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Review

Implication of Alpha-Synuclein Phosphorylation at S129 in Synucleinopathies: What Have We Learned in the Last Decade?

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Abstract. Abnormal accumulation of proteinaceous intraneuronal inclusions called Lewy bodies (LBs) is the neurpathological hallmark of Parkinson's disease (PD) and related synucleinopathies. These inclusions are mainly constituted of a presynaptic protein, α-synuclein (α-syn). Over the past decade, growing amounts of studies reported an aberrant accumulation of phosphorylated α-syn at the residue S129 (pS129) in the brain of patients suffering from PD, as well as in transgenic animal models of synucleinopathies. Whereas only a small fraction of α-syn (<4%) is phosphorylated in healthy brains, a dramatic accumulation of pS129 (>90%) has been observed within LBs, suggesting that this post-translational modification may play an important role in the regulation of α-syn aggregation, LBs formation and neuronal degeneration. However, whether phosphorylation at S129 suppresses or enhances α-syn aggregation and toxicity *in vivo* remains a subject of active debate. The answer to this question has important implications for understanding the role of phosphorylation in the pathogenesis of synucleinopathies and determining if targeting kinases or phosphatases could be a viable therapeutic strategy for the treatment of these devastating neurological disorders. In the present review, we explore recent findings from *in vitro*, cell-based assays and *in vivo* studies describing the potential implications of pS129 in the regulation of α-syn physiological functions, as well as its implication in synucleinopathies pathogenesis and diagnosis.

Keywords: Phosphorylation, kinases, membrane binding, degradation, subcellular localization, biomarker, toxicity, animal models, cell-based assays

INTRODUCTION

Parkinson's disease (PD) and related synucleinopathies, including dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), are characterized by the progressive loss of vulnerable neuronal populations in the brain [1–3] and the presence of intraneuronal α-synuclein (α-syn)-rich inclusions, called Lewy bodies (LBs) [4–7]. Converging evidence from neuropathological, *in vivo* and *in vitro* studies support a causal role of α-syn in the pathogenesis of synucleinopathies [6, 7]. However, the molecular and cellular mechanisms controlling α-syn aggregation and toxicity remain in part non-elucidated.

In the last few years, an increasing number of studies reported that α-syn within LBs is subjected to several post-translational modifications (PTMs), including phosphorylation, ubiquitination,

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cross-linking, truncations and nitration, suggesting that these modifications may play a key role in the regulation of α-syn aggregation and toxicity *in vivo* [8]. Among these PTMs, a growing interest has been focused on the phosphorylation at residue S129 (pS129) and its possible implication on α-syn-induced neurodegeneration [8, 9]. Indeed, under normal conditions, only a small fraction (>4%) of α-syn is constitutively phosphorylated at S129 in the brain [10–12], whereas a dramatic accumulation (<90%) of pS129 has been observed in the brains of patients suffering from synucleinopathies [12–15], as well as in transgenic animal models of PD [16–19]. These findings strongly support the hypothesis that phosphorylation at S129 may play an important role in the control of α-syn normal functions, as well as the regulation of its aggregation, LBs formation and neurotoxicity.

Despite increased efforts to identify the kinase responsible for α-syn phosphorylation, and to understand the consequences of such modifications on the biophysical and biochemical properties of α-syn, the questions on how phosphorylation modulates α-syn normal functions and whether pS129 suppresses or enhances α-syn toxicity *in vivo* remain the subjects of heated debate. The answer to this question has important implications for understanding the exact role of α-syn phosphorylation in the pathogenesis of PD and related disorders and may lead to the identification of new therapeutic targets for the treatment of these neurological disorders.

Phosphorylation is an important molecular switch for the regulation of α-syn proprieties and physiological functions

Although the exact native state of α-syn remains the subject of active investigation and debate [6, 20, 21], converging *in vitro* [21–23] and *in vivo* [21, 24, 25] data suggest that this protein behaves as an unstructured and intrinsically disordered protein. In particular, α-syn carboxy-terminal (C-terminal) region exists in a disordered conformation in monomeric, fibrillar and membrane-bound states [23, 26–28] and plays an important role in the control of α-syn proprieties, notably its interactions with other proteins [29–34], metal ions [35, 36] and other ligands (e.g. dopamine and polyamines) [37].

