ROLE OF THE MICROTUBULE-ASSOCIATED TPPP/p25 IN PARKINSON'S AND RELATED DISEASES AND ITS THERAPEUTIC POTENTIAL

ABSTRACT

Introduction: The discovery and development of therapeutic strategies for the treatments of Parkinson's disease (PD) and other synucleinopathies are limited by a lack of understanding of the pathomechanisms and their connection with different diseases such as cancers.

Areas covered: The hallmarks of these diseases are frequently multifunctional disordered proteins displaying moonlighting and/or chameleon features, which are challenging drug targets. A representative of these proteins is the disordered Tubulin Polymerization Promoting Protein (TPPP/p25) expressed specifically in oligodendrocytes (OLGs) in normal brain. Its non-physiological level is tightly related to the etiology of PD and Multiple System Atrophy (TPPP/p25 enrichment in inclusions of neurons and OLGs, respectively), multiple sclerosis (TPPP/p25-positive OLG destruction), as well as glioma (loss of TPPP/p25 expression). The established anti-proliferative potency of TPPP/p25 may raise its influence in cancer development. The recognition that whereas too much TPPP/p25 could kill neurons in PD, but its loss keeps cells alive in cancer could contribute to our understanding of the interrelationship of "TPPP/p25 diseases".

Expert commentary: The knowledge accumulated so far underlines the key roles of the multifunctional TPPP/p25 in both physiological and diverse pathological processes, consequently its validation as drug target sorely needs a new innovative strategy that is briefly reviewed here.

KEYWORDS

α-synuclein, cancer, CNS diseases, drug target, microtubule, protein chameleon, tubulin deacetylases, Tubulin Polymerization Promoting Protein/p25, validation.

1. INTRODUCTION: PRESENT STATE OF PARKINSON RESEARCH

Parkinson's disease (PD), the second most common neurodegenerative disorder belongs to the family of synucleinopathies, which includes dementia with Lewy bodies (LBD) and multiple system atrophy (MSA). Classic motor signs of PD can be attributed mainly to the substantial loss of dopamine-containing neurons in the substantia nigra pars compacta [1]. L-Dopa, dopamine agonists and amantadine as well as new MAO-B and catechol-o-methyltransferase inhibitors appear to be efficacious treatments of motor complications [2,3]. Indeed, L-Dopa appears as the most effective therapy, it is considered as a "gold-standard"; however, after 4-6 years the patients frequently suffer from severe side effects such as dyskinesia [4]. During the past decades pharmacotherapy, deep brain stimulation and physiotherapy have been evaluated for the symptomatic treatment of PD as well. The major problem with the pharmacotherapy is the lack of drugs that halt the progression of the disease. There are new drugs and other therapies such as glia cell line-derived neurotrophic factor (GDNF); however, these treatments failed in phase 3 trials to translate the successful results from preclinical to clinical studies [3,4] due to the application of inappropriate preclinical models (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine) as well as targets often representing late stage of PD [4].

In the recent years, the Parkinson research on new drugs focuses on α-synuclein (hallmark protein of the disease), its small oligomers and related proteins [4]. α-synuclein is involved in the

control of neurotransmitter release and re-cycling as well as in synaptic and structural plasticity [5]; its aggregation can affect synaptic function and axonal firmness, which is supported by the observation that neuronal susceptibility in PD is related to axonal length, axonal caliber and the degree of myelination [5]. Immunizations against α-synuclein and inhibitors or modulators of α-synuclein aggregation are under clinical trials [4]. The disordered α-synuclein is degraded mainly by the ubiquitin–proteasome system, whereas more complex conformations, including aggregates, are removed by the autophagy–lysosomal pathway [6-8]. In fact, pathogenic depletion of proteasome components and lysosomes observed in brains of PD patients has underlined the notion that defects in the protein quality-control mechanism contribute to PD pathogenesis [9-10].

Progressive neuronal cell loss and widespread aggregation of α-synuclein forming Lewy bodies and Lewy neurites are the major characteristics of PD and other synucleionopathies [11]. After the initial appearance of Lewy neurites, additional aggregates are formed and rapidly accumulate leading to oligomers, protofibrils and finally insoluble Lewy bodies and Lewy neurites [12]. Although the staging/categorization systems (such as Braak's staging) are appropriate in most cases; a significant number of patients with widespread α-synuclein aggregation classified in Braak stage 5-6 do not have a diagnosis or symptoms of PD [13,14]. In fact, until the recent past the α-synuclein aggregate was considered as a major drug target; nowadays it seems to be justified that the small, soluble oligomeric forms are regarded as the fatal species [15], the assembly/co-assembly of which leads to the formation of aggregates. In this complex process the mutations of α-synuclein also play a role. The identification of mutations in the gene encoding α-synuclein (SNCA) in families with PD had a substantial effect on PD research [16]. Clinical mutations of human α-synuclein are Ala53Thr, Ala30Pro, Glu46Lys, His50Gln, Gly51Asp and Ala53Glu; and the presence of two major phosphorylation sites, Ser87 and Ser129 in Lewy bodies has been reported to be characteristic for PD pathology [17,18]. However, it is well documented that wild type α-synuclein aggregates were detected in the brain inclusions of patients suffering from sporadic PD, which accounts for about 90% of all cases [3]. In order to prevent/arrest the pathological assembly of α-synuclein, the not-yet discovered mechanism driving the etiology of PD needs to be established, in this relation the microtubule system is a key player.

