Molecular Science

Review

Pin1 and neurodegeneration: a new player for prion disorders?

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Abstract: Pin1 is a peptidyl-prolyl isomerase that catalyzes the *cis/trans* conversion of phosphorylated proteins at serine or threonine residues which precede a proline. The peptidyl-prolyl isomerization induces a conformational change of the proteins involved in cell signaling process. Pin1 dysregulation has been associated with some neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease. Proline-directed phosphorylation is a common regulator of these pathologies and a recent work showed that it is also involved in prion disorders. In fact, prion protein phosphorylation at the Ser-43-Pro motif induces prion protein conversion into a disease-associated form. Furthermore, phosphorylation at Ser-43-Pro has been observed to increase in the cerebral spinal fluid of sporadic Creutzfeldt-Jakob Disease patients. These findings provide new insights into the pathogenesis of prion disorders, suggesting Pin1 as a potential new player in the disease. In this paper, we review the mechanisms underlying Pin1 involvement in the aforementioned neurodegenerative pathologies focusing on the potential role of Pin1 in prion disorders.

1. Introduction

Proline-directed protein phosphorylation is a key event in the regulation of cell signaling that has gained attention thanks to the recent discovery of Pin1, a member of the peptidyl-prolyl *cis/trans* isomerase (PPIase) family. Pin1 controls the *cis/trans* isomerization of protein phosphorylated at specific serine or threonine residues preceding a proline (pSer/Thr-Pro), participating in the regulation of their functions [1, 2].

A deregulation of proline-directed protein phosphorylation has been associated to an alteration of vital cellular functions in several neurodegenerative disorders [3, 4]. In Alzheimer's disease (AD), aberrant amyloid precursor protein (APP) and tau phosphorylation occurs, leading to extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) formation made of toxic amyloid-beta peptides and hyperphosphorylated tau, respectively. The accumulation of these deposits causes synaptic dysfunction and loss of neurons [5]. Moreover, α-synuclein (α-syn) phosphorylation is altered in Parkinson's disease (PD) resulting in insoluble and fibrillar α-syn inclusions that cause neuronal death [6]. A recent paper showed the effect of phosphorylation on protein misfolding, confirming the influence of this mechanism in triggering protein aggregation and amyloid formation [7]. Also in Huntington's disease (HD), proline-directed protein phosphorylation plays a crucial role in determining neuronal apoptosis by acting on p53 tumor suppressor gene [8].

Several papers suggest a key role for Pin1 in the pathogenesis of these diseases, although the detailed molecular mechanism is not yet well understood [9, 10]. So far, Pin1 involvement in AD has been the most extensively investigated. Indeed, decreased Pin1 levels were observed in the brain of AD patients resulting in the accumulation of *cis* tau at the early stage of the disease, as recently revealed by the development of antibodies specific for *cis* and *trans* isomers of pThr231-Pro motif in tau [11].

Besides the role of proline-directed protein phosphorylation on tau, APP, α-syn, and p53, a recent finding supports the possibility that phosphorylation could represent a physiological mechanism of prion protein conversion. Indeed, phosphorylation of prion protein at Ser-43-Pro induces the formation of proteinase K resistant aggregates *in vivo* [12]. This result suggests that Pin1 could have a crucial function not only in AD, PD, and HD, but also in prion disorders. However, this hypothesis requires further investigation. In this article, we review Pin1 involvement in neurodegenerative pathologies focusing on the potential impact of this PPIase in the development of prion disorders.

2. Pin1 structure and function

Pin1 was identified in 1996 by Lu and colleagues as a protein interacting with NIMA, a mitotic kinase involved in cancer [1]. The discovery revealed the ability of Pin1 to suppress NIMA activity and brought attention to this new mitotic regulator enzyme able to conformationally modify phosphorylated proteins into different foldings. This molecular switch is commonly known as *cis/trans* isomerization, an important mechanism that determines the fate of phosphoproteins [13]. Moreover, transition from *cis* to *trans* or vice versa depends on the structure of the target protein [10].

Pin1 catalyzes conformational changes of specific pSer/Thr-Pro motifs that are phosphorylated by so called "proline-directed protein kinases," such as mitogen-activated protein kinases (MAP kinases), glycogen synthase kinase-3 (GSK-3), and cyclin-dependent kinases (CDKs) [10]. Pin1-catalyzed isomerization is allowed by the interesting stereochemistry of proline, consisting of a 5-membered ring on its chain of peptides [10].