Interestingly, α-syn C-terminal tail contains the majority of PTMs sites, including phosphorylation (Y125, S129, Y133 and Y136), truncation (D115, D119, P120, E130 and D135), ubiquitination (K96) and tissue transglutaminase cross-linking (Q109) [8, 38] (Fig. 1), suggesting that these PTMs may regulate α-syn structure and physiological functions. In this section, I will discuss how phosphorylation at S129 may act as a molecular switch for the regulation of α-syn functions and proteostasis.

Phosphorylation at S129 modulates α-syn-membrane binding

α-syn interaction with vesicles of different lipid compositions has been extensively studied (see reviews [39–41]) and recently, a great deal of attention has been focused on the role of phosphorylation at S129 in the regulation of α-syn-membrane interaction.

In vitro assessment of pS129 effect on α-syn-membrane binding has yielded controversial observations. Whereas some studies reported the absence of effect of S129 phosphorylation on α-syn-membrane interaction [42, 43], two independent groups showed that GRK-mediated authentic phosphorylation and S129→E (mimicking the phosphorylation state) decrease α-syn affinity to bind phospholipids [44, 45]. On the other hand, cellular and animal studies, based on the use of the phosphomimic strategy, reported an inhibitory effect of S129 phosphorylation on α-syn-membrane interaction. In yeast and worm models of PD, S129→A substitution (to block phosphorylation) increases α-syn membrane-bound fraction, however the phosphomimic mutation (S129D) inhibits its association with membranes [46, 47]. A similar observation has been reported in an adeno-associated virus (AAV)-based rat genetic model of PD where immuno-electron microscopy analysis detected the majority of the non-phosphorylatable α-syn mutant (S129A) associated with cellular membranes [48]. Collectively, *in vitro* and *in vivo* data support the hypothesis of an inhibitory effect of S129 phosphorylation on α-syn membrane binding.

It is worth noting that a non-negligible fraction of α-syn in cells is associated with membranes (i.e. mitochondrial membrane, synaptic vesicles) [39, 49] and this α-syn-associated fraction is also subjected to phosphorylation by membrane-associated kinases, namely the G protein-coupled receptor kinases (GRKs) [49]. A recently described role of membrane-associated α-syn phosphorylation is the regulation of neurotransmitter uptake, notably the dopamine [49]. In a cell-based assay, Hara and colleagues reported that GRK-mediated S129 phosphorylation enhances the ability of α-syn to increase dopamine uptake,