The microtubule network plays crucial roles in diverse processes such as cell division, differentiation, intracellular trafficking as well as in pathological inclusion body formation; its dynamic instability is central to these functions allowing them to rapidly reorganize, differentiate spatially and temporally in accordance with environmental signals/factors [19,20]. The microtubules, their major posttranslational modifications (tyrosination, acetylation) and microtubule associated proteins (parkin, PTEN induced putative kinase 1 (PINK1), leucine-rich repeat kinase 2 (LRRK2) and α-synuclein) regulate long-distance intracellular cargo trafficking along axons and dendrites; the dysfunction of the interplay between cytoskeletal components is reflected in PD. The fragmentation of stable microtubules and the depletion of dynamic ones coupled with impairment of axonal transport seem to be common in the early phases of the pathology of synucleinopathies [21,22]. In PD experimental models, an earlier microtubule destabilization followed by the enrichment of acetylated and detyrosinated microtubules and the block of axonal transport were often observed. Microtubules are necessary in pathological inclusion body formation [23], since aggregated protein species are delivered to the aggresome by a retrograde transport on microtubules [24]. However, the relationship between microtubules and α-synuclein is complicated [21,22]. Tubulin can promote α-synuclein fibrillation in vitro, but there are conflicting data on whether destabilization of the microtubule network facilitates or prevents the aggregation in vivo, or α-synuclein influences microtubule formation. Recently α-synuclein has been found as a foldable microtubule dynamase regulating nucleation and dynamics of microtubules [25].

In conclusion, to date the exact mechanisms of progressive dopaminergic cell loss remain to be unraveled. It is a real question what induces aggregation of α-synuclein. Lewy bodies contain a variety of molecular components such as tubulin, microtubule associated proteins, ubiquitin, heat shock and autophagosomal proteins [26] and Tubulin Polymerization Promoting Protein (TPPP/p25) [27-30] that could be the initiator of α-synuclein aggregation. Another important, but not well-studied aspect of PD pathology is the concomitant beta-amyloid and/or tau aggregates in a significant number of patients [14,31,32]. No disease-modifying treatment is available [21,33,34], consequently, therapeutic development should focus on the hallmark proteins. In reality, there is a real stagnation in the development of new drugs. The crucial bottleneck is the lack of primary endpoints, which reflect the progression of PD when the substantia nigra is not or only very mildly affected. Our new innovative strategy to validate an anti-Parkinson drug target makes attempt to contribute to this issue.

2. MOONLIGHTING AND CHAMELEON FEATURE OF TPPP/p25

TPPP/p25 has been identified besides α-synuclein as hallmark of PD and synucleinopathies [27-29]. It does not have a well-defined 3D structure; it is an intrinsically disordered protein [35,36]. It modulates the dynamics and stability of the microtubule system *via* its bundling and tubulin acetylation promoting activities [37,38]. These physiological functions are mediated by its direct associations with tubulin/microtubules as its primary target. In vitro TPPP/p25 is able to induce the polymerization of tubulin into normal and double-walled microtubules, tubulin aggregates and microtubule bundles as shown by electron microscopy [37] (Fig. 1). The co-localization of TPPP/p25 with the microtubule network was observed by immunofluorescence microscopy in various cell lines expressing the protein either endogenously (CG-4 cells) or after transient transfection (HeLa cells), and its bundling activity protects the microtubules against depolymerizing agents [23,38] (Fig. 1).

Mechanism by which TPPP/p25 can stabilize the microtubule network manifests itself by its dimerization capability; the dimerization induces partial folding and the exposed unstructured terminals associate with distinct microtubules resulting in their cross-linking as demonstrated by a special sandwich enzyme-linked immunosorbent assay, pelleting experiments and bimolecular fluorescence complementation assay [39].

