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Proteolytic clearance of extracellular α-synuclein as a new therapeutic approach against Parkinson disease

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Many neurodegenerative diseases such as Alzheimer disease and Parkinson disease show similar characteristics. They typically show deposits of protein aggregates, the formation of which is considered important in their pathogenesis. Recently, aggregation-prone proteins have been shown to spread between cells and so may contribute to the pathogenesis of diseases like prion disease. Such a pathogenesis pathway is possibly common to many neurodegenerative diseases. If confirmed, it could allow the development of therapeutic interventions against many such diseases. In Parkinson disease, α-synuclein, a major component of cytosolic protein inclusions named Lewy body, has been shown to be released and taken up by cells, which may facilitate its progressive pathological spreading between cells. Accordingly, inhibition of spreading by targeting extracellular α-synuclein may represent a new therapy against Parkinson disease. Research into the intercellular spreading of extracellular protein aggregations of α-synuclein and its clearance pathway are reviewed here with a focus on the proteolytic clearance pathway as a therapeutic target for the treatment of Parkinson disease. Considering the similar characteristics of aggregation-prone proteins, these clearance systems might allow treatment of other neurodegenerative diseases beyond Parkinson disease.

Keywords: α-synuclein, Parkinson disease, proteolysis, protease, plasmin, neurosin, matrix metalloproteinase, neurodegenerative disease, prion, aggregation-prone proteins

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Introduction

Age-related progressive neurodegenerative diseases such as Alzheimer (AD) and Parkinson (PD) diseases are increasingly affecting the world's aging population. Despite much research, their pathogenesis still remains insufficiently understood to allow the rational design of therapeutic interventions that reduce their progression.

Interestingly, many neurodegenerative diseases involve protein aggregate inclusions, despite displaying different symptoms. PD shows cytosolic Lewy bodies or Lewy neurites composed of mainly α-synuclein (α-syn). AD shows extracellular senile plaques of mainly Aβ and cytosolic neurofibrillary tangles comprising mainly hyperphosphorylated tau. Moreover, polyglutamine diseases, amyotropic lateral sclerosis (ALS) and prion disease show typical protein inclusions composed of mainly mutated polyglutamine expanded proteins, mutated superoxide dismutase (SOD)-1 and PrPsc, respectively.[1] Furthermore, the process of protein aggregate formation is considered to be significant in the pathogenesis of neurodegenerative diseases and aggregation-prone proteins are promising therapeutic targets for the treatment of such neurodegenerative diseases.

Recently, these aggregation-prone proteins have been observed to show unexpected yet interesting similar characteristics. Their regional and intercellular spreading has been observed irrespective of cytosolic or extracellular proteins and these processes are suspected to be significant in the pathogenesis of neurodegenerative diseases. Therefore, the inhibition of these proteins spreading may represent a new possible treatment of such diseases.

This review discusses the intercellular spreading of extracellular α-syn and the proteolytic clearance of extracellular α-syn as a new therapeutic target for PD. The characteristics of α-syn are compared with those of other aggregation-prone proteins implicated in neurodegenerative diseases.

The Relationship Between PD and α-Syn

PD is the second most common neurodegenerative disease after AD. It is characterized by a progressive dopaminergic neuronal loss in the substantia nigra pars compacta. Also characteristic is the presence of intracytoplasmic protein aggregates, Lewy bodies or Lewy neurites, as observed during other similar neurodegenerative diseases associated with aggregation-prone protein.[2]

The intracytoplasmic protein aggregates observed in PD patients are mainly composed of α-syn, a small protein that is expressed abundantly in neuronal cells; it is localized mainly in the presynaptic nerve terminals.[3] Mutations of α-syn gene (A30P, E46K and A53T) and multiplications of the wild-type gene have been found to be associated with familial cases of early onset PD. Moreover, genetic variations in both the promoter and the region of the SNCA gene encoding the α-syn protein have been found to increase the susceptibility to PD.[4] Recent genome-wide association studies (GWASs) have identified variants of the SNCA gene that are coupled to increase PD susceptibility.[5,6] Overall, a clear link has been found between this protein and idiopathic and familial PD. In support of these genetic studies, animal models with transgenic overexpression of α-syn have been shown to mimic several aspects of PD.[7] These observations in addition to several in vitro studies have firmly established the involvement of α-syn in the pathogenesis of PD.[7]

Extracellular α-Syn and the Spreading of α-Syn Pathology Between Cells

α-Syn is normally considered a cytoplasmic protein and its function in cells' cytoplasm has been received most attention. However, this view is beginning to change.

