Protein aggregates stimulate macropinocytosis facilitating their propagation

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ABSTRACT. Temporal and spatial patterns of pathological changes such as loss of neurons and presence of pathological protein aggregates are characteristic of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis, Frontotemporal Dementia, Alzheimer’s disease and Parkinson’s disease. These patterns are consistent with the propagation of protein misfolding and aggregation reminiscent of the prion diseases. There is a surge of evidence that suggests that large protein aggregates of a range of proteins are able to enter cells via macropinocytosis. Our recent work suggests that this process is activated by the binding of aggregates to the neuron cell surface. The current review considers the potential role of cell surface receptors in the triggering of macropinocytosis by protein aggregates and the possibility of utilizing macropinocytosis pathways as a therapeutic target.

KEYWORDS. amyotrophic lateral sclerosis, alzheimer’s disease, macropinocytosis, neurodegenerative disease, prion, propagation, protein misfolding, protein aggregate, Parkinson’s disease, SOD1

Neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), Alzheimer’s disease, and Parkinson’s disease have temporal and spatial patterns of pathological changes such as loss of neurons and presence of pathological protein aggregates. The patterns that have been identified in neuropathological studies are have been suggested to be consistent with propagation of protein misfolding and aggregation reminiscent of the prion diseases.5

Evidence from in vitro studies suggests that large protein aggregates of a range of proteins (SOD1, tau, α-synuclein, and proteins containing expanded polyglutamine repeats) are able to enter cells via an incompletely understood
mechanism and propagate misfolding and aggregation.\textsuperscript{6-14} Animal studies demonstrate that injection of brain or spinal cord extracts from transgenic mice expressing human tau, SOD1 or \(\alpha\)-synuclein can seed pathology in transgenic mice at the sites of injection and initiate spread of pathology to other regions of the nervous system.\textsuperscript{14-17} These data combined with the patterns of neurodegeneration provide strong evidence of prion-like protein misfolding propagation in neurodegenerative diseases such as Parkinson’s disease and ALS. The precise mechanism of cell-to-cell propagation of protein misfolding \textit{in vivo} remains unclear. There are several possible scenarios that may explain the propagation of protein misfolding, the most commonly proposed of which is the release of protein aggregates in to the extracellular space and subsequent uptake in to nearby or connected naive neurons where seeding can then take place. In this model both aggregate release and subsequent uptake are requisite. Misfolded or aggregated material may be released from cells via an active mechanism, either within exosomes or naked, or alternatively, aggregates may be passively released from dead or dying cells. In reality, within a complex biological system it is likely that the release of aggregated protein may be a combination of each of the possible release mechanisms. This makes therapeutic targeting of aggregate release difficult, moreover, reducing the burden of misfolded protein by active release may be beneficial to the cell and thus its blockade detrimental. One aspect of protein misfolding propagation that may provide a more tractable therapeutic target is the entry of protein aggregates in to cells.

Work to date suggests that macropinocytosis may be responsible for the uptake of protein aggregates associated with various neurodegenerative diseases.\textsuperscript{6,11,18-20} A range of studies examining several different types of protein aggregates, including those made from \(\alpha\)-synuclein, Huntingtin (exon1Q44), and tau are consistent with an unconventional, bulk endocytosis route being responsible for their uptake.\textsuperscript{12,18,21-24} A systematic study using SOD1 aggregates by Münch et. al concluded that protein aggregates enter into N2A cells \textit{via} macropinocytosis.\textsuperscript{6} These findings were subsequently corroborated in work that showed uptake of both extracellular wildtype and mutant SOD1 soluble forms into NSC-34 cells can be inhibited by small molecule inhibitors of macropinocytosis EIPA and rottlerin.\textsuperscript{11,19} EIPA (5-(N-Ethyl-N-isopropyl)amiloride) is an amiloride analog that inhibits Na\(^+\)/H\(^+\) exchangers which suppresses macropinocytosis due to the vulnerability of GTPase Rac1 to pH changes.\textsuperscript{25} However, the small molecule inhibitors generally utilized to define macropinocytosis, such as EIPA, are not specific and have been shown to prevent various forms of endocytosis.\textsuperscript{26} As a result, further work is required to more precisely define the precise mode of endocytosis responsible for protein aggregate uptake.

Macropinocytosis is a type of endocytosis that is partly defined by pinosome size, macropinosomes can be several micron in diameter. Macropinocytosis can be constitutive or a transient process, generally triggered by growth factors but has been observed to be activated by particles such as bacteria, apoptotic bodies and viruses.\textsuperscript{27} Its activation leads to formation of large membrane ruffles that can fold back on the cell allowing the internalisation of fluid, membrane and other particles into large vacuoles several micron in diameter.\textsuperscript{28} The role of stimulation of macropinocytosis in aggregate uptake remains unclear.

