the comprehensive explanations of pathogenesis and permanent cure are not yet established, and therapeutic and surgical procedures offer provisional aids [7, 8].

One of the most commonly accepted notions for the onset of symptomatic features of PD is the resultant interplay of the environmental factors, increased age, and genetic susceptibility of an individual [3, 5, 9]. Administration of 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), reserpine, methamphetamine, rotenone, maneb, zinc, manganese, paraquat, and cypermethrin in rodents develop many symptomatic features mimicking PD [9–14]. These chemicals either alone or in combination inhibit mitochondrial function resulting in depleted energy metabolism, free radical generation, and neuroinflammation leading to programmed cell death and selective neurodegeneration [15–19]. Some environmentally relevant chemicals directly cross the blood–brain barrier (BBB) and enter the brain owing to their lipophilic nature, such as MPTP and rotenone. Others, those are hydrophilic in

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nature, require either specific transporters, such as paraquat that requires a neutral amino acid transporter or needs to be imported directly in the target tissue, such as 6-OHDA [9, 14]. Environmental factors could contribute notably, if the genetic factors clutch them appropriately, as every synthetic heroin (which contains MPTP) user does not develop PD-like symptoms. Pesticides and other environmental chemicals, when enter the body and subsequently in the brain, get converted by the phase I and II xenobiotic metabolizing enzymes either to more or less toxic intermediary metabolites. The bioconversion is directly proportional to the catalytic activity, protein expression, and DNA sequence of the coding and non-coding regions of a gene, which encode the enzyme [20]. Twin analysis, omics, and RNA interference (RNAi) further supported the genetic bases in a few cases and deciphered autosomal pattern of inheritance of many PARK genes [5, 21–23]. Omics and RNAi also deduced many indefinable aspects, which include the identification of molecular fingerprints and molecular explanations of PD pathogenesis [7, 22]. Although amalgamation of modern techniques and multiple rodent models were expected to offer novel clues to disease pathogenesis and molecular biomarkers, clinical and phenotypic symptoms are still used as the gold standard to diagnose PD [7]. Despite noteworthy and extensive endeavors made by rodent models and molecular tools to pinpoint the biochemical, clinical, pathological, epidemiological, and molecular bases of disease pathogenesis, the complete molecular machinery of PD pathogenesis is still mysterious [21, 22, 24].

Salient characteristics, achievements, and limitations of various rodent models and/or molecular tools employed for understanding PD pathogenesis have been comprehensively reviewed elsewhere [3–5, 10, 21, 22, 24–27]. This article updates the contributions made by imperative rodent models, omics, and RNAi approaches altogether based on the information available in the literature along with the reasons why such sincere efforts could not yet embrace the desired success. Attempts are also made to portray likely explanations for lack of comprehensive translation of the information generated from rodents to humans.

Contributory Factors of PD

Since most of the rodent models are based on the alleged causative factors, it is worthwhile to discuss about the undeniable and suspected contributory factors of sporadic PD. Undoubtedly, aging is the main perpetrator, as it has been found to increase the incidences of PD in humans. It is estimated that approximately 1 % of the population of 50–60 years of age may develop PD, and the incidences may go up further in elderly individuals [5]. The effect of age is reflected even in the experimental rodent models, as the aged rats have been found to be more susceptible to a chemical that induces the nigrostriatal dopaminergic neurodegeneration [13]. Environmental factors that include pesticides exposure, rural living, well water drinking, and head trauma could be other contributory factors, as evidenced from epidemiological studies and/or rodent experimentations [3–5].

Appearance of the disease warning signs in an early age has been limited; however, onset of the disease in the individuals below 50 years of age gave a notion that genetic factors could play a critical role in PD pathogenesis. Now, the familial PD is well recognized by the twin analyses and case studies [5]. Several genes have been mapped and are suspected to manipulate PD pathogenesis in the genetically susceptible persons owing to point mutations and inappropriate epigenetic regulation [28–30]. Despite the fact that the majority of the patients do not possess familial history, absence of cardinal differences between the sporadic and familial forms and the lack of symptoms in a few patients carrying defective genes, point out the influence of heritable factors on the age of onset, particularly when the appropriate environmental conditions are available [5, 24, 30, 31].

Rodent Models

Many chemicals, which include pesticides and metals, turn out to be inseparable parts of the environment, and their involvement in PD pathogenesis could be incredible. A few pesticides and metals may lead to PD in humans and also reproduce PD-like symptoms in the exposed experimental rodents [5, 25, 32, 33]. Salient characteristics and pros and cons of a few extensively used rodent models are described below.

6-OHDA

Even after several decades of history of its use for developing PD-like features in rats, 6-OHDA is still widely used for the same purpose [21]. The severity of the symptoms produced by 6-OHDA depends on the site of its administration in the substantia nigra and the extent of the lesions [32]. The 6-OHDA model provided new insights to understand PD pathogenesis and validated pre-existing information gathered from sporadic cases and also from other rodent models. 6-OHDA offered multifaceted confirmations of the degeneration of cell bodies of dopaminergic neurons in the substantia nigra and fibers in the striatum [34], the fundamental features of sporadic PD in humans. Mitochondrial dysfunction has been widely accepted as the priming event leading to oxidative stress and thereby the nigrostriatal dopaminergic neurodegeneration. 6-OHDA mimics sporadic PD in the sense that it inhibits mitochondrial electron transport chain complex I (nicotinamide adenine dinucleotide-ubiquinone

reductase), generates free radicals, and induces programmed cell death [4].

6-OHDA induces neurodegeneration by multiple means. Oxidative stress is critical in PD pathogenesis whether it is generated by the mitochondrial complex I inhibition or owing to other means. Although growth arrest and DNA damage-induced gene 153 is also reported to act as a neuronal cell death mediator, a null mutation of the gene results in reduction of apoptosis in the 6-OHDA model [35]. Similarly, free radicals produced owing to 6-OHDA exposure causes several changes in dopaminergic neurons that mediate neurodegeneration. Fos expression also mediates some of the abnormal sensory circuits of neurodegeneration in 6-OHDA-treated rats [36]. Proteases, such as caspases, are known to regulate apoptotic machinery and induce toxicity in dopaminergic neurons. Caspase-3 mediated proteolytic cleavage and activation of protein kinase C-δ is reported to be decisive in dopaminergic neurodegeneration and cleavage resistant form of the protein is found to protect against apoptosis in 6-OHDA-treated rodents [37]. 6-OHDA augments unfolded protein stress, causes cytochrome-c release and upregulates pro-apoptotic proteins, which include caspases and p53 [38]. 6-OHDA downregulates tumorous imaginal discs 1 protein, which hampers functional and structural compensation and exacerbates neurodegeneration [39]. Moreover, the 6-OHDA model highlighted the fact that auto-oxidation of dopamine into free radicals may also lead to PD-like features [32].

6-OHDA neither induces the formation of the Lewy bodies nor produces the similar degree of phenotypic symptoms in all experimental rodents [24, 32]. Owing to inability of 6-OHDA to cross the BBB and directly enter the brain [4], the nigrostriatal administration is required, which is tricky and cumbersome. Due to lack of its environmental occurrence and direct human exposure, and inconvenient delivery in brain the 6-OHDA model could not be considered very ideal for extrapolation of the results to humans and is now becoming less popular as compared with its popularity in the past [24].