Pin1 is an 18 kDa protein made up of an N-terminal WW domain and a C-terminal catalytic domain that are connected by a flexible linker [14]. The WW domain allows Pin1 to bind its substrates while the C-terminal domain catalyzes their *cis/trans* isomerization [2]. This classic "tag-and-twist" mechanism has been recently revised by Matena and colleagues. They postulate a more complex scenario in which the two domains of Pin1 are not completely independent from one

another and suggest transient domain interactions that enhance the affinity of WW domain towards phosphorylated substrates [15]. Although the exact catalytic mechanism by which Pin1 accelerates the isomerization is still unknown, a recent study has shown how Pin1 is able to reduce the free energy barrier for the *trans* form in order to obtain an almost barrier free reaction. More specifically, pThr motif allows substrate anchoring at Pin1, while Cys-113 and Ser-154 residues in the enzyme influence the isomerization reaction of the *cis* and *trans* forms, respectively [16].

Pin1 is differently expressed by mitotic cells and neurons. In the first case, Pin1 levels are associated with the cell's proliferative capacity. While in neurons, Pin1 levels are much higher during developmental stages than in the adult, suggesting a role for Pin1 in cortical neuronal differentiation [11]. However, the physiological function of prolyl isomerase in neurons is not fully understood. Evidence has shown that, thanks to its ubiquitous expression, Pin1 regulates the isomerization of several substrates such as c-Jun, NF-κB, p53, beta-catenin, c-Myc, IRAK1, gephyrin, myeloid cell leukemia sequence-1 (MCL-1), tau, and APP [9, 17 ,18]. Although it is expressed mainly in the nucleus, Pin1 also localizes in other subcellular compartments, such as the cytoplasm and mitochondria, depending on substrate localization. For instance, it co-localizes with the full length APP protein at the plasma membrane, suggesting an involvement of the isomerase in APP turnover [17].

3.   Misfolded proteins and neurodegeneration

The accumulation of misfolded and aggregated proteins is a common hallmark of several neurodegenerative diseases. Indeed, amyloid plaques and NFTs accumulate in AD, Lewy bodies in PD, inclusions containing Htt in HD, and prion plaques in prion disorders [19]. These amyloid-like protein deposits are in dynamic equilibrium with the soluble species resulting in variable solubility, stability, and size [20]. Contrasting hypotheses have been proposed regarding their neurotoxicity. However, current evidence indicates that these insoluble inclusions might exert a neuroprotective mechanism by sequestering and isolating toxic misfolded intermediates [21]. Although to date, the toxic question remains unresolved, significant efforts are now being made to address another important point: the mechanisms governing the formation and propagation of protein aggregates. Protein aggregation can be regulated by several cellular events including covalent modifications such as nitration, ubiquitination and phosphorylation [22]. Importantly, phosphorylation can affect protein conformation, function, as well as localization, and its role in neurodegeneration will be fully discussed below.

Moreover, an emerging hypothesis has tried to explain the propagation of disease-associated proteins on the basis of recent findings on the transmission of several amyloidogenic proteins. Contrary to the traditional hypothesis supporting cell-autonomous development of protein aggregates, a growing number of studies provided convincing evidence of cell-cell propagation of these deposits. The proposed mechanism resembles that of the pathogenesis of prion diseases demonstrating a common role for prions in neurodegeneration. Thus, a prion-like self-propagating process is shared by a wide range of pathological aggregates including Aβ, tau, α-syn, and Htt. Several *in vitro* and *in vivo* studies showed the transmissibility of non-prion protein deposits, suggesting that they might act as "seeds" to initiate aggregate formation by recruiting additional soluble species into elongating fibrils [23]. In addition, it has been shown that aggregates can spread, by prion-like mechanism, through anatomical connections throughout the brain [24]. To date, it is unclear what determines the selective vulnerability of different brain regions.