Monomeric and oligomeric α-syn have been continuously reported to be present in both healthy and diseased human cerebrospinal fluid (CSF) and blood plasma.[8-10] In addition, α-syn and its aggregates have been shown to be secreted from neuronal cells by a non-classical exocytic pathway[11] and its release is increased under various protein misfolding stress conditions.[12] α-Syn has also been reported to be secreted in a calcium-dependent manner by exosomes,[13] and lysosomal dysfunction increases exosome-mediated α-syn release.[14] These studies imply that extracellular α-syn is not only due to cellular leakage by cell death, but also its release from cells. This phenomenon is proposed to be probably part of a cellular quality control mechanism for the removal of damaged and harmful proteins by exocytosis.

Uptake of α-syn into cells has also been observed.[15-18] Although the exact mechanism of α-syn uptake has not been established, 11-amino acid imperfect repeats found in the α-syn sequence have been shown to be critical.[16] α-Syn has been shown to be internalized via GM1 and hitherto unknown protein receptors via a lipid raft-dependent endocytosis mechanism,[18] suggesting that cytosolic α-syn can be released from cells and taken up by other cells. In addition to studies of its release and uptake, extracellular α-syn has been reported to have effects on neurotoxicity[15,19,20] and inflammation.[14,18,21-24]

Recent observations that the transplants grafted into the brain of PD patients displayed Lewy bodies[25,26] were considered to be connected with Braak et al.'s proposal that Lewy body pathology spreads from one brain area to another according to a stereotypic pattern in specific stages.[27] Consequently, more recent in vitro and in vivo experiments[28-33] have shown that α-syn aggregates released from neuronal cells can be transferred to neighboring neurons and form Lewy body-like inclusions, providing a mechanistic basis for the spread of α-syn pathology in PD patients and a hypothesis that a prion-like mechanism may underlie the progression of PD.

Interestingly, other cytosolic proteins, such as tau, SOD-1 and polyglutamine expanding proteins, implicated in the pathogenesis of other neurodegenerative diseases have also been observed to have similar characteristics to α-syn.

Tau, a major component of cytosolic neurofibrillary tangles observed in AD, has been found in both healthy and AD CSF.[34,35] It is secreted from cells by unconventional exocytosis or exosome[36-38] and its aggregates are taken up by cells via a hitherto unknown mechanism.[39] In addition, extracellular tau has been shown to be toxic to cultured neuronal cells,[40] and prion-like intercellular spreading of tau aggregates has been reported both in vivo and in vitro.[41,42]

Native and misfolded SOD-1 have also been observed in the CSF of control and ALS patients.[43] Once secreted,[44,45] it causes neurotoxicity and glial cell activation.[45,46] Prion-like propagation of SOD-1 aggregates have also been reported both in vivo and in vitro.[47,48]

Polyglutamine peptide aggregates observed in polyglutamine diseases have also been reported to be internalized by cells and become co-sequestered in aggreasomes with cytosolic proteins,[49] implying the possibility of the intercellular spreading of polyglutamine aggregates.

Accordingly, the prion-like characteristics of aggregation-prone proteins responsible for many neurodegenerative diseases may be a common pathogenic mechanism and thus the reason for the recent growing interest (see reviews in refs. 1 and 50–54 for more details).

Proteolytic Clearance as a Therapeutic Approach Against PD and Other Diseases

Although this hypothesis needs further proof, it has led to the investigation of therapies that slow the progression of neurodegeneration by preventing the intercellular spreading of these proteins.