MPTP

MPTP itself is not as toxic as its intermediary metabolite, 1-methyl-4-phenylpyridinium cation (MPP^+), which is highly reactive and severely neurotoxic [4, 16, 25, 40]. Monoamine oxidase B (MAO-B) of the astrocytes synthesizes MPP^+ from MPTP [4, 25, 41]. The organic cation transporter (Oct)-3 present in the astrocytes (glial cells) and dopamine transporter (DAT) localized on neurons regulate the entry of MPP^+ into dopaminergic neurons (Fig. 1) [42]. The MPTP rodent model supported the notion that the mitochondrion is an epicenter of PD pathogenesis [40]. Although it is not yet clear whether mitochondrial depolarization or free radical generation after the mitochondrial complex I inhibition is the most critical event, the MPTP model elucidated the contributions made by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, microglial cells, neuronal inflammation, and secondary signaling molecules, including p38 mitogen activated protein kinase, c-Jun N-terminal kinase (JNK) and tumor suppressor protein 53, in PD pathogenesis [10, 25, 43]. MPTP triggers the activation of JNK, which is regulated by GST Pi protein via protein–protein interactions [44]. Although the role of free radicals and microglial activation in the MPTP model is established, a recent study has shown that MPTP-induced dopaminergic neurodegeneration primarily depends on the free radical outburst and activation of NADPH oxidase in dopaminergic neurons, and the activation of microglial NADPH oxidase takes place at later stage [45]. Furthermore, the MPTP model showed that phosphorylated Akt/protein kinase B depletion hinders with the normal functioning of cell survival [46].

PINK 1 and DJ-1, two known neuroprotective molecules, modulate the dopaminergic sensitivity towards MPTP-induced toxicity [24]. This assumption gave a conduit of how genetic factors play important roles even in toxin-induced disease pathogenesis. It is validated by a recent study in which loss of PARK 7 (DJ-1), a cellular target, is found to be associated with the modulation of MPTP-induced PD phenotype. In this study, a cell permeable Tat-DJ-1 protein is reported to protect dopaminergic neurodegeneration by reducing the effects produced by MPTP-mediated oxidative stress [47]. MPTP model elucidated the inputs of peroxisome proliferator-activated receptor γ in dopaminergic neuroprotection and treatment outcomes [48]. Moreover, the MPTP model deciphered the roles of heat shock proteins (HSPs), such as HSP1b, in the regulation of neurodegeneration, as knocking down the *HSP1b* gene increases the vulnerability of dopaminergic neurons [49]. Neurotrophic factors and apolipoprotein could encounter PD pathogenesis and help in the repairing of dopaminergic neurons, as their expressions are increased in MPTP-induced rodent models [50, 51]. The MPTP model also improved our awareness about the contribution of environmental factors, the mechanisms of PD pathogenesis and therapeutic strategy to encounter the disease.

MPTP develops an acute rodent model; low doses and long-term exposures may help in reproducing chronic PD phenotype [52]. Despite species to species variation, absence of slow and progressive degeneration and distinct Lewy body formation, the MPTP model is widely used to understand pathogenesis and to appraise the efficacy of anti-PD chemical entities [25, 40, 41, 52].

Paraquat and Maneb

Initially, 1,1′-dimethyl-4,4′-bipyridinium dichloride (paraquat), which has a structural similarity to MPP^+ and produces neurotoxicity, was assessed for neurodegenerative potential in the experimental rodents [53, 54]. Paraquat

enhances alpha (α)-synuclein-induced disruption of membrane integrity and increases the conductance, as a result of increased oxidative stress [55]. Later on, a fungicide, manganese ethylene bis-dithiocarbamate (maneb) was also tested for its potential in experimental animals either alone or with paraquat [15]. Structural and functional anomalies in the endoplasmic reticulum (ER) and mitochondrion are found to be associated with PD pathogenesis. Paraquat slows down mitochondrial complex I activity and augments the microglial activation and free radical production through NADPH oxidase, while maneb reduces mitochondrial complex III activity and both of them together or individually may lead to oxidative stress, DNA damage, defective energy metabolism, and cellular apoptosis (Fig. 1) [4, 10, 15, 25, 56]. Roles of ER and mitochondrion in PD pathogenesis are also validated in the paraquat alone induced dopaminergic neurodegeneration model in a study. ER stress and mitochondrial dysfunction were found to trigger caspase-12 activation, hydrogen peroxide release, and PARK 13 (HtrA2/Omi) activation; however, minocycline, a microglial activation inhibitor, encountered such alterations [57].

Toxicant responsive genes, such as cytochrome P450 (CYP/Cyp) 2D6, play critical roles in PD pathogenesis and treatment outcomes. The expression of Cyp2d22, a human CYP2D6 ortholog in the mice, is increased in maneb and/or paraquat-induced PD phenotypes, showing the role of detoxification machinery in the disease pathogenesis [20]. Toxicity of pesticide-induced PD depends on the metabolic conversion of pesticides in to too high or too low toxic species. The conversion of paraquat dication to paraquat cation and subsequent transport to dopaminergic neurons mainly depend on Oct-3 and DAT [58]. Maneb and paraquat induce apoptosis through Bak-dependent pathway when administered alone; however, on combined systemic exposure, they follow the Bax-dependent apoptotic pathway [59]. Although it is known that males are often at the higher risk for PD as compared with females, supportive evidence from an animal experimentation is provided in a recent study in which the effect of paraquat was monitored. Paraquat administration was found to increase the brain-derived neurotrophic factor (BDNF) expression in the hippocampus of female rats, which was responsible for reduced susceptibility of the females towards the nigrostriatal