TPPP/p25 can affect the acetylation level of the microtubule network by its direct association of the tubulin deacetylases such as histone deacetylase 6 (HDAC6) and the NAD^+-dependent deacetylase sirtuin-2 (SIRT2) [38,40]; while the classic HDAC6 inhibitors, TSA and SAHA, result in maximum acetylation of the microtubule network at micromolar concentration, AGK2, a SIRT2 specific inhibitor, appears to be ineffective even at a 10-fold higher concentration (Fig. 2). Compound 9, a bis hydroxamate compound is less active than the classic inhibitors likely due to its poor permeability as demonstrated by cellular ELISA experiments [41]. Thus it was concluded that such in vitro experiments performed with HeLa cells using HDAC6 inhibitors with distinct potency are suitable for functional studies at controlled acetylation levels.

HDAC6 displays a tubulin deacetylase activity although it also modulates microtubule dynamics without altering acetylation as proved by using a catalytically inactive enzyme [42]. Moreover, HDAC6 also plays a role in the transport and autophagic clearance of misfolded proteins [43]. The integration of TPPP/p25 to the tubulin-associated HDAC6 significantly reduces the deacetylase activity, TPPP/p25 functions as a HDAC6 inhibitor (Fig. 2). However, this inhibitory potency is not originated from the release of the HDAC6 from the tubulin in the presence of TPPP/p25 rather its structural reorganization within the ternary complex [40]. The binding of TPPP/p25 to HDAC6 and/or SIRT2 as physiological

partner proteins results in increased microtubule acetylation, in addition to its bundling activity, which influence the stability, dynamics, growth velocity of the microtubules as well as the microtubule-derived cell motility [38]. All these functions of TPPP/p25 contribute to the control of cell division; the uncontrolled cell proliferation is one of the major characteristic of the tumor progression.

The multifarious functions of TPPP/p25 are also regulated by post-translational modifications. The phosphorylation mediated by ERK2 and cyclin-dependent kinase 5 (on Thr14, Ser18 and Ser160) resulted in the loss of TPPP/p25-induced microtubule assembly [44]; LIM kinase 1-induced phosphorylation of the Ser residue(s) reduced the polymerization promoting potency of TPPP/p25 [45]. The Rho-associated coiled-coil kinase phosphorylates TPPP/p25 (on Ser32, Ser107 and Ser159), without affecting its tubulin polymerization promoting activity, however, the phosphorylation inhibits its interaction with HDAC6 resulting in increased HDAC6 activity coupled with decreased microtubule acetylation [46]. Therefore, the phosphorylation of distinct sites(s) by different kinases may play a crucial role in its multifunctional functions such as its mitotic regulatory activity [47].

TPPP/p25 is a microtubule associated disordered protein, which is related to its physiological functions; however, it displays pathological ones as well that are determined by its interacting partners (α-synuclein). TPPP/p25 is the prototype of Neomorphic Moonlighting Proteins [48,49] (Fig. 3). The functional binding studies with different truncated, deletion mutants and fragments of TPPP/p25 proved that the binding segment(s) of the full-length TPPP/p25 could be replaced by other segments resulting in distinct specificities and/or binding affinities [50]. This intriguing phenomenon was denoted *neomorphic chameleon* feature, in this case the functional plasticity is due to alterations at gene level [50]. Obviously, the high conformational plasticity with the unique amino acid composition and sequence can ensure exceptional functional resilience.

3. INNOVATIVE STRATEGY FOR PD THERAPY

In addition to α-synuclein [51], the well-established hallmark protein of PD and other synucleinopathies such as MSA, TPPP/p25 has been found to be enriched and co-localized with α-synuclein in the pathological human inclusions functioning as a biomarker protein [27-29,52,53]. TPPP/p25 was demonstrated in Lewy bodies of substantia nigra pars compacta of LBD and PD by immunohistochemistry by confocal microscopy using highly specific mono- and polyclonal antibodies [27-30]. In addition, the presence of TPPP/p25 in isolated Lewy bodies and Lewy neurites was established by double immunofluorescence confocal laser-scanning microscopy [29]. MSA also belongs to synucleinopathies, which is characterized by glial cytoplasmic inclusions [53], in which TPPP/p25 was found to be co-localized with α-synuclein [27,52-54] in spite of the fact that this latter protein is expressed in neurons [55,56]. The potency of TPPP/p25 to induce oligomers and protofilaments of α-synuclein has been reported [29,57,58]. Both TPPP/p25 and α-synuclein can be taken up by HeLa and CHO cells from the medium resulting in their intracellular co-aggregation as detected by immunofluorescence microscopy [57,58]. The precise mechanism how the aberrant protein-protein interactions of these disordered hallmarks convey aggregates leading to the formation of pathological inclusions in brain has not been discovered in details; however, it has been accepted that TPPP/p25 is a promoting factor of the formation of pathological aggregates [29,57-59]. Yet, neither α-synuclein nor TPPP/p25 can be considered as an anti-Parkinson drug target since both proteins display physiological functions as well [48,60]. In addition, as demonstrated very recently drug targeting of moonlighting and/or chameleon proteins involved in neurodegeneration is highly challenging [50].