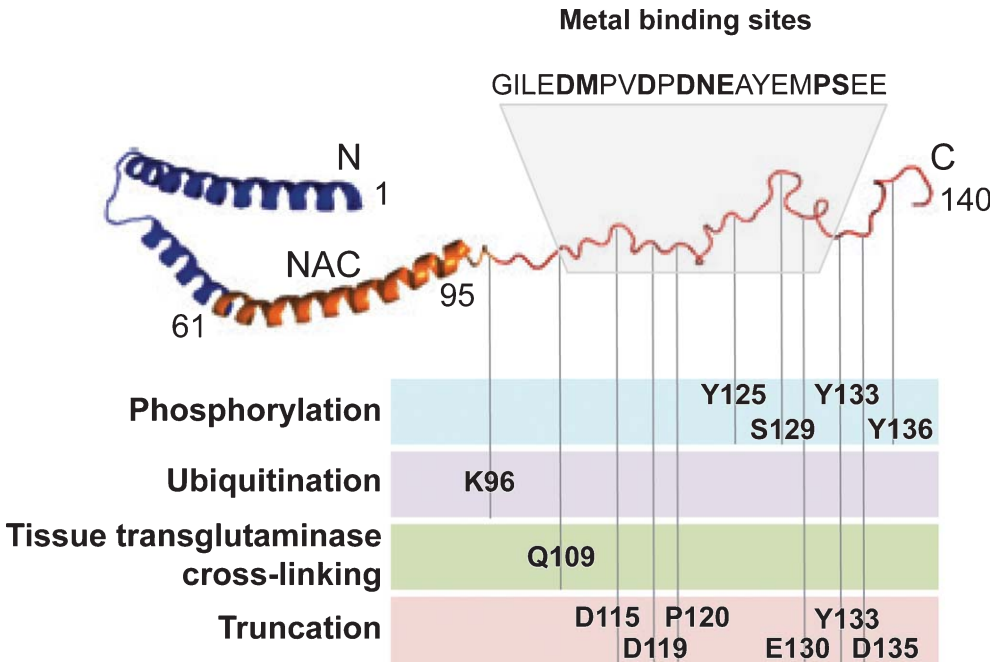

Fig. 1. α-syn C-terminal residues subjected to post-translational modifications or implicated in α-syn interaction with metalions. Schematic representation of α-syn (Protein Data Bank ID: 1XQ8) [129] with the N-terminal region, the NAC region and the C-terminal region are colored in blue, orange and red, respectively. In the upper part of the scheme are represented the binding regions (amino acids in bold) with bivalent metal ions. In the lower part of the scheme are represented the potential sites, in α-syn C-terminal region, subjected to post-translational modifications, including phosphorylation, ubiquitination, tissue transglutaminase cross-linking and truncation.

without affecting cell surface expression of dopamine transporter [49]. These data suggest that pS129 plays an important role in the regulation of α-syn functions at the synaptic terminals. However, a question remains open on how phosphorylated α-syn modulates synaptic plasticity despite its low level [12] and short physiological life [43]. Hints for answering this question has been in part provided by Hirai and collaborators who reported that α-syn phosphorylation state is tightly regulated by physiological stimuli (i.e. stress) and proposed that pS129 may play an important role in the stress-induced synaptic plasticity [50].

Phosphorylation at S129 enhances α-syn interaction with metal ions

Several studies reported that α-syn C-terminal region is implicated in its interaction with metal ions [35, 36, 51] and the localization of S129 in a very close proximity to the putative metal binding sites [52–54] suggests that phosphorylation at this residue may modulate α-syn-metal ions binding (Fig. 1).

The effect of S129 phosphorylation on metal binding has been investigated *in vitro* using synthetic C-terminal peptide. Using Terbium (Tb^{3+}) as a luminescent probe of metal binding and isothermal titration calorimetry, Liu and colleagues showed that S129 phosphorylation has no effect on α-syn peptide (residues 119–132) interaction with trivalent ions [52]. In a more recent study, the use of a larger peptide fragment corresponding to the entire α-syn C-terminal region (residues 107–140) confirmed the absence of pS129 effect on α-syn interaction with trivalent ions, however it showed an increased binding affinities to divalent ions (Cu^{2+}, Pb^{2+} and Fe^{2+}) [53]. Moreover, tandem mass spectrometry analysis revealed that S129 phosphorylation affects ions binding sites. For example, the residue D119 involved in the binding of bivalent ions (Fe^{2+} and Pb^{2+}) to the non-phosphorylated peptide is not implicated in the interaction with pS129 peptide [53]. This observation suggests that pS129 may significantly affect α-syn conformation and redistribute metal ions binding sites.

It is widely confirmed that interaction with metal ions promotes α-syn fibrillization [55–57] and the question on how phosphorylation at S129 affects this process remains to be explored. In a recent study, Nubling and collaborators reported that mimicking phosphorylation by S129→E substitution facilitates oligomer formation in the presence of trivalent metal

ions (Fe^{3+} and Al^{3+}), as compared to wild type protein [45]. This result is in contradiction with the observations described above reporting that pS129 has no effect on α-syn interaction with trivalent metal ions [52, 53]. This apparent discrepancy could be due to the fact that the authentically phosphorylated peptide and the phospho-mimic peptide may behave differently in the presence of metal ions. In summary, these results suggest that phosphorylation at S129 play an important role in the regulation of α-syn interaction with metal ions and could significantly affect metal ions-mediated α-syn structure and aggregation properties.