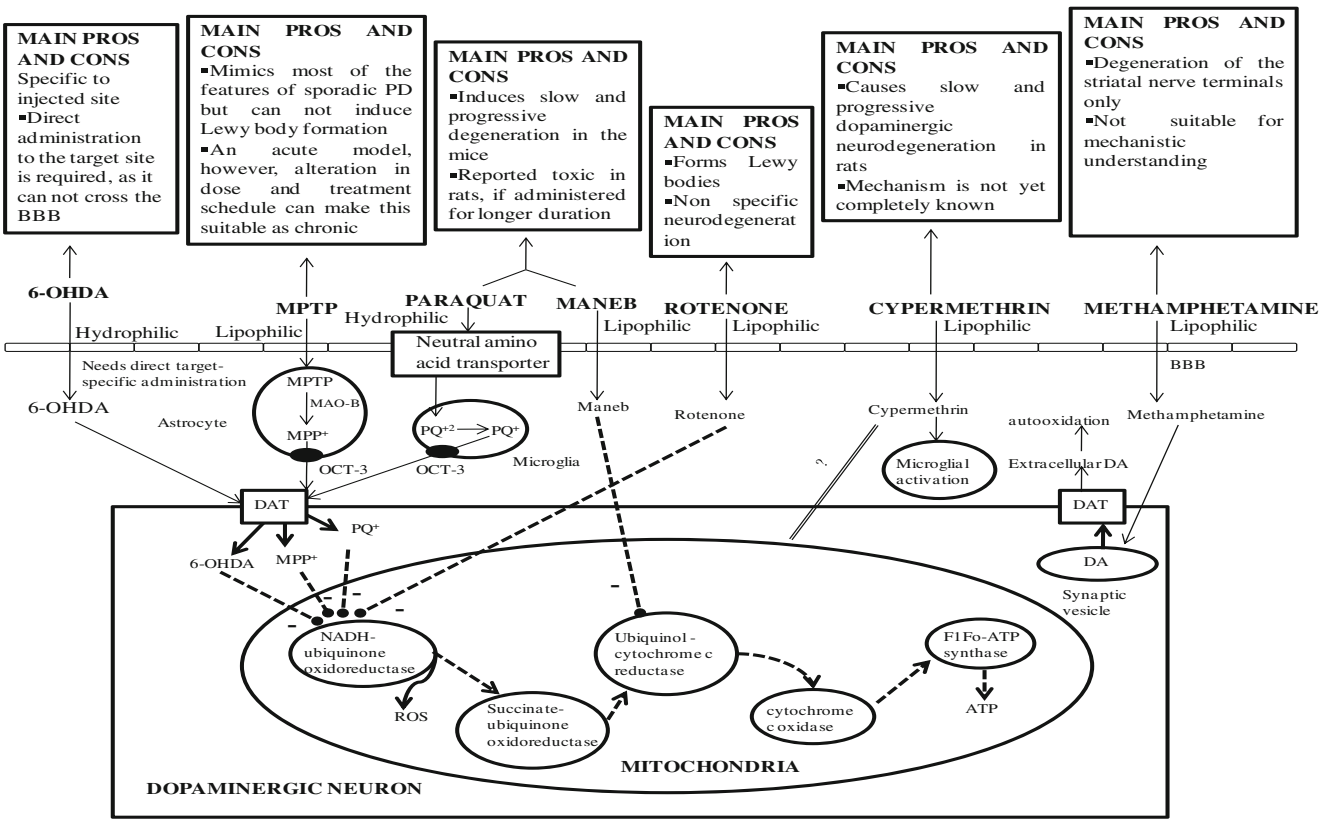

Fig. 1 Routes of entry of the major PD-inducing chemicals in the brain, their enzymatic/non-enzymatic conversion into active radicals in glial cells, subsequent entry into dopaminergic neurons, and the inhibitory effects produced by them at the level of mitochondrial complexes or dopamine auto-oxidation [4, 10, 24, 25, 42, 58, 68]. 6-OHDA, MAO-B, Oct-3, DAT, PQ^{2+}, PQ^{+}, MPTP, MPP^{+}, NADH, DA, ATP, BBB, and ROS abbreviate 6-hydroxydopamine, monoamine transporter B, organic cation transporter-3, dopamine transporter, paraquat dication, paraquat cation, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1-methyl-4-phenylpyridinium cation, reduced nicotinamide adenine dinucleotide, dopamine, adenosine triphosphate, blood–brain barrier, and reactive oxygen species, respectively

dopaminergic neurodegeneration as compared with males, as decreased BDNF expression was noted in males [60].

Despite mild to severe toxicity of paraquat and/or maneb administrations in rodents after prolonged exposure [9, 61], the use of their combination in rodents is widely accepted and offers two main advantages—inevitable human exposure that makes them environmentally relevant and ability of progressive and slow neurodegeneration [61]. Epidemiological studies have also shown the direct relevance of maneb and paraquat co-exposure to humans [25, 62].

Rotenone

Like other animal models, the rotenone model also exhibits mitochondrial complex I inhibition and induction of oxidative stress and apoptosis [4]. The release of cytochrome c is found to be independent of caspase activation in rotenone-induced dopaminergic neurodegeneration. This phenomenon exists even in the presence of cytoplasmic cytochrome c release after mitochondrial complex I inhibition [63]. Matrix metalloproteinase-3 (MMP-3) is also implicated in dopaminergic neurodegeneration induced by rotenone, which is regulated by multiple mechanisms. MMP-3 requires activation from proMMP-3 by an intracellular serine protease. Under stress, HtrA2/Omi, a mitochondrial serine protease, translocates into the cytosol, causes MMP-3 activation, and triggers apoptosis in dopaminergic cells [64].

Although rotenone readily enters the brain, inhibits mitochondrial complex I, generates free radicals, exhibits Lewy bodies formation and other anatomical and phenotypic symptoms of PD (Fig. 1) [65], it is not as popular as MPTP or 6-OHDA, owing to its non-specificity, high mortality rate, and severity of the lesions [9, 10, 41].

Other Pesticides

Epidemiological studies showed the contribution of a few other pesticides in increased incidences of PD [5, 33, 66, 67]. Several classes of pesticides, which include, pyrethroids, dithiocarbamates, organochlorines, and organophosphates have been reported to induce PD-like symptoms in the experimental rodents [68, 69]. As these pesticides are commonly used globally, therefore, could be of major health concern. Dieldrin and cypermethrin induce neurodegeneration in the adult experimental animals after prolonged exposure [13, 69]. Postnatal pre-exposure of cypermethrin is also found to enhance the susceptibility of the animals, when re-exposed upon adulthood [13]. Despite the fact that little is known about the mechanism of cypermethrin-induced neurodegeneration, the neuronal loss is specific to dopaminergic neurons of the nigrostriatal pathway [68]. Moreover, its effect on microglial activation is known, but prolonged opening of ion channels or mitochondrial dysfunction could possibly be the most important events involved therein [19, 68].

Methamphetamine

Methamphetamine produces dopamine depletion leading to temporary or permanent disturbance in the dopaminergic system after chronic or intermittent exposure and has established the role of growth factors, particularly glial derived neurotrophic factor [10, 70]. Methamphetamine selectively reduces phasic, but not tonic, dopaminergic signaling in the striatum [71]. Methamphetamine-induced response is found to be age dependent in the primates owing to age-related changes in the neurotrophic capacity of the striatal dopamine system [72]; however, in rodents, such information has not yet been reported. As methamphetamine depletes dopamine level in the striatum, the effects of such drugs have also been studied in the exposed humans to assess the actual risk. Drug users are found to have a high risk for developing PD as compared with unexposed individuals [73]. Methamphetamine-induced neurotoxicity also involves the striatal vasoconstriction leading to hypoxia and dopamine reduction in the exposed individuals [74].

In general, the methamphetamine model lacks many fundamental features of PD and is considered as a dopamine depletion model rather than a true PD model.