In rational drug design, molecular docking and crystallography have been usually applied to target globular proteins. In the cases of α-synuclein pathology, in silico high-throughput structure-based

docking screen method combined with experiments was suggested leading to fragment-like molecule design [61]. However, this strategy does not take into account the *neomorphic moonlighting* and *neomorphic chameleon* features of the disordered proteins involved in the inclusion formation in the case of synucleinopathies. Recently the challenges associated with targeting of chameleon proteins have been explored using the TPPP/p25-α-synuclein complex under conditions which is unfavorable for the oligomerization as a case study [50]. The binding segments on TPPP/p25 involved in the physiological and pathological interactions have been identified at molecular and cellular levels [57,58]; accordingly the unstructured C-terminus interacts with tubulin, while the flexible middle CORE segment associates with the C-terminus of α-synuclein [57,58]. The dissimilarity of the binding motives involved in the physiological and pathological interactions has significant innovative impact in anti-Parkinson drug research. Therefore, the interface of the TPPP/p25-α-synuclein complex has been recently identified and validated as a specific drug target and this innovative strategy has been suggested for anti-Parkinson drug development [50,57,58] (Fig. 4).

It is worth noting that in normal brain tissues α-synuclein and TPPP/p25 are expressed predominantly in distinct cell types, neurons or oligodendrocytes (OLGs), respectively [55,56,62,63]. Originally, TPPP/p25 was identified from rat brain; it is localized predominantly in OLGs, in neuropil and fiber-like structures of the CA3 hippocampal region [62]. Subcellular proteomics revealed that besides α-synuclein TPPP/p25 is also a component of neuromelanin granules of substantia nigra neurons [64], and it has also been found in various synaptic preparations as a component of the postsynaptic density, which suggests it may occur in neuronal cells at a very modest level [65]. Cell-to-cell transmission of α-synuclein by different mechanisms as well as the uptake of both hallmark proteins by eukaryotic cells from the media has been reported [57,66]. α-synuclein (as well as its oligomeric or the Ser129 phosphorylated forms) has been detected in the cerebrospinal fluid of PD patients [67,68]. The presence of TPPP/p25 in the cerebrospinal fluid has been detected in some patients suffering from multiple sclerosis [69]. The fact that both proteins have been detected in cerebrospinal fluid [67-69] rationale their co-existence and co-aggregation in both neurons and OLGs of diseased cells. Consequently, the TPPP/p25-α-synuclein pathological complex could be potential drug target; the interface of the complex should be targeted by competitors/foldamers to impede or to destruct the intracellular TPPP/p25-α-synuclein complex with no or minimal side effect.

4. UNCONTROLLED MICROTUBULE DYNAMICS AND CANCER

TPPP/p25 is expressed predominantly in differentiated (but not in the dividing progenitor) OLGs of normal human brain [62,70]; the differentiation is coupled with the expression of TPPP/p25 [63]. OLGs are the main constituents of the myelin sheath wrapping the axons, therefore these cells are indispensable for the proper transmission of signal along axons. Indeed, in the case of multiple sclerosis, which is a demyelinating disease, loss of TPPP/p25-positive OLGs in the brain and increased TPPP/p25 levels in the cerebrospinal fluid of patients were found, respectively [69,71]. Moreover, TPPP/p25 appears as a potential factor of mitotic process in the course of cell cycle and differentiation. In the case of glioma, a brain tumor, the expression of TPPP/p25 is negligible [72]. In agreement with this observation, the microinjection of TPPP/p25 into cleavage *Drosophila* embryo was found to inhibit mitotic spindle assembly and nuclear envelope breakdown without affecting other cellular events [73]. More recent data have shown that TPPP/p25 is involved in the regulation of astral microtubules and spindle orientation during mitosis [74], it reduces cell proliferation via inhibition of the G_1/S-phase transition and the progression of cells into G_1 from G_2/M-phase [47]. Overexpression of TPPP/p25 was found to significantly reduce, while its RNAi-mediated knockdown significantly increased the rate of

cell proliferation in osteosarcoma (U2OS) cell line [47]. These physiological and pathological data concern with the lack/low level of TPPP/p25 expression in cancer cells such as HeLa or neuroblastoma and suggest its anti-proliferative potency (Fig. 5). Rather surprisingly, TPPP/p20 (a homologue of TPPP/p25 lacking its N-terminus) also displays tubulin polymerization promoting and microtubule bundling activities [75], but it is enriched in certain lung carcinomas positively associated with tumor size, metastasis, and poor survival [76,77]; its depletion suppresses the uncontrolled cell proliferation coupled with mitotic abnormalities [78].