4. Pin1 and AD

AD is the most common neurodegenerative disorder among people over 65 years old. It is characterized by progressive neuronal loss and synaptic dysfunction that lead to cognitive decline and changes in mood and personality [25]. Growing evidence supports the concept that AD is a multi-factorial disorder, hence several pathogenic factors contribute to the disease [26]. Although, to date, AD pathogenesis is not completely clear, the accumulation of intracellular NFTs and extracellular amyloid plaques are universally considered the major pathogenic determinants of the disease [5]. NFTs are composed of hyperphosphorylated tau, high molecular-weight microtubule associated proteins (MAPs), medium and high molecular weight neurofilament proteins (NFM/H), and vimentin [27]. On the other hand, amyloid plaques consist of amyloid-beta peptides generated from APP processing alteration, resulting in increased amyloidogenic pathway [28]. Tau, NF, and APP processing are regulated by phosphorylation under physiological conditions; therefore, a dysregulation of this mechanism induces pathogenic proteins generation. Notably, increased proline-directed phosphorylation seems to affect tau and NF, as well as APP functioning in AD; several works show the involvement of Pin1 in the pathology [3, 17]. Indeed, Pin1 knockout mice develop AD-related features such as amyloid and tau pathology together with age-dependent motor and behavioral deficits [3, 17, 29]. Moreover, a reduction of Pin1 activity has been observed in human brain regions notoriously prone to degenerate in AD [3]. The low amount of Pin1 was not only due to decreased protein levels, but also to increased protein oxidation. Importantly, Cys-113-oxidized Pin1, a catalytically inactive form, has been found to be significantly increased in AD mouse models as well as in human AD brains [30-32].

Overall, Pin1 reduction greatly affects isomerization of phosphorylated tau, NF, and APP, impairing the equilibrium between *cis* and *trans* conformations. Consequently, the pathogenic *cis* isomer will be mainly produced, leading to an alteration of tau and APP function, which in turn results in the appearance of NFTs and amyloid plaques.

4.1. Pin1 and tau protein

Tau is a microtubule-associated protein (MAP) and plays a key role in stabilizing microtubular structures essential for connecting the neuronal cell body and synapse. A finely regulated balance between phosphorylation and dephosphorylation controls tau binding to microtubules, contributing to axonal outgrowth and neuronal plasticity [33]. When this balance is disrupted, tau becomes hyperphosphorylated, dissociates from microtubules, and starts to self-aggregate. This forms NFTs that destabilize the cytoskeleton, leading to neuronal death [34].

Interestingly, tau hyperphosphorylation occurs mostly at Ser/Thr-Pro sites that are targeted by protein kinases particularly active in AD such as GSK3β and Cdk5 [35]. Accordingly, AD-related Pin1 deficiency inhibits dephosphorylation at Cdk5-mediated sites by protein phosphatase 2A (PP2A), which is downregulated in AD [36]. These observations suggest a neuroprotective role of Pin1 in AD, which has been further investigated by analyzing the ability of Pin1 to bind pSer/Thr-Pro tau.

The first result revealed the interaction of the isomerase with two tau phosphorylation sites: Thr-212 and Thr-231 [37]. In particular, *cis* pThr-231-Pro tau was observed as the earliest pathogenic sign in mild cognitive impairment (MCI) brains, detected by an antibody developed by Nakamura

and colleagues. They generated two antibodies that allowed them to discriminate between *cis* and *trans* pThr-231-Pro tau isomers [11] and, consequently, a specific immunotherapy against *cis* pThr-231-Pro tau has been proposed [38].

However, only two Pin1 binding sites cannot protect against tau hyperphosphorylation, requiring further investigation. Recently, Kimura and colleagues showed that Pin1 binds tau at all Cdk5-mediated sites: Ser-202, Thr-205, Ser-235, and Ser-404. Furthermore, the binding was stronger to Ser-202 and Thr-205 compared with Ser-235 and Ser-404 [35]. Thus, Pin1 should be able to accelerate *cis* to *trans* isomerization of the aforementioned phosphorylated sites that would then be correctly dephosphorylated by PP2A. However, the analysis of remaining pSer/Thr tau sites would be useful to understand Pin1 involvement in AD pathology and to develop a therapeutic strategy to restore tau physiological function.