Preventing the spread of extracellular α-syn may be a suitable means of halting the progression of PD. The level of extracellular α-syn depends both on the rate of α-syn release from neuronal cells and the rate of its removal through various clearance pathways such as cell-mediated

clearance, proteolytic degradation, chaperone-mediated clearance and active/passive transport out of the brain. Therefore, the treatment of PD could be achieved through targeting the regulation of α-syn release and uptake, or the removal of extracellular α-syn by a variety of clearance systems.

Proteolytic clearance is possible using any of several proteases that have been identified to be able to cleave and degrade α-syn: these include neurosin,[55] matrix metalloproteinases (MMPs),[56] calpain,[57] cathepsin D[58] and plasmin.[59] Among them, neurosin, MMPs and plasmin have been reported to cleave and degrade extracellular α-syn. Neurosin, a serine protease, is preferentially expressed in neurons and oligodendrocytes in the brain.[55] It was first observed to be colocalized in some senile plaques in AD patients as well as Lewy bodies in PD patients.[60] It has also been reported to degrade intracellular α-syn, but less efficiently A53T α-syn and also to inhibit α-syn polymerization.[55] Tatebe et al. later demonstrated in vitro that secreted neurosin degrades extracellular α-syn.[61] Recently, the viral mediated delivery of neurosin has been shown to promote the clearance of α-syn and reduces pathology in an α-syn model,[62] implying that neurosin may be a new therapeutic target for PD.

MMPs, particularly MMP-3, have also been reported to cleave extracellular α-syn.[56] Sung et al. demonstrated that oxidative injury induces the cleavage of extracellular α-syn released from neuronal cells and this is eventually cleaved by MMPs. However, the cleavage of α-syn by MMP-3 further induces its aggregation,[56] lessens any likely effects on PD of cleaving extracellular α-syn by MMPs.

The plasmin system may be a therapeutic target for preventing the intercellular spreading of extracellular α-syn. It is one of the proteases that can cleave extracellular α-syn.[59] Plasmin is an extracellular serine protease that is important in fibrinolysis. It is derived from its inactive form, plasminogen, by tissue type plasminogen activator (tPA) or urokinase plasminogen activator (uPA).[63] Although plasmin is synthesized mainly in the liver, it has also been detected in the CNS and is mainly expressed in neurons and astrocytes. Additionally, plasmin in the CNS is physiologically and pathologically important in such as neuronal development, synaptic plasticity and excitotoxicity through the cleaving of extracellular matrix components such as fibronectin, laminin and MMPs in addition to fibrin.[64-66] Plasmin cleaves monomeric and further oligomeric and fibrillar forms of α-syn irrespective of the familial type of point mutation, unlike neurosin. However, tPA, uPA and thrombin do not cleave α-syn. Plasmin also inhibits the intercellular spreading of α-syn released from neuronal cells and glial activation by extracellular α-syn by cleaving the N-terminal region of α-syn into small fragments. This suggests that the plasmin system in the CNS may prevent the progression of PD through inhibiting extracellular α-syn's detrimental intercellular spreading and glial activation.[59]

Interestingly, plasmin can also degrade several forms of Aβ and block Aβ-induced toxicity, which contribute to the progression of AD,[67,68] suggesting that it could also act against other neurodegenerative diseases besides PD. Overall, proteolytic enzymes such as neurosin and plasmin which cleave extracellular α-syn appear to be potential therapies against PD.