There is a growing family of microtubule-associated proteins such as oncogenes, tumor suppressors, and apoptosis regulators that alter the microtubule dynamics which is considered as one of the critical events in tumorigenesis and tumor progression [79]. Many of them are seemingly unrelated proteins that share a common microtubule-related function, thus these microtubule associated proteins with their microtubule-stabilizing/destabilizing potency may play crucial role in the rational microtubule-targeting cancer therapy [79]. Natural and synthetic compounds that disrupt microtubule dynamics are among the most successful and widely used cancer chemotherapeutic agents [79]. There is accumulating evidence that a family of cellular proteins that are associated with and alter the dynamics of microtubules can determine the sensitivity of cancer cells to microtubule-targeting agents and play a role in tumor cell resistance to these agents; such as the pro-apoptotic BIM protein [80].

The relationship between dementia and cancer is complex and far from the agreement. There are observations based upon mostly population and case-control studies that suggest reduced risk of certain cancers and increased risk of malignant melanoma in PD [81-83]. Common factors such as aging, chronic inflammation and immunosenescence have been implicated. However, the pathophysiological pictures are further complicated by the facts that certain cancer therapy agents have neurotoxic effects, while others reduce neurodegeneration [22,84]. Shared genetic pathways seem to be a major focus, particularly those favoring apoptosis and cell proliferation [83]. In fact, evidence has steadily emerged on intriguing relationships between cancer and neurodegenerative diseases, both disorders of aging; however, the signals are mixed that make difficult the straightforward mechanism [81,85]. The mechanisms underlying these associations are far from clear, in part because of the heterogeneity of both cancer and neurodegeneration.

Molecular modulation of microtubule dynamics and stability seems to be the most effective way to affect cancerous processes as demonstrated by treatment of cancer with paclitaxel and Vinca alkaloids acting as stabilizer and destabilizer of the microtubule system, respectively. Nowadays microtubules are also promising targets in PD [84]. Epothilone D (which stabilizes microtubules and can cross the blood-brain barrier) rescued microtubule defects and diminished MPTP-induced degeneration; davunetide (a microtubule-interacting peptide) improved motor functions and reduced the aggregation of α-synuclein in mouse model of PD [21,22]. Tyrosine kinase Abelson (c-Abl) has been implicated in human leukemia, but it also phosphorylates hallmark proteins of PD (α-synuclein, parkin) in dopaminergic neurons [86]. c-Abl kinase inhibitors are applied to treat cancer, and now these molecules (such as nilotinib) are also in the focus of PD therapy. Nilotinib can cross the blood-brain barrier and has been found neuroprotective in animal models of PD; it improved motor behavior of mice and also increased the concentrations of dopamine and its metabolites [87,88]. Further investigations are necessary to study its effects on dopaminergic neurons in models resembling more closely human patients.

The anti-proliferative potency of TPPP/p25, hallmark protein of synucleinopathies, may raise its influence also in cancer development. The recognition that whereas too much TPPP/p25 could kill neurons in PD, but its loss keeps cells alive in cancer could contribute to our understanding of the

interrelationship of "TPPP/p25 diseases". Therefore, the deeper understanding of its physiological and pathological interactions and regulation of its expression levels is of great importance.

5. EXPERT COMMENTARY

Although the motor symptoms of PD can be alleviated by using various therapies; still, no neuroprotective or neurorestorative therapy exists for the treatment of this chronic disorder [3,4,21,33]. The discovery and development of therapeutic strategies for the treatments of PD and other CNS diseases have been limited by a lack of understanding of the mechanisms driving protein aggregation and inclusion body formation which result in progressive dopaminergic cell loss. Hallmark proteins of the conformational diseases such as PD are usually disordered proteins without well-defined 3D structures. These proteins, such as TPPP/p25, often display moonlighting and chameleon features [48,50,89,90], thus the classic bioinformatics (e.g. docking) and experimental methods (e.g. crystallography) are inefficient to develop specific drugs with negligible side-effects. Disordered proteins as drug targets have been rarely studied, but novel experimental and computational methods are emerging to investigate their druggability [91,92]. Targeting of neomorphic moonlighting proteins is a challenging task since the pathological interactions should be prevented/impeded without influencing the physiological ones. This issue has not been taken into consideration in most of the Parkinson research.

In a case study focusing on the TPPP/p25-α-synuclein complex, the potential initiator of the inclusion formation, the dissimilarity of TPPP/p25 binding segments involved in its physiological and pathological interactions could solve the specificity of drug targeting as a new innovative strategy for anti-Parkinson drug development [57,58]. The interfaces of the TPPP/p25 complexed with tubulin and α-synuclein have been recently identified and validated at molecular and cellular levels [57,58]; nevertheless, multinuclear NMR studies would contribute to the identification of the segments at atomic level that renders it possible the design and development of specific drugs such as peptidomimetic foldamers with specific foldamer potency.