Phosphorylation at S129 regulates α-syn turnover

While several PTMs, notably ubiquitination [9, 58–61], sumoylation [62] and phosphorylation at Y39 [9, 63, 64], have been reported to regulate α-syn degradation via different proteolytic pathways, little is known about the implication of the phosphorylation at S129 on α-syn turnover. The first evidence of a cross-talk between phosphorylation at S129 and α-syn degradation has been reported by Chau and collaborators [65]. In their study, the authors observed that inhibition of the ubiquitin-proteasome system induced a significant increase of pS129 levels in human neuroblastoma [65]. A subsequent study confirmed this observation and reported that blocking the autophagy-lysosomal degradation pathway also induces a massive accumulation of pS129 in human neuroblastoma and rat cortical primary cultures [66]. Using a pulse-chase analysis and *de novo* protein synthesis inhibitor (cycloheximide), the authors observed that pS129 half-life time is significantly shorter ($t_{½} = 54.9 \pm 6.4$ min) compared to the non-phosphorylated form ($t_{½} > 240$ min), suggesting that the phosphorylated form is selectively targeted for degradation [66].

More recently, our group reported that the overexpression of Polo-like kinase 2 (PLK2), the main kinase responsible for α-syn phosphorylation in the brain [67–69], enhances α-syn turnover *via* the autophagic degradation pathway [70]. This cell process is unique to the synuclein family (α and β-syn) and is governed by PLK2 kinase activity and by the direct interaction between PLK2 and α-syn [70] (Fig. 2). A similar observation has been reported in a yeast model of PD where S129→A substitution compromised the clearance of α-syn *via* the autophagic degradation pathway [71]. Although the physiological relevance of PLK2 and α-syn interaction remains unknown, a number of converging evidence suggest a synergistic role of these two proteins in the

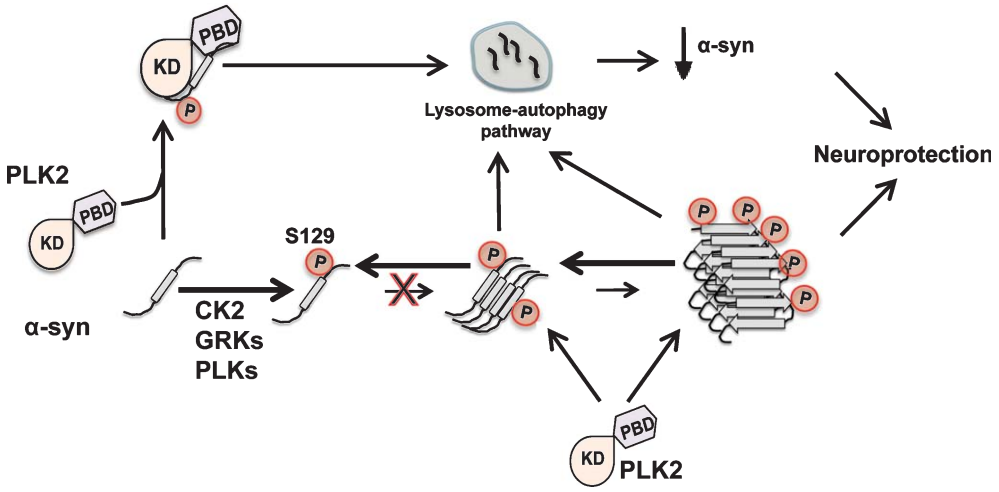

Fig. 2. Potential role of phosphorylation at S129 in the regulation of α-syn clearance, aggregation and toxicity. Converging lines of evidence supports the implication of pS129 in the regulation of α-syn turnover. In our recent report we showed that PLK2 phosphorylates, interacts with and enhances α-syn clearance via the lysosome-autophagy degradation pathway [70], thereby it suppresses its toxicity *in vivo*. In another hand, *in vitro* assays revealed that authentic phosphorylation at S129, inhibits α-syn fibrillogenesis [42], suggesting that this post-translational modification may reduce α-syn fibrillogenesis and aggregation-related toxicity. Finally, α-syn oligomers and fibrils are good substrates for several kinases, notably PLK2 [116], suggesting that this event could occur after LBs formation. It is plausible that S129 phosphorylation may represent an active process promoting LBs disaggregation and/or clearance. Collectively, these observations suggest that phosphorylation at S129 may play a protective role against α-syn toxicity. PLKs: Polo like kinases, CK2: Casein kinase 2, GRKs: G protein-coupled receptor kinases, KD: Kinase domain, PBD: Polo box domain.

regulation of the synaptic transmission [6, 72–74] and cell response to oxidative stress [75–77]. Of note, our finding describing PLK2-mediated α-syn clearance offers new opportunities for the development of therapeutic strategies for the treatment of PD aiming at reducing, in a specific manner, the toxic levels of α-syn.