Proteolytic systems against Aβ are actively being studied for the treatment of AD. Several proteases including neprilysin, insulin degrading enzyme and MMPs as well as plasmin have been identified to cleave Aβ.[69-72] Ex vivo gene delivery of neprilysin has been reported to reduce amyloid plaque burden in AD models.[73] Also, the inhibitor of PAI-1, which inhibits tPA activity and further plasmin activity, augments the activity of the plasmin system, thereby reducing the Aβ level and restoring memory deficit in AD models.[74] Consequently, several in vitro and in vivo studies have implicated amyloid degrading enzymes as new therapeutic targets against AD (see reviews in refs. 75–77 for more details). With regard to prion disease, cysteine proteases such as cathepsin B and L have been reported to degrade prions in $CD11c^{+}$ dendritic cells and in GT1-1 neuronal cells.[78] Efforts to find proteases that can degrade PrPsc and further inhibit the amplification of pathologic effects of PrPsc are still ongoing.

In addition, dysregulation of these protease systems has also been observed to be associated with neurodegenerative diseases. In PD, reduced expression of neurosin has been observed in the brain of an animal model of PD and in patients with dementia with Lewy bodies.[62] Alterations of MMPs have also been observed in neurodegenerative diseases other than PD,[79] and extracellular α-syn has been reported to regulate the activity of MMPs.[22,80,81] Although the association between the plasmin system in the CNS and PD has yet to be established, the reduction of tPA activity by extracellular α-syn in primary astrocytes and microglia has been reported.[80] Extracellular α-syn has also been shown to increase PAI-1 expression in neurons, astrocytes and microglia and thus may inhibit plasmin activity,[59] suggesting that the plasmin system may be dysregulated in PD. Furthermore, the association between the plasmin system and other neurodegenerative diseases has been well reported. In AD, decreased tPA activity has been observed in AD models and its activity has been proposed to be controlled by a substantial increase of PAI-1.[82] Increased PAI-1 has been observed in APP transgenic mice[83] and in the CSF of AD patients.[84] Brain plasmin activity is also reduced in AD brains.[70] In prion disease, tPA accelerates the cleavage of prion protein by plasmin, implying that the plasmin system may be involved in the pathogenesis.[85] Dysregulation of neprilysin and insulin degrading enzyme, major Aβ degrading enzymes, has also been observed in AD.[86-88] Therefore, dysregulation of the proteolytic clearance systems may be a common pathologic mechanism of neurodegenerative diseases beyond PD.

Other Clearance Systems as Therapeutic Approaches Against PD and Other Diseases

In addition to proteolytic degradation, other clearance pathways could represent potential therapeutic targets. Cell-mediated clearance pathways including endocytosis or phagocytosis have been reported to clear extracellular α-syn.[17] Immunization against α-syn can improve α-syn pathology, possibly due to increased cell-mediated clearance.[89,90] As

a chaperone-mediated clearance pathway, HSP70 was reported to reduce extracellular α-syn oligomer formation and related toxicity.[91]

A variety of clearance pathways of Aβ in AD have been actively studied.[92] Microglia and astrocytes have been reported to be able to phagocytose Aβ and immunization with Aβ was shown to promote clearance,[93] which are currently considered the most effective therapeutic targets against AD.[94] Clusterin has also been reported to bind to Aβ and enhance Aβ clearance as a chaperone.[93] Passive immunization against prion has shown to decrease CNS pathology.[95] Immunization strategies against tau and mutant SOD-1 could also be used to treat AD and ALS, respectively.[96-98] However, it remains uncertain whether their primary targets are cytosolic or extracellular proteins.

Concluding Remarks

Several common characteristic of neurodegenerative diseases are coming to be known. Precise understanding of these diseases' pathogenesis could aid the development of common therapeutic interventions to stop their progression. In this sense, the spreading of aggregation-prone proteins, particularly cytosolic proteins such as α-syn, tau, SOD-1 and polyglutamine expanding proteins, into neighboring cells is potentially important in pathogenesis. Furthermore, interventions against their spreading could form the bases of new treatments. Accordingly, the proteolytic clearance system and other clearance systems which block the proteins' spreading and hence their detrimental effects could serve as good targets for treatment. There may be more proteolytic enzymes responsible for the degradation of aggregation-prone proteins and efforts to find them and so regulate them directly or indirectly should aid the elucidation of the pathogenesis of many neurodegenerative diseases and hence help the development of therapeutic strategies of them.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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