6. FIVE-YEAR PERSPECTIVE

A new, exciting and emerging area is the relationship of neurological disorders such as PD with cancer [81-83]. At first, it is hard to imagine two diseases more different than PD and cancer. Tumorigenesis and neurodegeneration have been described as two sides of the same coin [93], and cancer (abnormal cell proliferation) and neurodegeneration (abnormal cell death) can be considered as the opposite ends of a spectrum regarding genes which control the cell cycle [81]. TPPP/p25, besides its potency to induce α-synuclein aggregation, displays anti-proliferative (anti-mitotic) activity as well [47,63,72,73]. Therefore, it seems that the uncontrolled (non-physiological) level of TPPP/p25 leads to distinct diseases: its enrichment leads to PD and other synucleinopathies due to overexpression [27-30,52,53], while its low level or lack is coupled with uncontrolled cell division characteristic for glioma, a brain tumor [72]. In the years to come, our ability to understand the interrelationship of "TPPP/p25 diseases" is expected to grow, and this new area of the medical sciences should be in the focus of the near future researches.

7. KEY ISSUES

- The discovery and development of therapeutic strategies for the treatments of Parkinson's disease (PD) and other synucleinopathies are limited by a lack of understanding of the pathomechanisms driving protein aggregation and inclusion body formation leading to progressive dopaminergic cell loss.

- The hallmarks of these diseases are frequently disordered proteins displaying unique (neomorphic) moonlighting and/or chameleon features, which are challenging drug targets.

- The disordered Tubulin Polymerization Promoting Protein (TPPP/p25), a neomorphic moonlighting protein, displays distinct functions by day and at night; it modulates the dynamics and stability of the microtubule system at physiological condition *via* its bundling and tubulin acetylation promoting activities; however, at pathological conditions it is either co-enriched and co-localized with α-synuclein (in neurodegeneration) or omitted (in cancer).

- As a new innovative strategy in the Parkinson research, the interface segments of TPPP/p25-α-synuclein complex has been validated as potential drug target.

- The recognition that whereas too much TPPP/p25 could kill neurons leading to neurodegeneration, while its loss keeps cells alive in cancer suggesting its anti-proliferative potency; thus these diseases could be classified as "TPPP/p25 diseases".

LIST OF ABBREVIATIONS

dementia with Lewy bodies, LBD; histone deacetylase 6, HDAC6; multiple system atrophy, MSA; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP; oligodendrocyte, OLG; Parkinson's disease, PD; NAD^+-dependent deacetylase sirtuin-2, SIRT2; Tubulin Polymerization Promoting Protein, TPPP/p25.

DECLARATION OF INTERESTS

The authors report no conflicts of interest.

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** Of considerable interest

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***** This was the first study which proved the co-localization of TPPP/p25 and α-synuclein in inclusion bodies characteristic for PD and MSA suggested TPPP/p25 as a hallmark protein of synucleinopathies.***

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**** This study provided evidence for the chameleon feature of TPPP/p25.***

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***** This study suggested a new innovative strategy for PD, and validated the interface of the moonlighting TPPP involved in its pathological interactions as a drug target.***

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FIGURE LEGENDS

Figure 1. Effect of TPPP/p25 on the organization and functions of the microtubule system. A and B: electron microscopic images of TPPP/p25-promoted assembly and bundling of microtubules [73]. Bar is 100 nm in A and 50 nm in B. C: TPPP/p25 (green) aligned along the microtubule network (red) at low (1) and high (2) expression levels [23], bar is 10 μm. D and E: Inhibition of mitotic spindle formation in tubulin-GFP-expressing cleavage Drosophila embryo by TPPP/p25 microinjection at the posterior pole (right) [73]. A-B and D-E are modified from [73], Copyright (2003) National Academy of Sciences.

Figure 2. Relationship of HDAC6 inhibition and the acetylation level of the microtubule system. A: Scheme of the consequence of the interaction of HDAC6 with TPPP/p25. B: Effect of deacetylase inhibitors on the intracellular microtubule acetylation (cellular ELISA) [40,41]. TSA: Trichostatin A, SAHA: suberoyl anilide hydroxy amide, 9: N^1,N^8-dihydroxyoctanediamide [41], AGK2: 2-Cyano-3-[5-(2,5-dichlorophenyl)-2-furanyl]-N-5-quinolinyl-2-propenamide. C: Microtubule network is not acetylated in HeLa cells that do not express TPPP/p25 [38]. D: The microtubule network is acetylated in CG-4 (oligodendrocyte) cells that express TPPP/p25 endogenously [38].

Figure 3. TPPP/p25 is a neomorphic moonlighting and a neomorphic chameleon protein.
Categories of the multi-structural and multi-functional proteins: ***moonlighting protein (MP)****:* displays multiple independent functions without alteration at gene level [94]; ***neomorphic MP***: distinct

functions at physiological and pathological conditions [48,49]; ***chameleon protein (CP):*** high conformational plasticity at protein level [89,90]; ***neomorphic CP***: functional plasticity by alteration at gene level [50].

