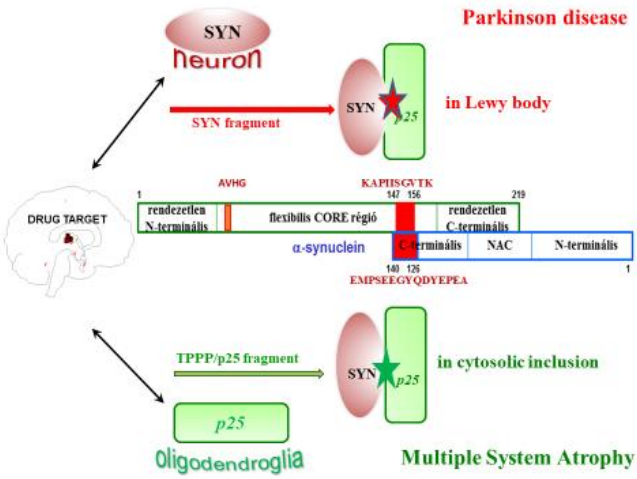
With the aging of society, neurological disorders, especially the *proteopathies*, cause serious socio-economic problem. Currently there is no appropriate therapy for the healing of these protein conformational disorders, a dominant representative of which is Parkinson's disease (PD). The development of therapeutic strategies is limited due to the lack of understanding of the pathomechanisms and the validation of specific drug targets. Two intrinsically disordered proteins with high conformational plasticity, alpha-synuclein (SYN) and Tubulin Polymerization Promoting Protein (TPPP/p25) are regulators of the microtubule network and hallmarks of synucleinopathies. They are expressed in distinct cell types, in neurons and oligodendrocyte cells, however, they are enriched and co-localized in both cell types leading to the etiology of PD and multiple system atrophy. In addition, both SYN and TPPP/p25 display physiological and pathological functions; all these issues indicate that they cannot be considered potential drug targets. Since the small, soluble assemblies of SYN are fatal with respect to the etiology of synucleionpathies, a new innovative strategy has been established (cf. scheme) below, for the identification and validation of specific drug target that is the key to the development of the pharmaceutical intervention.



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The degradation of unfolded proteins and aggregates plays an important role in protein quality control regulated by the proteasome machinery and autophagy cooperating with the dynein/dynactin-mediated trafficking pathway. The cargo aggregates are transported on the acetylated microtubule (MT) network for their clearance by the aggresome-autophagy pathway. The inadequate operation of these defensive systems can lead to various diseades including Parkinson's disease. The disordered moonlighting and chameleon TPPP/p25 modulates the dynamics and stability of the MT network by its tubulin acetylation enhancing and MT bundling activities; however, its enrichment leads to the formation of aggresomes at cellular level as well as inclusions co-localizing with ◎-synuclein in Parkinson's disease,

respectively. HDAC6, a tubulin deacetylase, is a key player in the trafficking and degradation of cargo proteins.

We provided evidence for the direct interaction of DYNLL/LC8 dynein light chain with the microtubule-regulatory proteins, TPPP/p25 and HDAC6, which was visualized in living HeLa cells by immunofluorescence microscopy coupled with bimolecular fluorescence complementation technology; in addition, the localization of the labelled LC8-TPPP/p25 complex was detected on the microtubule network. The interaction of LC8 isoforms to TPPP/p25, characterized by biochemical and immunological methods using human recombinant proteins, was revealed (Kd = 12 nM). LC8 did not affect the TPPP/p25-derived assembly of the tubulins/MTs, but slightly reduced the dimerization of TPPP/p25; however, TPPP/p25 promoted the association of LC8 to tubulin and its polymerized form. The concentration-dependent ratio of the binary and the ternary complexes of tubulin with TPPP/p25 and LC8 was evaluated by mathematical modelling that allows the prediction of the functional consequences of the multiple associations at different cellular situations The hetero-association of LC8 and HDAC6 was detected by ELISA using HeLa cell extract, and shows that LC8 did not affect HDCA6 activity. Our data suggest that the multiple associations of the microtubule network with its regulators and DYNLL/LC8, an integral subunit of the dynein motor complex (cf. Scheme below), could influence the efficiency of the aggresome-autophagy pathway.