Phosphorylation at S129 modulates α-syn protein–protein interaction

The implication of α-syn C-terminal tail on its interaction with different proteins partners [29–34] raised the question on how phosphorylation at S129 may regulate α-syn interactome. McFarland and collaborators were the first to address this question using targeted functional proteomics approaches [78]. In this study, the authors showed that the non-phosphorylated α-syn peptide mainly interacts with proteins related to mitochondrial electron transport (complex I, III and IV proteins of the electron transport chain) [78], however the phosphorylated peptide had more affinity to certain cytoskeletal proteins and presynaptic proteins implicated in the synapse transmission and vesicle trafficking [78]. In a recent study, Yin and collaborators showed that the C-terminal region is implicated in α-syn interaction with Rab GTPases (Rab8a), a small guanine nucleotide binding proteins implicated in coordinating vesicle trafficking [79]. Using a battery of biophysical and cell culture approaches, the authors reported that phosphorylation at S129 promotes α-syn binding to Rab8a and modulates Rab8a-mediated α-syn toxicity [79]. These observations suggest that pS129 could serve as a molecular switch to control α-syn interaction with different protein partners and therefore modulates its functions. However, further investigations are required to assess the impact and the physiological consequences of S129 phosphorylation on α-syn interaction with other proteins, such as SNARE proteins [80, 81], cytoskeletal proteins (i.e. tubulin) [82, 83] and other amyloidogenic proteins (i.e. tau) [31, 32].

Phosphorylation at S129 regulates α-syn subcellular localization

α-syn exhibits different subcellular localizations (nuclear, cytoplasmic, neurites), suggesting that this protein may play a specific role in each cell compartment. This subcellular localization is regulated by different factors notably PTMs (i.e. monoubiquitination [84]), PD-linked mutations [85], protein sequence (i.e. α-syn N-terminal region) [85, 86] and implication of proteins partners (i.e. importin αa [86]). Despite the absence of nuclear targeting sequence, a growing number of cell-based assays and *in vivo* studies reported an important accumulation of phosphorylated α-syn in the nucleus. In cell culture, using anti-pS129 antibodies and immunocytochemistry approaches, several groups detected α-syn in the nucleus of mammalian cell lines [67, 87] and primary neuronal culture [67, 88, 89]. *In vivo*, α-syn nuclear localization has been observed in α-syn-transgenic mice [90–94], AAV-based rat model of PD [48] and α-syn-transgenic drosophila [16]. Furthermore, a biochemical approach has also helped in detecting pS129 expression in the nuclear fraction extracted from mice brains [95, 96]. Together, these observations suggest that phosphorylation at S129 may play a central role in the control of α-syn nuclear translocation.

This hypothesis was confirmed by Goncalves and Outeiro using a photoactivation-based approach to track α-syn intracellular dynamics in cell culture [85]. In their study, the authors observed a continuous α-syn trafficking between the cytoplasm and nucleus, which is in part regulated by α-syn phosphorylation state. Indeed, S129→A substitution to block phosphorylation significantly reduces α-syn nuclear translocation [85], suggesting that pS129 may act as a tag for α-syn targeting to the nuclear compartment.

Moreover, recent observations suggested that phosphorylation might act in synergistic pairs with PD-linked mutations to control α-syn nuclear localization. In a cell-based assay, Fares and collaborators showed that the newly described PD-linked mutation, G51D, is associated with an increase of pS129 levels and its accumulation in the nucleus of mammalian cells and primary neuronal cultures [97].

Interestingly, some specific kinases catalyzing α-syn phosphorylation at S129 may also play a role in pS129-mediated α-syn trafficking between the cytoplasm and nucleus. In cell culture, overexpression of GRK5 promotes α-syn translocation to the nucleus, while PLKs (PLK2 and PLK3) potentiate α-syn trafficking from the nucleus to the cytoplasm [67, 85].