Figure 4. Potential targets for development of specific drugs for Parkinson's disease and cancer.

Figure 5. Interrelationship between neurological disorders and cancer type diseases. MT: microtubule, SM: multiple sclerosis.

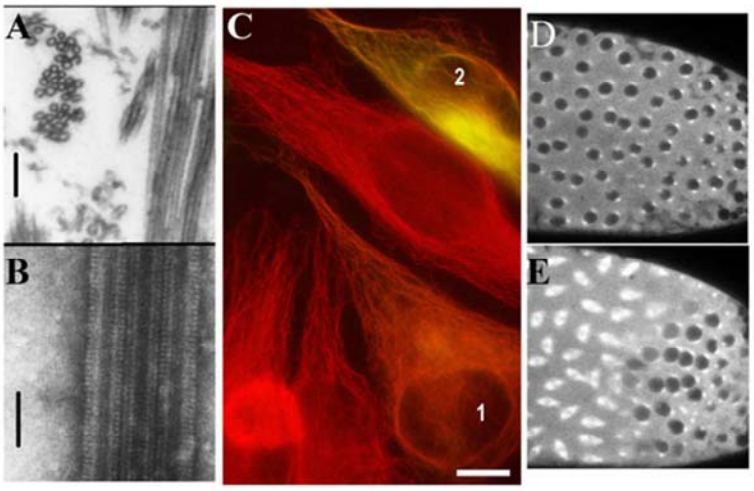

Figure 1

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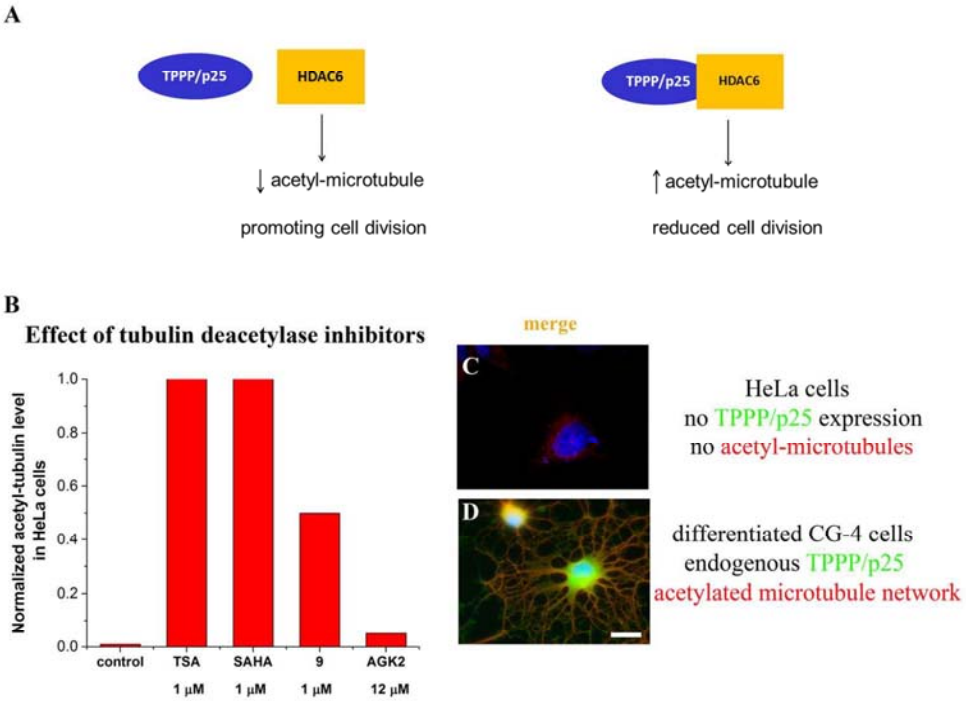

Figure 2

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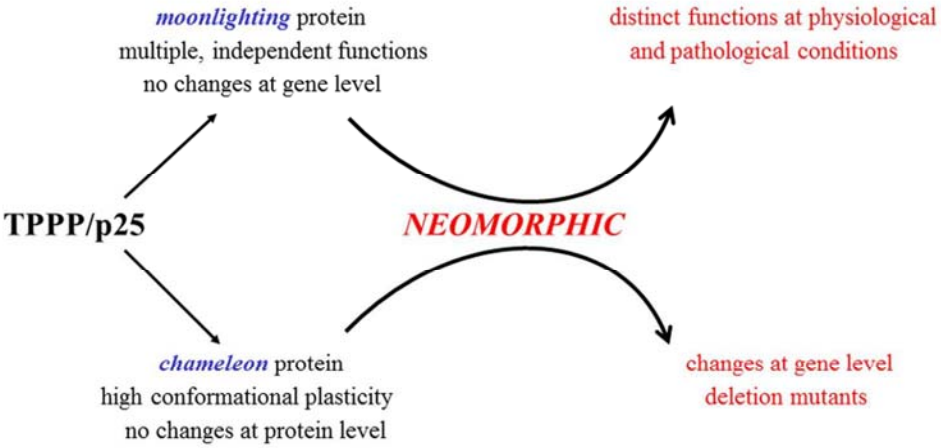