Metals

Apart from chemicals, several metals, including iron, lead, zinc, and manganese have been found to be associated with PD pathogenesis, as abnormal metal exposures or accumulations have been associated with the increased incidences of PD in humans or PD-like pathology in experimental animals [12, 75, 76]. “Metal accumulation may lead to PD” came into existence, when the postmortem brain of PD patients was seen to possess high level of metals [75]. Epidemiological studies revealed that zinc, lead, and iron are increased in the substantia nigra of PD patients, whereas the copper level is decreased. Like pesticides, metals induce free radical biosynthesis leading to the degeneration of dopaminergic neurons of the nigrostriatal pathway [77]. Metals catalyze the formation of free radicals either by Fenton’s reaction or directly by reacting with macromolecules, which lead to the generation of fatty acid radicals and 4-hydroxynonenal causing DNA damage and apoptosis. Additionally, the metals, such as cadmium, arsenic, and lead bind to sulphydryl group of proteins and lead to depletion of glutathione [76]. Zinc, another metal, also induces oxidative stress via the activation of NADPH oxidase and depletion of glutathione, which in turn activate the apoptotic machinery leading to dopaminergic neurodegeneration similar to paraquat [11, 78]. Although metal-based rodent models have not

yet been fully understood, the contributions of metals in increasing oxidative stress and reducing antioxidant defense system cannot be straightforwardly ignored [11, 76].

Like other chemical-induced PD models, metal models lack many fundamental features of sporadic disease. However, more exposure time is required to develop a few cardinal features of the disease in rodents like that of sporadic PD in humans.

Some Other Toxins

In addition to the above-mentioned toxins, many chemicals contribute to PD pathogenesis as reported in epidemiological investigations and/or from animal experimentations. Majority of such chemicals inhibit the mitochondrial complex I or elicit neurotoxicity by multiple mechanisms [79–81]. Trichloroethylene, a complex I inhibitor, is reported to cause PD-like features in humans upon exposure and also elicit motor impairment in experimental animals. Its oral exposure to experimental animals for 6 weeks selectively inhibits complex I and leads to the nigrostriatal dopaminergic neurodegeneration [79]. Annonacin, another mitochondrial complex I inhibitor, causes the nigrostriatal dopaminergic neurodegeneration by impairing the energy metabolism [81]. Several bacterial neurotoxins, from which humans and aquatic animals are exposed, could also elicit PD-like features. β-Methylamino-L-alanine, a cyanobacterial neurotoxin, elicits a few features quite similar to PD [80]. Isoquinoline derivatives, such as tetrahydroisoquinoline, elicit PD-like features in the animals possibly because of its structural similarity with MPTP [82, 83]. Similarly, haloperidol, a dopamine D2 receptor blocker; epoxomixin, a proteasome inhibitor; and 3,4-dihydroxyphenylacetaldehyde, a dopamine metabolite, also cause some or the other characteristic feature of PD [84–86]. Such agents although not yet very widely studied, could be tested across multiple studies either alone or in combination with established and widely studied toxins for developing better rodent models to understand PD pathogenesis and to assess the efficacy of neuroprotective agents.

Genetic Models

The role of genetic factors becomes vital when the mutated gene carrier is exposed to pesticides and heavy metals. The genetic theory of PD came into existence from the studies related to close relatives and twins who were diagnosed with an early onset of the disease [5]. The inheritance of genes, critical for an early onset of the disease, follows either autosomal dominant or autosomal recessive pattern [87]. Early onset PD-related genes are mapped in the specific chromosomal locations, together designated as the PARK loci [88, 89]. α-Synuclein protein is encoded by PARK 1 and 4, Parkin by PARK 2, ubiquitin carboxyl terminal hydrolase (UCH) L-1 by PARK 5, PTEN-induced kinase 1 (PINK1) by PARK 6, DJ-1 by PARK 7, leucine-rich repeat kinase 2 (LRRK2) by PARK 8, ATP13A2 by PARK 9 and Omi/HtrA2 by PARK 13 genes [88, 89]. Moreover, many other genes of elusive nature or function have also been mapped as PARK 3, PARK 10, PARK 11, and PARK 12 [87–89]. Apart from PARK genes, synphilin 1, paired like homeodomain transcription factor 3 (Pitx 3) and nuclear receptor-related factor 1 genes, etc., have also been mapped at various chromosomal locations and could possibly influence the genetics of the disease [28, 29].

Genetic models proved to be quite useful in understanding familial PD pathogenesis. Owing to absence of most of the critical phenotypic symptoms altogether and the roles of all the genes involved in PD make little sense of genetic models in understanding the sporadic PD.

Dual Models

Several rodent models of the genetically linked PD have been developed by knocking down the critical gene(s) responsible for familial forms of the disease [65, 90, 91]. By and large, the genetic models provided evidence-based proofs of the molecular mechanisms elucidated by the toxins-induced rodent models [24, 65, 90]. Recently, dual/fusion/combinational/two-hit rodent models have been generated to test the hypothesis that the environmental factors mainly act on the genetically susceptible individuals, as apt environmental factors and suitable genetic makeup together could be decisive for an early onset of PD [92]. Dual rodent models are mainly based on the principle of the combination of two contributory factors for creation of a new animal category to study the multifactorial etiology and to reiterate the major cardinal disease symptoms [24, 92, 93]. Generation of such model is an animated tool to comprehend PD in the conditions, which maximally imitate idiopathic PD [92, 94]. For example, lipopolysaccharide (LPS) induces several neuroinflammatory molecules that include nitric oxide, cytokines, and interferons, while α-synuclein dysfunction is associated with its abnormal accumulation, but low-dose of LPS and dysfunctional α-synuclein together may cause all the above-mentioned events and significantly mimic idiopathic PD features [24, 94].

Since dual models could be chronic and progressive in nature as far as dopaminergic neurodegeneration is concerned, they prove to be quite useful to investigate the mechanistic and therapeutic aspects of idiopathic PD [24, 94]. Fusion models need to be developed across laboratories all over the world employing major causative gene(s) and

toxin(s) for wider acceptance and real implication in understanding sporadic PD.

Advantages and Limitations of Rodent Models Over Other Models

Despite the fact that nonhuman primate models proved to be very useful in understanding a few decisive aspects of PD pathogenesis, rodents have many advantages over primates owing to several reasons. Convenient availability, housing, maintenance, handling, and plausible use of a large number of experimental animals per set for generation of much reliable data, make rodents the preferred choice over primates [95]. Additionally, it is possible to test multiple toxins alone as well as in combination to assess their neurodegenerative potential in the limited time. Although nonhuman primates are close to humans, generation to generation studies to assess the role of a particular gene [96], or the effect of toxins could be easily performed in rodents within the short span as compared with nonhuman primates. Nonetheless, the short life span of rodents does not always reflect an advantage, as the sporadic disease in humans generally appear very late and after long-term exposure to environmental factors. Owing to such advantages and disadvantages, many suspected toxins have been widely tested to check neurodegenerative potential in rodents, but limited studies are available with primates [32, 97]. Although lower animal models that include, *Drosophila*, zebra fish, nematodes have been continuously used to reveal the familial PD and genetic bases of onset and progression of the disease [98], successful application of such models for more than 90 % of the cases of the disease, which are sporadic in nature, is difficult. Furthermore, the genetic makeup of lower animals does not share a noticeable resemblance with humans, if a comparison is made with rodents or primates [99], leading to a possibility that data obtained from the toxicological response to a PD toxin or pharmacological response of anti-PD compounds could vary significantly and data extrapolation in humans would become more difficult.