It is important to note that studies of pS129 subcellular localization present certain limitations: 1) some anti-pS129 antibodies exhibit non-specific cross-reactivity to other unknown antigens present in the nucleus, since certain antibodies show a nuclear pS129 signal in α-syn-knockout tissue [96]; 2) the majority of α-syn subcellular localization analysis was performed in α-syn overexpressing systems, either transfected cells, viral based gene delivery

or in transgenic animals. These observations raise the question on the physiological relevance of the used systems, and urge the development of optimized models to decorticate the exact role of S129 phosphorylation on α-syn nuclear translocation, its role in this subcellular compartment and its impact on cell survival.

Implication of α-syn phosphorylation at S129 in synucleinopathies pathogenesis and treatments

Abnormal accumulation of pS129 in synucleinopathy-diseased brain [12, 15] and the increase of its levels during ageing [99, 100], the greatest risk factor for PD, suggest that this PTM may represent a key player in the pathogenesis of PD and related disorders. However, the exact implication of S129 phosphorylation on α-syn aggregation and toxicity *in vivo* remains under debate.

Does phosphorylation enhance or suppress α-syn toxicity in vivo?

Investigation of the relative implication of pS129 on α-syn toxicity *in vivo* has yielded controversial results [8, 48, 101–103]. This apparent controversy is in part due to the fact that phospho-mimics (S129D/E) do not replicate the exact properties of the authentically phosphorylated α-syn [8, 42, 66]. Faced with this limitation, our group and others sought to address this question by overexpressing α-syn with its natural kinases, directly in the rat brain. Among the kinases responsible for the α-syn phosphorylation at S129 (casein kinases [14], the G protein-coupled receptor kinases (GRKs) [44], LRRK2 [104] and Polo-like kinases (PLKs) [67, 68]), two independent groups investigated the effect of the overexpression of GRK2 and GRK6 on α-syn toxicity in fly [18] and rodent genetic models of PD [105], respectively. In both models, GRKs overexpression was associated with an increase of pS129 levels and an enhanced cellular loss [18, 105]. It is important to note that GRK6-mediated phosphorylation moderately increased α-syn toxicity, however it significantly accelerated this process [105]. Together these observations suggest that GRK-mediated phosphorylation of α-syn exacerbates its toxicity *in vivo.* In a more recent study, our group reported an opposing result after overexpressing α-syn with another kinase, PLK2 [70]. In this study, we showed that AAV-mediated overexpression of PLK2 in the rat midbrain induced a 3-fold increase of pS129 levels in the infected neurons, a significant reduction of α-syn-associated dopaminergic neuronal loss and an alleviation of the hemi-parkinsonian motor impairment [70]. This effect is governed by PLK2 kinase activity and α-syn phosphorylation at S129 [70]. At the molecular level, our study demonstrated that PLK2 overexpression enhances α-syn clearance via autophagic degradation pathway, and suggest that PLK2-mediated neuroprotection effect is probably due to the reduction of intra-neuronal α-syn protein levels under the toxic threshold [70] (Fig. 2). The discrepancy between PLK2- and GRKs-mediated effects on α-syn toxicity could be in part due to the superiority of PLK2 to efficiently phosphorylate α-syn *in vivo* [70, 106]. Moreover, PLK2-mediated α-syn turnover depend on PLK2 and α-syn protein-protein interaction, suggesting that PLK2 may play the role of a co-chaperone to assist α-syn autophagic clearance. Collectively, these *in vivo* data demonstrate that the effect of phosphorylation on α-syn toxicity is governed by the kinase responsible for its phosphorylation, suggesting that kinases rather than the phosphorylation *per se* are key regulators of α-syn toxicity *in vivo.*

Is phosphorylation at S129 required for α-syn aggregation and seeding in vivo?

The impact of S129 phosphorylation on α-syn aggregation has been extensively studied *in vitro* and the data support the hypothesis of an inhibitory effect this PTM may have on α-syn fibrillogenesis [8, 42] (Fig. 2). However, the question on whether pS129 controls α-syn aggregation and seeding *in vivo* is still elusive.