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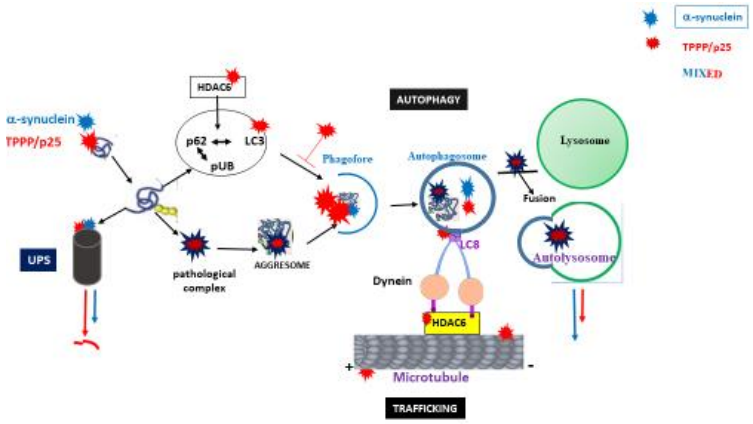
The pathological hetero-association of SYN and TPPP/p25 is a key factor in the etiology of PD and multiple system atrophy (MSA). In normal brain, SYN and TPPP/p25 occur in neurons and oligodendrocytes, respectively, while at pathological conditions they are enriched in both cell types likely due to cell-to-cell transmission. The selective degradation of the pathological SYN by autophagy has been extensively searching; our objective is to extend the research to the elimination of the SYN complexed with TPPP/p25. For this purpose a human cell model (HeLa) was that expresses these hallmark proteins at low level, if at all, the high pathological level was proceeded by their uptake from the medium mimicking extracellular situation. The effect of the TPPP/p25 on the SYN-derived proteolytic degradation was characterized by fluorescence microscopy and fluorescence-activated cell sorting technology (FACS) using HeLa cells expressing fluorescent-tagged LC3 (mRFP-EGFP-LC3). These experiments showed that the SYN-derived autophagy maturation is suspended by the presence of TPPP/p25; no enhancement of autolysosme could be detected. This effect is likely related to the inhibitory effect of TPPP/p25 displaying against to the SYN-promoted LC3-p62-derived vesicle (phagofore) formation. The autophagy maturation appeared to be inhibited by TPPP/p25 likely due to its tight binding to LC3. The quantification of the hallmark proteins by Western blot using specific anti-SYN and anti-TPPP/p25 antibodies showed that the SYN assembled

with TPPP/p25 is failed to be degraded. The combination of this finding with our recently published one suggests that the destruction of the pathological complex by interface-targeting agents can be achieved thus the degradation of the excess SYN could be proceeded.



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The microtubules play crucial roles achieved in both physiological and pathological processes by their decoration with proteins/enzymes as well as by posttranslational modifications. The microtubule associated protein, TPPP/p25 display regulatory functions by day and pathological functions at night. Physiologically, the moonlighting TPPP/p25 modulates the dynamics and stability of the microtubule network by bundling microtubules and enhancing the tubulin acetylation due to the inhibition of tubulin deacetylases. The optimal endogenous TPPP/p25 level is crucial for its physiological functions, to the differentiation of oligodendrocytes, which are the major constituents of the myelin sheath. Pathologically, TPPP/p25 forms toxic oligomers/aggregates with ◎-synuclein in neurons and oligodendrocytes in Parkinson's disease and Multiple System Atrophy, respectively; and their complex is a potential therapeutic drug target. TPPP/p25-derived microtubule hyperacetylation counteracts uncontrolled cell division. All these issues reveal the anti-mitotic and alpha-synuclein aggregation-promoting potency of TPPP/p25, concerning with the finding that Parkinson's disease patients have reduced risk for certain cancers.