No Model Is Absolutely Close to Ideal to Understand PD Pathogenesis

None of the rodent models developed so far can be said to be perfectly ideal since an ideal animal model should be an indicator of the multifactorial etiology and reproduce slow, progressive, and exposure-dependent onset of the phenotypic, behavioral, biochemical, and anatomical impairments along with the secondary changes associated with sporadic PD [24]. The major achievements of rodent models have been the confirmation of the contributory roles of pesticides and heavy metals in PD pathogenesis. Rodent models also validated the contributory roles of environmental factors, which were reported from the epidemiological studies [5, 11, 25]. Rodent models have shown that susceptibility to environmental neurotoxins is age dependent, as in sporadic PD. The postnatal exposure enhances the vulnerability upon adulthood in the rats [13] also validates the theory that age is the most important contributory factor. Although rodent models gave a validation of the major contributory factors reported through epidemiological or clinical investigations, rodent models fail to offer the role of newer contributory factors or all the culprits of PD [100, 101].

Chemically induced rodent models established the role of mitochondrial dysfunction, defective ubiquitin proteasomal pathway and energy metabolism, apoptosis, neuroinflammation, microglial activation, dopamine autoxidation and oxidative stress in the pathogenesis of PD [4, 10, 101]. Many chemicals used to mimic PD symptoms in rodents lead to α-synuclein aggregation (but lack of defined Lewy body formation, except in rotenone) and subsequent cell death owing to their ability to inhibit proteasomal and mitochondrial functions. The results obtained from rodent models substantially mimic the results obtained from the clinical samples and postmortem brain of PD patients [101]. Owing to such similarities, rodent models are also used to test the therapeutic efficacy of several drugs and natural products and also to design new modes of therapy for the disease [52]. Despite all possible efforts, none of the current models completely mimics sporadic PD [100, 102]. The difference in the life span from humans could also contribute to the absence of cardinal pathogenic features of sporadic PD, such as Lewy body formation in rodents. Furthermore, lack of a neuromelanin and distinct regulatory pattern of tyrosine hydroxylase (TH) makes rodent models less reliable [101, 102]. Although immunohistochemical and biochemical observations exhibited the similar pattern of results, the degree of differences is still enormous not only between sporadic and chemically induced PD but also among various rodent models [100]. A few of them develop PD symptoms after acute exposure contrary to the chronic progression of sporadic and familial forms of PD in the humans [68, 100, 102]. A few others though chronic in nature, are either not yet established unequivocally or reported from specific laboratories or do not produce the cardinal features of PD (Fig. 1) [9]. For example, rotenone is nonspecific, MPTP mainly leads to rapid neurodegeneration, and paraquat may lead to death of many experimental animals at the concentration and time of exposure, which induce PD phenotype in mice. The development of defined Lewy body, one of the basic hallmarks of sporadic PD, is reported only in a rotenone-induced rodent model [25, 32]. Moreover, reserpine and amphetamine do not produce significant

degeneration of dopaminergic neurons and pesticides, and metals possess one or the other drawbacks detailed above [52, 65, 100]. Dual rodent models are relevant to PD pathogenesis and could be more appropriate for assessing the efficacy of therapeutic agents [92]; however, developing such models in experimental rodents is difficult, cumbersome, and cost-consuming.

While it seems quite difficult to translate findings of rodent models in humans, as most of rodent models possess different chromosomal localization of a few identified causative genes with significant degree of differences in behavior, environment and anatomy of the brain and responses towards a toxin as compared with humans, it is expected that after the advancement of newer tools and development of an ideal animal model, it would become easier [100–102]. Most of the neuroprotective agents, which succeeded in preclinical investigations in rodents, could not be successfully translated into clinical interventions. For proper translation of the mechanistic observations and extrapolation of rodent data to humans, it is essential to develop and validate newer rodent models that could help to overcome the disparity and drawbacks in connection with the current animal models.

Omics and RNAi in Understanding PD Pathology

Omics tools, such as genomics, proteomics and transcriptomics, are large-scale technologies used to generate a plethora of information based on genetic variations and global expression profiles of genes and proteins and to identify the differentially expressed transcripts/proteins. Omics-driven information provided clues to identify the individuals at high risk, develop molecular fingerprints for diagnosis as well as for discrimination of various stages of PD pathogenesis (Fig. 2) [3, 26, 103, 104].

Single Nucleotide Polymorphisms

Genome-wide association studies of several single nucleotide polymorphisms (SNPs) with PD-linked genes are reported [105]. For example, SNPs in various forms of synuclein (SNC-A, SNC-B, and SNC-G), Parkin, UCHL1, PINK1, DJ1, LRRK2, ATP13A2, and Omi/HtrA2 genes and their association with PD are recently established [105–107]. Polymorphism in mitochondrial DNA-encoded complex I gene validated the role of mitochondria in PD pathogenesis [108]. As most of such studies are performed in humans rather than rodents, such