In mammalian cell lines and primary neuronal culture, the addition of small amounts of exogenous α-syn pre-formed fibrils (Pffs) seeds the formation of intracellular α-syn aggregates recapitulating the main features of LBs [107, 108]. This cellular process is governed by protein seeding, a nucleation-dependent mechanism in which the α-syn Pffs provide a template for the assembly of soluble monomeric α-syn and lead to the formation of highly ordered protein aggregates [109]. Using this cell-based assay, Luk and colleagues showed that phosphorylation is not required for the formation of intracellular LB-like inclusions [107]. In this study, the authors transduced cells with α-syn S129A Pffs in cells stably overexpressing full length α-syn. Strikingly, immunocytochemistry revealed the presence of intracellular inclusions resembling those formed after wild type α-syn transduction, suggesting that phosphorylation at S129 is not required for inclusions seeding [107]. Moreover, the addition of truncated

α-syn (1–120) Pffs to cells stably overexpressing wild type α-syn induced the formation of pS129-positive inclusions [107, 108]. Interestingly, this phospho-signal, which does not occur within the truncated Pffs, originates from the newly recruited endogenous α-syn and implies that phosphorylation may occur after inclusion formation [107, 108]. Collectively, these data demonstrate that phosphorylation is not a limiting factor for α-syn aggregation and seeding *in vivo,* raising the question on whether accumulation of pS129 is an early or a late event in synucleinopathies pathogenesis.

When does phosphorylation at S129 occur during PD pathogenesis: An early or late event?

Until today, the available data concerning pS129 accumulation in the brain have been collected in post-mortem tissues and the question on whether pS129 accumulation occurs during the early or late stages of synucleinopathies remains ambiguous.

In a recent work, Walker and collaborators investigated how pS129 levels and solubility change in cingulate and temporal cortex of DLB patients, at different stages of the disease. Using biochemical analysis, the authors reported a progressive accumulation of pS129-immunoractive species in diseased brains, compared to the healthy controls, and a positive correlation between pS129 levels and the severity of the disease symptoms [110]. Moreover, accumulation of insoluble phosphorylated forms, as well as the formation of pS129-postive insoluble species became detectable only at the late stages of the disease (stage IV and V, according to the Unified Staging System [111]) [110]. A similar study, using brain samples form patients suffering from PD, also reported a dramatic accumulation of pS129-positive inclusions in different brain regions at the late stages of the disease [112]. Together, these results demonstrate that abnormal accumulation of insoluble α-syn phosphorylated forms is mainly observed at the advanced stages of synucleinopathies, and suggest that accumulation of this PTM could be a late event in the disease progression. Moreover, the co-localization of α-syn with several kinases within LBs, notably CK2 [113], GRK5 [114] and LRRK2 [115] and the ability of these kinases to efficiently phosphorylate fibrillar and aggregated forms of α-syn [67, 116, 117], suggest that these kinases may also catalyze α-syn phosphorylation after LB formation.

Together these findings support the hypothesis that α-syn phosphorylation may occur after LBs formation and suggest that pS129 accumulation in the brain could represent a late event in the disease progression. It is plausible that the aberrant phosphorylation of α-syn within LBs may reflect an active process whereby phosphorylation may promote LBs disaggregation and/or enhance their clearance and degradation (Fig. 2).

Phosphorylation at S129 is a reliable biomarker for the diagnosis of PD and related disorders

In the past few years, the detection of phosphorylated α-syn at S129 in human cerebral spinal fluid (CSF) and blood plasma has been considered as a promising potential biomarker for the diagnosis of PD and related disorders [118]. Since then, several groups investigated the potential utility of pS129 accumulation in human fluids and peripheral nervous system as a biomarker for PD and synucleinopathies.

pS129 accumulation in human fluids and peripheral nervous system is the pathological hallmark of synucleinopathies

In 2013, Foulds and colleagues conducted a longitudinal study to investigate the relative changes of pS129 levels in the blood plasma of patients suffering from PD [119]. In this study, the authors showed that, although the levels of total α-syn were similar between PD patients and control subjects, pS129 levels were significantly higher in PD samples [119]. Moreover, statistical analysis confirmed the utility of pS129 plasma levels in discriminating patients with PD from healthy controls. Furthermore, recent studies reported that detection of phosphorylated α-syn inclusions within structures of the peripheral nervous system might also be a useful diagnostic test for PD and related synucleinopathies. Using skin biopsies, two independent groups reported pS129 accumulation in small and large nerve fibers in the majority of patients suffering from PD, while no signal was detected in healthy controls [120, 121]. Importantly, this cutaneous pathology was correlated with the evolution of the disease symptoms, suggesting that this peripheral marker can reflect the disease progression and could serve as a biomarker to monitor the disease evolution [120]. Moreover, other studies reported the presence of pS129-immunoreactive signal in gastric, duodenal and colonic biopsies [122, 123]. This pS129 pathology in gastric biopsies can be detected several years prior to the onset of motor symptoms, and therefore could be used for the early diagnosis of PD cases [123].