4.2. Pin1 and NFs

NFs are the most abundantly expressed cytoskeletal proteins in myelinated axons and consist of three different molecular weight subunits (NF-L, NF-M, and NF-H) and α-internexin [39]. The carboxy terminal domains of NF-M and NF-H are rich in lysine/serine/proline (KSP) repeats that, under normal conditions, are phosphorylated in the axonal compartment. This post translational modification allows NFs to contribute to neuronal differentiation, axon outgrowth, regeneration, and guidance [40]. In the same manner as tau, NFs became hyperphosphorylated in neuronal cell bodies in AD [41] and the aberrant phosphorylation is the result of deregulation of several protein kinases and protein phosphatases, such as PP2A. In that respect, Pin1 has been shown to stabilize the AD-related stress induced hyperphosphorylation of KSP repeats that are mediated by ERK1/2 and JNK. In addition, Pin1 prevents PP2A-mediated NFs dephosphorylation in neurodegeneration, promoting the isomerization of phosphorylated NF proteins [4].

4.3. Pin1 and APP

APP is an ubiquitous expressed transmembrane protein that is abundant in neurons and regulates nervous system development, especially neuronal growth and survival [42]. Depending on its cellular localization, APP undergoes a non-amyloidogenic or amyloidogenic processing. In the first case, it is localized to the plasma membrane and cleaved by alpha and gamma secretases that prevent amyloid-beta peptide formation. However, when APP is internalized to the endosomes, amyloid-beta peptides are generated by beta secretases [43]. The amyloidogenic pathway is increased in AD and has been associated with APP phosphorylation of Thr-668-Pro residues motif that determines its isomerization from *trans* to *cis* [44]. Indeed, while *trans* conformation slows amyloid-beta production, the *cis* conformation is related to amyloidogenic processing. Normally, Pin1 was found to bind pThr-668-Pro, maintaining the balance between the two isomers. But the reduction of Pin1 levels and activity observed in AD brains slows the uncatalyzed *cis* to *trans* isomerization rate, leading to a higher concentration of the *cis* pThr-668-Pro motif for a longer time and promoting amyloid-beta generation [17].

Recently, another mechanism has been discovered by which Pin1 would be able to indirectly regulate APP processing. The new proposed model suggests that Pin1 inhibits GSK3β activity, an upregulated kinase in brains of AD patients. Pin1 binds GSK3β at pThr-330-Pro motif, suppressing

its kinase activity, leading to decreased toxic amyloid-beta peptide generation and increased APP turnover [45]. These data show the ability of Pin1 to regulate, directly or indirectly, the fate of APP and confirm its neuroprotective role in AD.

5. Pin1 and HD

HD is an adult-onset, inherited neurodegenerative disorder that results from expanded CAG repeats in exon 1 of the coding gene (Htt). The hallmark symptoms of HD can include motor and cognitive impairments which are caused by gradual loss of neurons especially in the striatum. It is hypothesized that HD is caused by either a loss-of-function of the wild type protein Htt or a toxic gain-of-function of mutant Htt. Indeed, mutant Htt leads to the formation of intracellular aggregates in the central nervous system, as well as in peripheral tissues [46, 47]. These inclusions trigger toxic cellular events such as mitochondrial dysfunction, reactive oxygen species generation, DNA damage, and inhibition of neural-specific transcription [48].

The p53 tumor suppressor gene mediates Htt-induced toxicity in *in vitro* and *in vivo* models of HD [49], and the underlying mechanism is being fully investigated. In fact, p53 plays a key role in the regulation of apoptosis in HD, in addition to its role in other neurodegenerative diseases, and its anti-apoptotic activity requires Pin1 involvement [50, 51]. Specifically, mutant Htt induces the activation of a stress-signaling cascade involving ATM, HIPK2, and PKCdelta kinases, leading to p53 phosphorylation on the Ser-46 motif. This event triggers the isomerization of pSer-46-Pro-47 site by Pin1, allowing for the dissociation of p53 from the apoptosis inhibitor iASPP and the consequent induction of apoptotic target genes [8].

Besides this mechanism, Pin1 also regulates a transcription-independent apoptosis pathway which may contribute to neuronal death in HD. In this case, the prolyl-isomerization of pSer-46 induces p53 monoubiquitination, its mitochondrial translocation, and direct apoptosis [52]. Moreover, the early activation of p53 is prevented by Pin1 genetic ablation in a mouse model of HD, suggesting the protective role of targeting Pin1 in mutant Htt-related neurodegeneration [8].