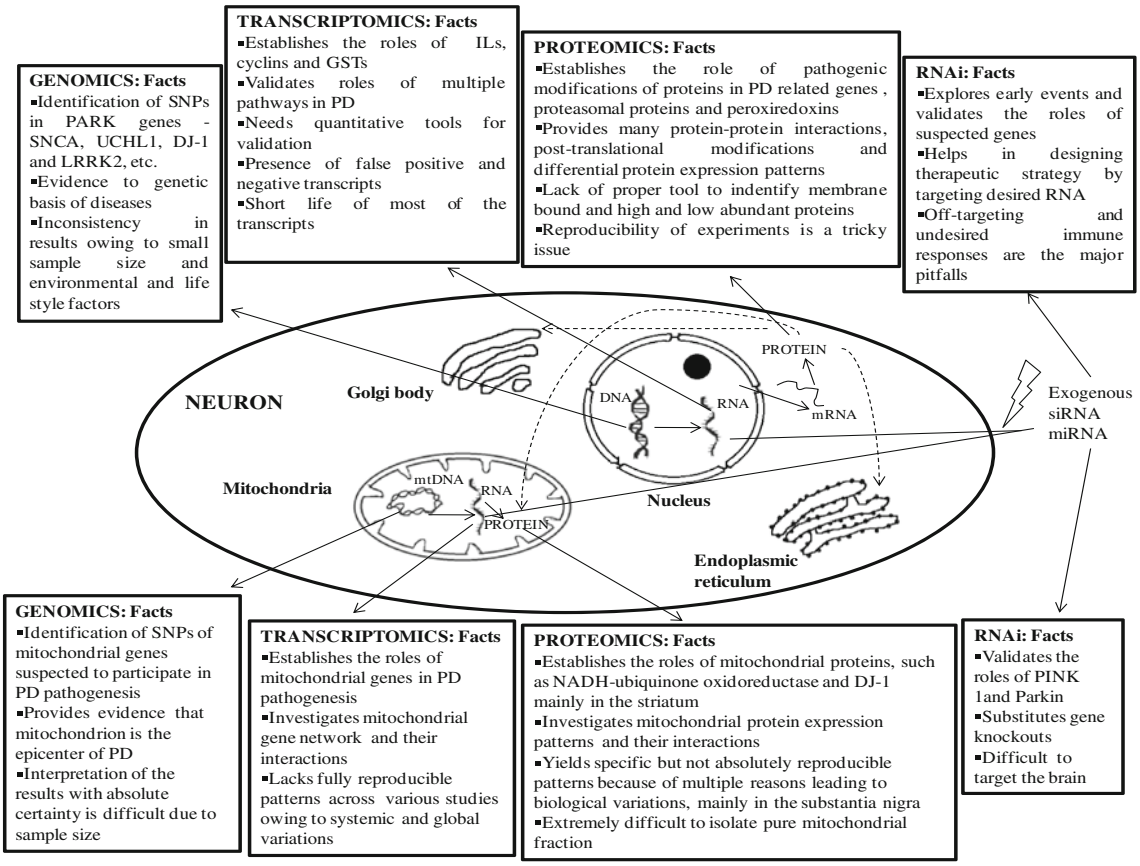

Fig. 2 Contribution of genomics, transcriptomics, proteomics, and RNAi in understanding PD pathogenesis at cellular and organellar levels and their pros and cons in understanding PD pathogenesis [3, 5, 21, 22, 26, 27, 106–108, 116, 137]

investigations are not being discussed in detail. In summary, conflicting reports are available in literature even in the same population. The results of a few investigations revealed the association of SNPs of the selected genes with PD; however, others have shown lack of such associations [105, 107].

Transcriptomics

The differential transcription profiling has been used to simultaneously assess the role of many transcripts in PD pathogenesis [109]. Several groups of investigators working in this area have generated enormous information employing various rodent models. The main biological pathways involved in PD pathogenesis, which could get consensus among the studies, are neurotransmission, dopamine metabolism, biodegradation and transportation, oxidative stress, mitochondrial function, energy metabolism, neuroinflammation, protein accumulation and degradation, and apoptosis (Table 1) [110–115]. Several studies concentrated on the role of transcripts derived from nuclear origin; however, studies are also available in which the role of the mitochondrial system is assessed. A study based on mitochondrial transcripts profiling has shown specific susceptibility of the striatum for oxidative phosphorylation deficiency [116]. Transcriptional profiling of brain and blood showed an alteration in SNC-A as a critical pathogenic feature [117]. Microarray has been extensively used to assess the changes in gene expression patterns in most of toxins-induced rodent models, except a few models that were recently reported [118–122]. Microarray data established the roles of many genes that are associated with familial and toxins-induced PD. For example, Parkin and α-synuclein are reported to play critical roles in the regulation of ubiquitin proteasomal pathway and subsequently in neurodegeneration and neuroprotection [110]. Although many studies have also been conducted using human sample, which can provide direct information about the alteration in the gene expression at the later stages of PD [109, 110], such information is not discussed in detail as the main purpose of the article is to discuss rodent models. Transcriptomics can even be done with the human samples as well, since the mRNA of the postmortem brain can be stable up to 36 h after death [123].

Proteomics

Classical proteomics and modern quantitative proteomics in combination with bioinformatics are used to analyze the expression levels and posttranslational changes in the proteins of the tissues and biological fluids [124, 125]. Nigrostriatal proteomics starting from the postmortem brain up to animal models not only transformed the understanding of the genetic basis of the disease but also provided information to develop therapeutic strategies to encounter PD by identifying specific targets [126]. Posttranslational modification-based molecular fingerprints could be identified and protein localization and translocation can be recognized by employing proteomic tools [127–129]. Furthermore, proteomics provided the clues to PD pathogenesis by generating novel information or by validating the traditional assumptions and established the roles of mitochondrial dysfunction, oxidative stress, kinase and autophagy modulators, and disturbances in protein aggregation and degradation [130–134]. Proteomic approaches, applied to various toxins-induced rodent models, demonstrated that the alteration in the proteins related to mitochondrial complex I, neuronal cytoskeleton, and ubiquitin proteasome system (UPS) was common among them (Table 2) [19, 114, 135–137].

Cellular or quantitative proteomics of blood and cerebrospinal fluid (CSF) is being used to identify, if any suitable biomarker exists for diagnosis of the disease [138]. Blood is considered to be the best sample because it is not only obtained using a less invasive tool but also is the most dynamic tissue of the body [139]. CSF is also an attractive and ideal body fluid to search for PD biomarkers [140, 141]. By proteomics-based approaches, neuromelanin, mortalin, DJ-1, haptoglobin derivatives, truncated globin, aggregated serum amyloid P component, and ion channel proteins have been identified as differentially regulated proteins in the blood or CSF of PD patients [21, 135, 140, 142–145]. Many proteins that include, α-synuclein and UCH L-1, are dysregulated in patients and are expected to help in understanding and solving a few mysteries of PD pathogenesis [146, 147]. Proteomic approaches confirmed that α-synuclein undergoes posttranslational modification, a triggering event for neurotoxicity [146].

RNAi

Silencing of autosomal dominant genes, which play critical roles in PD pathogenesis could act as novel therapeutic targets for treating PD. RNAi, used to silence the selected gene expression, is projected as a substitute for the gene knockout approach to study the complex molecular and biochemical interactions within the pathways known to involve in PD pathogenesis [148]. As mutation in the genes belonging to PARK loci, oxidative stress, dysfunctional xenobiotic metabolizing machinery, inflammation, autophagy, and apoptosis [4, 22, 88] are associated with PD pathogenesis, therefore, direct or indirect modulators of such genes could be astonishing targets of RNAi-based studies [149, 150]. Small interfering RNAs (siRNAs) and micro RNAs (miRNAs) have made substantial input towards understanding the early events implicated in the functioning of dopaminergic neurons. The siRNA knockdown studies elucidated that the expression of homeoprotein LIM homeobox transcription factor 1 α (Lmx1 α) is the prerequisite for

Table 1 Details of some transcriptomics studies conducted employing rodent models and list of genes and pathways involved in PD pathogenesis