Collectively, these observations demonstrate that detection of pS129 in human fluids or in the peripheral nervous system could offer new opportunities for the development of promising biomarkers for the diagnosis of synucleinopathies and for monitoring their progression.

Accumulation of pS129 discriminates between synucleinopathy cases

Besides it potential role as a biomarker for PD and related disorders, converging evidence suggest that pS129 could also discriminate between the different synucleinopathy affections. In a post-mortem study, analysis of phosphorylated α-syn levels revealed a significant accumulation of pS129 in DLB-diseased brains, compared to healthy controls, confirming the association of pS129 accumulation and synucleinopathies [124]. Importantly, this study also revealed a difference in pS129 levels and solubility between the different synucleinopathies. In addition to its aberrant accumulation in PD and DLB-diseased brains, pS129 levels are higher in the PD with dementia (PDD) and DLB groups compared to PD without dementia (PDND), suggesting that pS129 accumulation is closely associated with demented cases [124].

Furthermore, pS129 levels in human fluids and in the peripheral nervous system could also discriminate between the different synucleinopathies cases. In a post-mortem study, Foulds and colleagues observed that the concentration of insoluble pS129 in cerebrospinal fluids is significantly higher in MSA samples compared to the other synucleinopathies, including PD and DLB [125]. More recently, pre-mortem analysis of pS129 signal in skin sympathetic nerve fibres and dermal nerve fibres revealed an accumulation of phosphorylated α-syn in PD patients, while no signal was detected in MSA or essential tremor control subjects. Together, these observations demonstrate that accumulation of pS129, rather then total α-syn, might provide a reliable test to discriminate between synucleinopathies and can help with the patients' classification for a better management and recruitment for clinical trials.

Conclusions

Elucidation of the relative implication of S129 phosphorylation on α-syn aggregation, LBs formation and neurotoxicity is crucial for the understanding of synucleinopathies pathogenesis and the development of new disease-modifying treatments for PD and related disorders. However, some important questions remain unexplored: *1)* identification of the exact α-syn physiological functions regulated by phosphorylation at S129. For example, a number of studies suggested the implication of pS129 in the control of α-syn subcellular localization [92, 93] and the modulation of dopamine synthesis [126], however the exact mechanism underlying these processes are not yet clear; *2)* systematic investigation of the role of each kinase on α-syn neurotoxicity, with a focus on PLK2-mediated α-syn clearance as a viable target for the development of new therapeutic strategies for PD; *3)* investigation of the cross talk between pS129 and other PTMs, for instance the interaction with Y125 phosphorylation. Indeed, recent studies suggested that S129 phosphorylation is tightly controlled by phosphorylation at Y125 [127] and it may regulate its toxicity *in vivo* [128]; *4)* investigation of the synergistic role of PD-linked mutations and pS129 in the regulation of α-syn toxicity. This question was raised by a recent observation showing that E46K increases α-syn phosphorylation at S129 in cell culture, yeast and rodent models of PD [98]; *5)* evaluation of the electrophysiological consequences of α-syn phosphorylation and its exact role in the regulation of stress-related synaptic plasticity; *6)* assessment of the role of pS129 accumulation in the increased susceptibility of some brain regions to α-syn pathology. This question is motivated by the *in vivo* observation reporting a disparity in phosphorylated α-syn expression levels between different brain regions [50] and finally *7)* investigation of the possible implications of phosphorylation on α-syn cell-to-cell transmission and its pathological propagation in PD-diseased brains. Ultimately, answers to these questions will lead to the identification of novel and more tractable therapeutic targets for the treatment of PD and related synucleinopathies.

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CONFLICT OF INTEREST

The author has none to declare.

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