6. Pin1 and PD

PD is a chronic, progressive neurodegenerative disorder that mainly occurs in the population older than 60 years, but can appear in much younger people. PD is not only associated with an impairment of motor control, but also with non-motor symptoms such as cognitive deficits, neurobehavioral disorders, and autonomic dysfunction [53]. Currently, there is no cure for PD and only symptomatic pharmacological approaches to treat motor abnormalities are available. The appearance of these symptoms results from loss or degeneration of dopaminergic neurons in the midbrain and accumulation of neuronal inclusions, called Lewy bodies, in the surviving neurons of the *substantia nigra* [54].

Most α-syn in these inclusions is phosphorylated at Ser-129, suggesting the relevance of phosphorylation mechanisms in α-syn aggregation [6, 55, 56]. Phosphorylation can also indirectly influence α-syn aggregation, as shown by Ryo and colleagues. These authors showed that phosphorylation of synphilin-1, an α-syn-binding protein, allows the binding of Pin1 on its Ser-211-Pro and Ser-215-Pro motifs. This promotes synphilin-1-α-syn interaction and likely α-syn inclusions generation [57].

In addition, Pin1 has been detected in Lewy bodies of human PD brain tissues, linking it to the pathogenesis of the disease [57]. Moreover, a recent paper provided further details showing Pin1 up-regulation in cell culture, animal models of PD and in human PD brains. It also showed the pro-apoptotic function of Pin1 in dopaminergic neurons [58].

Interestingly, p53 dependent neuronal death has been reported also in *in vitro* and *in vivo* models of PD [59, 60], as well as increased p53 levels in PD human brains [61]. These results provide convincing evidence of the interplay between Pin1 and p53 in PD, but more efforts should be made to understand the exact mechanism underlying this interaction. In conclusion, Pin1 signaling may represent a potential target for neuroprotection in PD.

7. Pin1 and prion diseases

Prion diseases, or transmissible spongiform encephalopathies (TSE), are a group of infectious, genetic or sporadic pathologies that affect nervous system of animals and humans [62,63]. Animal prion diseases include scrapie in sheep and goats, as well as bovine spongiform encephalopathy (BSE), known as "mad cow disease". Human prion diseases are classified into Creutzfeldt-Jakob Disease (CJD), variant CJD, Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia, and kuru. Prion disorders are characterized by the accumulation of prion protein aggregates resistant to proteinase K digestion in the brain. These insoluble inclusions derive from the conformational transition of the normal, soluble, cellular prion protein (PrP^C),which are rich in α-helical structures, into scrapie prion protein (PrP^{Sc}) or prions, predominantly composed of β-sheet-structures [64, 65]. Although PrP^C conversion is a key event in the pathogenesis of prion diseases, the molecular mechanism involved has not been definitely clarified. Since, under normal conditions, the spontaneous conversion of PrP^C into PrP^{Sc} is prevented by the high-energy barrier between the two isoforms, many factors have been proposed to induce this process [66]. These include pathogenic mutations and several cofactors, such as glycosaminoglycans, lipids, nucleic acids, metals, and post-translational modifications. The latter include glycosylation, oxidation, advanced glycation end-product modification, and phosphorylation [66]. Several kinases phosphorylate PrP^C, including Fyn, casein kinase II, and Cdk5 [67]. Interestingly, neuronal Cdk5 phosphorylates PrP^C at Ser-43, inducing protein conformational change that leads to the formation of insoluble, proteinase K-resistant aggregates *in vitro* and also in PrP^{Sc}-infected mice brains. Thus, Cdk5-dependent phosphorylation directly determines prion protein conversion [12].

Accordingly, Cdk5 levels were observed to increase in PrP^{Sc}-infected animal brains [68], as was pSer-43 PrP^C in the cerebral spinal fluid of sporadic CJD patients [69]. Recently, pSer-43 was shown to cause or exacerbate PrP^C mutant conversion into amyloid structure [70], confirming the importance of phosphorylation in PrP^C conversion and also in the presence of PrP^C-related familial mutations. Ser-43 motif is one of two Ser-Pro phosphorylation sites in PrP^C and is located in the N terminus of the mature protein at amino acids Ser-43-Pro-44. The other site is in the glycosyl phosphatidylinositol-anchor signal peptide at Ser-237-Pro-238 [12]. Moreover, the N-terminal domain of the prion protein seems to be involved in PrP^C function, in addition to PrP^{Sc} formation. Indeed, this domain was shown to direct prion protein oligomeric association [71] during *in vivo* and *in vitro* aggregation [72, 73].