Serial no.	Toxin (rodent model)	Tissue	Affected pathways	Differential gene expression level	References
1.	6-OHDA (rat)	Striatum	Neurotrophic factors and neurotransmitter release	Increases neurotensin, neuromedin U receptor, Finkel–Biskis–Jinkins murine osteosarcoma viral oncogene cellular homolog, cyclooxygenase-2, follistatin, neuromedin U, platelet-derived growth factor-D, orphan nuclear receptor-1 and TAC 2 expressions Reduces TAC 1 expression	[115]
2.	MPTP (rat)	Striatum	Cell growth, differentiation, regeneration and survival	Upregulates cerebellin 1 precursor protein, galanin, nerve growth factor receptor, and signal transducer and activator of transcription 4 expressions Downregulates ciliary neurotrophic factor expression	[118]
3.	MPTP (mouse)	Nigro-striatum	Mitochondrial dysfunction, oxidative stress, and apoptosis	Augments cathepsin D and UCH-14 expressions Attenuates ATP synthase protein 8, glutathione *S*-transferase (GST) mu-5 (Gstm5), and NADH–ubiquinone oxidoreductase expressions	[114]
4.	MPTP (mouse)	Striatum	Inflammatory responses, cytokine and mammalian target of rapamycin-signaling pathways, activation of astrocytes and cellular stress	Increases heme oxygenase 1 metallothionein 2, uncoupling protein 2, growth arrest and DNA-damage-inducible-beta, nuclear factor of kappa light polypeptide gene enhancer inhibitor, FBJ murine osteosarcoma viral oncogene homolog B, DNA-damage-inducible transcript 4, CD9 antigen and heparin-binding epidermal growth factor-like growth factor expressions Reduces retinoid X receptor gamma and paired box gene 8 expressions	[119]
5.	Maneb and paraquat (mouse)	Striatum	Electron transport, lipid metabolism, cell cycle, oxido-reductase activity, ubiquitin proteosomal system, and apoptosis	Upregulates ubiquitin C and programmed cell death 10 expressions Downregulates cytochrome c oxidase subunit VI a polypeptide 1, diazepam binding inhibitor, growth arrest specific 5, superoxide dismutase 1, and clusterin expressions	[111]
6.	MPTP (mouse)	Substantia nigra	Apoptosis, proteasomal system, and energy metabolism	Increases lactate dehydrogenase 2 B chain, fibroblast growth factor 1, cytochrome P450 family 4 subfamily V polypeptide 3, RAS-like estrogen-regulated growth-inhibitor, and protein tyrosine phosphatase receptor type Z polypeptide 1 expressions Reduces lipoprotein lipase, cadherin 2, G protein-coupled receptor 83, neuropilin, cysteine-rich motor neuron 1 and growth hormone receptor expressions	[120]
7.	MPTP (mouse)	Nigro-striatum	Inflammation, oxidative stress, glutamate toxicity, cell cycle, and cell death processes	Increases interleukin (IL)-1b, IL-10, nuclear factor kappa B (NF-kB) p65, N-methyl D-aspartate (NMDA) adenosine A_{2A} receptor (A_{2A}-R), cyclin B2, tumor necrosis factor (TNF) α and parkin expressions	[121]
8.	MPTP (mouse)	Nigro-striatum	Cell cycle, oxidative stress, inflammatory processes, glutamate signaling, and neuronal differentiation	Increases Bax membrane isoform α, IL-2 receptor gamma, TNF-β and IL-1β expressions Reduces G2/M-specific cyclin B2, proliferation-associated protein 1, cytochrome P450 1A1, GST- A, inhibitor-kB α subunit and NF-kB p65 expressions	[122]

neuronal progenitors to determine a dopaminergic fate [22, 151]. Similarly, the mutual interaction between miRNA-133b and Pitx 3 is reported to regulate the differentiation and survival of the midbrain dopaminergic neurons [152]. The tiny non-coding RNAs carry out localized control of gene expression in the neurons and any anomaly in it may lead to PD [153]. Tiny non-coding RNAs play a fundamental role in neurodegenerative diseases in rodents, as evidenced by the complete deficiency of a particular miRNA expression during neuronal loss [154]. Downregulations of miRNA-34b and miRNA-34c in PD brains hypothesize the involvement of these tiny non-coding molecules in mitochondrial dysfunction [155]. Furthermore, a few miRNAs are also identified to regulate the synthesis of neurotransmitter substance P by the *tachykinin* (*TAC1*) gene [156]. Small non-coding RNAs validated the existing knowledge regarding various genes, such

Table 2 Details of a few proteomics studies conducted employing various rodent models and list of proteins and pathways involved in PD pathogenesis

Serial no.	Toxin (rodent model)	Used tissue	Affected pathways	Differential protein expression level	References
1.	6-OHDA (rat)	Substantia nigra	Mitochondrial function	Increases prohibitin and complex I 30-kDa subunit expressions	[135]
2.	6-OHDA (rat)	Striatum	Energy metabolism, calcium homeostasis, antioxidation, and cytoskeletal	Reduces calreticulin and calmodulin expressions Increases peroxiredoxin 2, mitochondrial complex I and III expressions	[136]
3.	MPTP (mouse)	Mitochondrial fraction of the striatum	UPS	Attenuates 19S proteasome ATPase Rpt6 expression Augments α-synuclein expression	[132]
4.	MPTP (mouse)	Striatum, cortex, cerebellum	Dopamine signaling, mitochondrial function, UPS, calcium signaling	Upregulates ubiquitin-specific protease expression Downregulates cytochrome c oxidase subunit Vic, vacuolar ATP synthase subunit F, and TH expressions	[133]
5.	MPTP (mouse)	Mitochondrial fraction of the substantia nigra	Mitochondrial function and oxidative stress signaling	Increases DJ-1 expression Reduces complex I expression	[137]
6.	MPTP and methampheta-mine (mouse)	Striatum	Mitochondrial function, oxidative stress, signaling, and UPS	Upregulates cytochrome c1, calpain-2, and ubiquitin ligases expressions Downregulates glutathione peroxidase-4 and GST-M5, F- and V-type ATPase and proteasome subunit protein expressions	[114]
7.	Maneb and paraquat (mouse)	Striatum	Neurotransmitter release and glycolysis	Decreases complexin I, α-enolase, and glia maturation factor-β expressions	[166]
8.	Cypermethrin (rat)	Striatum and substantia nigra	Mitochondrial function, neurotransmitter release, and cytoskeletal assembly	Reduces stathmin, nicotinamide adenine dinucleotide isocitrate dehydrogenase α-subunit, prohibitin, ubiquitin conjugating enzyme, nicotinamide adenine dinucleotide dehydrogenase 24 k chain precursor, heat shock protein (Hsp)-70, and synaptosomal associated protein-25 expressions Increases phosphoethanolamine binding protein-1, mitogen-activated protein kinase activated kinase-5, Hsp-60, and α-internexin intermediate filament expressions	[19]

as α-synuclein, TH, Parkin, PINK1 and LRRK2, which are implicated in PD pathogenesis and assisted to gain newer insights [149, 157–159]. Thus, siRNA-mediated depletion of the major players involved in PD pathology and progression may help in designing a new therapeutic strategy for PD. Additionally, miRNA mimetic or expression vector could be used to restore or overexpress miRNAs of interest [22, 160, 161].

Omics and RNAi in PD: Noteworthy Validation but Incomplete Applications

Genomic (SNP-based) studies have a number of limitations, including sample size, life style factors, data interpretation, and varying levels of environmental exposure. Indeed, genomics offered an initiative to assess the association of SNPs in a larger scale at a rapid rate employing a huge sample size and predicted the possible association of many genes in PD pathogenesis (Fig. 2) [162]. The major disadvantage is variation among studies even within the same population.