Figure 3

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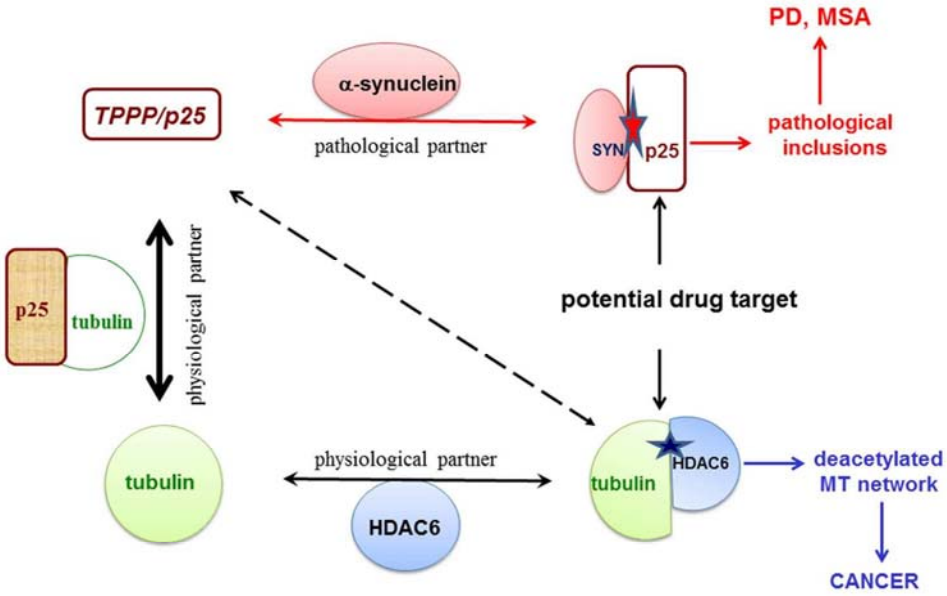

Figure 4

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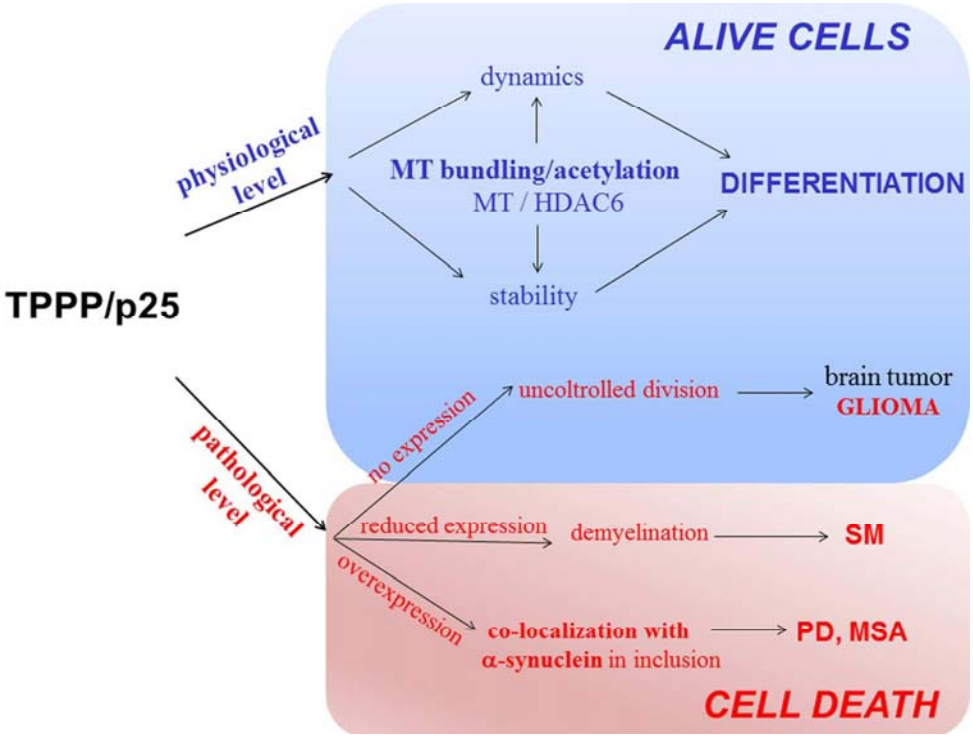

Figure 5

170x127mm (300 x 300 DPI)