In this context, it could be interesting to investigate the possible role of Pin1 in regulating the conversion of PrP^C into PrP^{Sc}. It can be envisioned that Pin1 may catalyze the conformational change

of pSer-43 at the N-terminal domain, influencing PrP^C isomerization and contributing to the physiological function of PrP^C. Pin1 dysfunction could alter this process leading to increased levels of pSer-43 and, consequently, to PrP^{Sc} generation. This scenario would resemble Pin1 involvement in tau or APP isomerization, and could promote deep investigation of the potential interplay between Pin1 and PrP^C. In fact, as for AD, HD, and PD, prion disorders could also have a link with the dysregulation of Pin1 activity that could be confirmed or refuted by measuring Pin1 levels in patients affected by prion diseases.

8. Conclusions

In this review we discussed the state of the art concerning the role of Pin1 in neurodegenerative disorders. Much evidence suggests that Pin1 regulation could represent a potential therapeutic target for AD, HD, and PD. The current therapeutic approach for AD consists of targeting the pathogenic *cis* isoform of tau by conformation specific antibodies [38]. Furthermore, the reduction of Pin1 oxidation may be a promising field to investigate in AD. In the case of PD, several inhibitors have been found to reduce MPP$^+$-induced Pin1 upregulation in cellular models of PD and notably, juglone treatment was able to induce neuroprotection against dopaminergic degeneration in a mouse model of PD [58]. Thereby, developing and testing pharmacological compounds to inhibit Pin1 may be an attractive therapeutic strategy in PD, as well as in HD. In fact, some evidence showed that small-molecule inhibitors of Pin1, such as PiB, protect from Htt-induced apoptosis [74].

Despite mechanisms underlying Pin1 involvement in these pathologies are not always yet clear, tight regulation of Pin1 could be useful to restore disease-related protein function. Indeed, Pin1 inhibition determines opposite effects in neurodegenerative disorders: on the one hand it leads to a reduction of inclusions in PD and decreased neuronal apoptosis in HD while, while on the other hand, it seems to exacerbate AD pathology. The discrepancy of Pin1 function in neurodegenerative disorders may be ascribed to its wide range of phosphorylated substrates and, likely, even to the specific phosphorylation motifs within the same substrates (Figure 1), depending on individual Ser/Thr-Pro site conformation after phosphorylation [2].
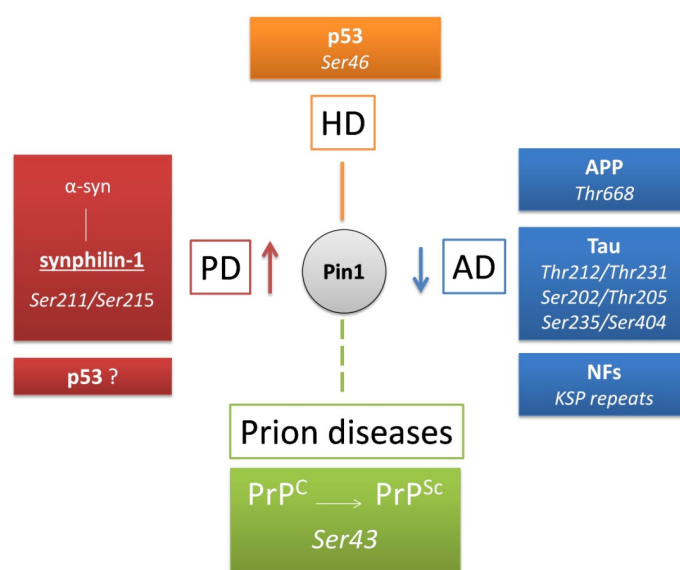

Figure 1. Pin 1 involvement in neurodegenerative diseases: potential Pin 1-binding targets.

Finally, the effect of Pin1 inhibition in prion diseases is yet unknown, but recent evidence promotes an investigation of the role of the PPIase in this pathology. It is intriguing to consider that the enzymatically driven conformational change of PrP^C to PrP^{Sc} may regulate prion conversion and accumulation. Thus, endeavoring to understand whether Pin1 is involved in PrP^{Sc} formation could eventually constitute Pin1 as a therapeutic target for prion diseases as hypothesized for other neurodegenerative diseases.

Conflict of Interest

All authors declare no conflicts of interest in this paper.

Acknowledgment

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