Evidences about the dysregulation of genes, associated with the neurotransmission, synaptic function, oxidative stress, mitochondrial function and energy production, protein misfolding and aggregation, UPS dysfunction, autophagy and apoptosis, etc., are known, however, the microarray data yielded inconsistent results across multiple studies [110–113]. Despite validation with secondary quantitative tools, transcriptomics of the nigrostriatal tissues could not help in projecting the role of infrequently expressed genes in PD pathogenesis [111–113]. One of the major reasons could be the loss of a substantial number of cell bodies of the dopaminergic neurons in the substantia nigra and related

fibers in the striatum, which are used for data generation and subsequent analysis. Many genes expressed at low levels and in a particular cell type make the implication of global study of the gene expression pattern questionable [163]. Therefore, the gene expression patterns of the striatum or substantia nigra, the ideal tissues used for microarray assessment of rodents with PD phenotype, need an error-free approach. The disagreement could also be due to the use of multiple arrays (mitochondrial genes specific, apoptotic genes specific, cytoskeletal genes specific, etc.), varying labeling procedures (direct or specific indirect methods), variable hybridization patterns (time of hybridization, types, and compositions of hybridization and washing buffers), and difference in the number and type of replicates (with or without dye swapping/use of single dye or two different dyes for controls and experimental sets) [26]. Therefore, microarray data generated and analyzed from the rodents across multiple studies would offer invaluable insights, if all such variables are kept unchanged. Furthermore, the RNA sample of the striatum or substantia nigra represents RNAs of multiple neuronal origins (such as dopaminergic, serotoninergic, glutaminergic, cholinergic, etc.); therefore, the representation of the RNAs of the dopaminergic neurons are compromised, which makes it complicated to distinguish [26]. Employing a microarray technique, several novel transcripts involved in PD pathogenesis, and treatment outcomes are identified [109]. But the transcripts getting upregulated or downregulated do not necessarily mimic the level of protein expressions, which are the actual regulators of neurodegeneration, as many genes are transcribed but not translated [27, 114]. Similarly, microarray profiling of blood offered important clues to assess the role of specific genes in PD pathogenesis [164]; however, in blood microarray patterns, several regulating factors could be compromised. Overall, the identification of causative and modulatory genes improved our understanding of the underlying etiology and mechanisms of pathogenesis, prevention and cure are perhaps at the same stage as those were before the application of these tools in PD research. A microarray technique is not more than a semiquantitative tool even after considering all the available corrective measures and validation of data with real-time PCR or other secondary tools is mandatory [27, 111]. Despite significant contribution of microarray tool in assessing the putative mechanism of the disease pathogenesis, the technology still fails to provide accurate and complete mechanism of sporadic PD. Overall, genomic approaches generated huge amounts of data, the main drawbacks remain to be elusive etiology and pathogenesis, as little success could be achieved in translating the information.

Several groups of investigators have identified a few differentially expressed proteins, which could not be tested across multiple laboratories due to lack of expensive tools and requirement of expert technical skills [124]. As observed in the clinical investigation [165], reproducibility across multiple studies could have been a major concern even in the rodents due to lesser sample size and varying conditions of animals storage and maintenance as well underlying experimental procedures [9, 21]. One of the most important reasons of failure is the lack of standard experimental procedures for the collection, storage and processing of samples, and lack of common strategies across various studies for the removal of the highly abundant proteins [21, 142, 166]. Proteomics has not yet delivered an expected breakthrough in PD diagnosis and identification of potential and efficacious counteractive measures [21]. The major reasons have been the huge discrepancy in the translation of rodent proteome data to human and most importantly difference in the expression patterns of the identified proteins from one rodent stock to another [102, 167]. The difficulty is more critical in rodents where the variation could be observed from a rodent to another one as well as between the inbred and outbred stocks of the same rodent. Owing to the BBB, many proteins are not able to enter the blood stream [168] and the proteins identified to date are the outcomes of pathogenesis rather than the triggering response and therefore cannot serve the purpose of a genuine biomarker [21]. In summary, proteomic approaches identified hundreds and hundreds of proteins that are differentially expressed but have not yet satisfactorily met the goal of developing biomarkers or molecular fingerprints suitable for real application in clinics.

RNAi elucidates mainly the role of already suspected genes/proteins in PD and mainly acts as a validation tool [22]. RNAi could be very successful for monogenic dominant genetic disorders, as only one gene is responsible for the overall consequence of that disease. But for PD, which has a multifactorial etiology and elusive pathogenesis, only combinational approaches, such as silencing of multiple targets, could possibly help in developing therapy. Similarly, the drugs developed from the synthetic tiny non-coding RNA molecules need to be tested for their toxicity, tolerability, and undesired off-target effects before they are used for clinical interventions [22]. Tiny non-coding RNA-mediated interference elicits immune responses and produces pro-inflammatory cytokines and interferons that may affect the disease progression [169–171], which tip off a caution for its use in treating PD. This technique may get desirable success beyond laboratory only when the undesired immune responses could be defeated. RNAi tools are still in their infancy and need to be continuously exploited to gain certain novel information regarding elusive aspects of PD pathophysiology.

The exact translation of omics and RNAi-generated data employing rodent models is not possible because of many primary reasons. The total number of genes and transcripts,

translated proteins, and posttranslational modifications varies from rodent to rodent and from rodent to human [27, 102]. Although these sophisticated tools gave the role of multiple genes/proteins/transcripts and validate the role of the most important ones (Fig. 2), lack of consensus among the results obtained from many studies and their subsequent translation without fail in humans are limited owing to lack of an ideal rodent model [102, 167]. Many proteins do not perform the similar functions across multiple species/genus [102]. Apart from such limitations, there are many additional hurdles for the application of sophisticated tools in understanding the disease pathogenesis. Such studies need to be performed in the multiple rodent models and postmortem human brains at extensive scale across laboratories, globally. But the drawbacks of the recent molecular tools are lack of cheap consumables, need of high technical expertise, and requirement of highly specialized and costly equipments [7]. Many more questions are still unresolved even with the availability of these sophisticated techniques as the relationship between protein aggregation and the molecular events leading to neurodegeneration has not yet been clarified [131].

Future Possibilities

Despite extensive efforts, rodent models and molecular tools could not identify the realistic biomarkers and still face barricading to pick up the fingerprints for an early diagnosis and unbiased assessment of treatment outcomes. Although lower animal models are beneficial to understand the role of genetic factors in PD, development of only an appropriate rodent or primate model could help in understanding the pathogenesis by employing modern molecular tools and RNAi. Success of a rodent or nonhuman primate model is purely dependent on the availability of epidemiological information across multiple populations, followed by appropriate statistical analyses. If everything goes all right, an ideal rodent or nonhuman primate model can be developed. Once all genuine causative factors would be identified, an authentic dual model may be developed to understand the precise pathology of sporadic PD. A combinational approach of employing an ideal model with multifaceted omics and RNAi could be expected to offer the pragmatic mechanistic understanding of PD pathogenesis and successfully be translated to sporadic PD. Theoretically, the dual or interactive model approach would offer the best prospect to understand sporadic PD pathogenesis; however, practical conquest lies in the development of a suitable combination approach.

Acknowledgments We sincerely thank the Council of Scientific and Industrial Research (CSIR), New Delhi, India/University Grants Commission, New Delhi, India for providing research fellowships to Sharawan Yadav, Sonal Agrawal, Garima Srivastava, Anand Kumar Singh, and Anubhuti Dixit. The CSIR-IITR communication number of the article is 3012.

Conflicts of Interest None declared.

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