Our recent work shows that SOD1 aggregates can bind to the cell surface and trigger activation of membrane ruffling in NSC-34 cells and iPSC generated human neurons\textsuperscript{20} consistent with stimulated rather than constitutive macropinocytosis. The engulfment of aggregates in to macropinosomes could explain the ease by which aggregates enter the cytosol. Macropinosomes are considered ‘leaky’ due to their lack of physical structure compared with coated vesicles.\textsuperscript{29} Indeed, we observed vesicle rupture upon entry of SOD1 aggregates in to NSC-34 cells.\textsuperscript{20} A characteristic signaling molecule associated with actin mobilization during macropinocytosis in the Rho GTPase Rac1. We found that application of SOD1 aggregates to NSC-34 cells resulted in an increase in the amount of activated Rac1, and also observed
that the uptake of aggregated SOD1 into NSC-34 and human iPSC derived motor neurons was suppressed by the Rac1 inhibitor W56.20 These data are consistent with a triggering of macropinocytosis that involves an activation of Rac1. Moreover, our data indicate that aggregates made from other proteins such as α-synuclein, huntingtin-polyQ and TDP-43 also trigger membrane ruffling and macropinocytosis.20 Indeed, this seems to be a generic response to cell surface interaction with protein aggregates since aggregates made from α-lactalbumin, that are not involved with disease, also trigger this response.

The question of how protein aggregates trigger macropinocytosis remains unanswered. To begin to shed light on the answer to this question one can turn to the virus uptake literature as the mechanisms of macropinocytosis activation have been more closely studied in this context. There are several viruses27 that have been shown to utilize macropinocytosis pathways to enter cells, including the Japanese encephalitis virus in neurons.30 Receptor complexes on the cell surface are vital to the binding of viral particles and activation of macropinocytosis. Integrins such as β1,31 αv,32 and the heparan sulfate proteoglycans33 have been reported to be involved in particle binding, while receptor tyrosine kinases such as ErbB-134 and EphA235 have been implicated in the triggering of the signaling cascade that stimulates macropinocytosis (Fig. 1A). Little is known about the receptors that mediate protein aggregate induced macropinocytosis in neurons. However, heparan sulfate proteoglycans have been identified as an important part of the mechanism that allows tau, α-synuclein and prion protein aggregate uptake18,36,37 suggesting that the receptor complexes involved may be similar to that involved in virus particle uptake.

While little is known about the neuronal receptor complexes associated with binding of protein aggregates, a wide range of receptors have been shown to be involved in the binding of protein aggregates to the cell surface of microglia.38,39 A group of receptors, known as pattern recognition receptors, are often responsible for this process. These receptors bind to pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs) that may be responsible for the recognition of protein aggregates. It is possible that these pattern recognition receptors bind to the repetitive patterns found on aggregates formed from various proteins, indeed the fibrillar structure of amyloid fibrils are thought to have a generic core structure. The binding of particles to these microglial receptor complexes results in activation of signaling cascades, including activation of MAP kinase and ERK1/2, subsequently resulting in cytokine release.30 Scavenger receptors CD3641 and SR-A,52 the receptor for advanced glycation end products (RAGE),43,44 integrin α6β1-integrin and integrin associated CD47, formyl peptide receptor 2/formyl peptide receptor-like 1 (FPR2/FPRL1),45 CD33,46 CD14 and Toll-like receptors (TLR) 2/447 have all been shown to be involved in microglial binding of protein aggregates made of Aβ peptide. In addition, we have previously shown that microglia binding of aggregated SOD1 is suppressed by pre-treatment with methyl-β-cyclodextrin (MβCD), fucoidan and lipopolysaccharide (LPS) suggesting that binding is dependent on lipid raft formation, scavenger receptors and CD14.48 The binding of fibrils to microglial cell surface is mediated not by individual receptors but though large receptor complexes (Fig. 1B) involving signaling receptors such as TLR2/4. This process of receptor complex formation, in some cases, is dependent on lipid raft formation to allow membrane protein organization.49

Neurons express heparan sulfate proteoglycans, RAGE,51 CD36,52 integrins and receptor tyrosine kinases such as EphA2 and EphA4.53 This is consistent with neurons having all the machinery to bind aggregates and to trigger macropinocytosis with a receptor complex similar to that involved in viral uptake and somewhat similar to that of microglial cell surface recognition (Fig. 1C). Indeed, a macropinocytosis-like process regulates growth cone membrane recycling, growth cone
Future work will be needed to further characterize the composition of the neuron specific set of cell surface proteins that are responsible for the binding, triggering of macropinocytosis and uptake of protein aggregates. Given that blocking all macropinocytosis in the brain, including microglial and astrocytic, may be detrimental this knowledge could be used to define a specific target to block neuronal uptake of protein aggregates. Blocking uptake may effectively suppress propagation of protein aggregation, as the cytoplasmic invasion of protein aggregates, like those of SOD1, are a necessary part of the prion-like propagation process. However, due to the role of a macropinocytic process in growth cone and axon development, the effect of inhibition of macropinocytosis on neurogenesis should be studied. Regardless, a receptor tyrosine kinase EphA4 has been implicated in ALS disease progression. In humans with ALS, EphA4 expression was found to inversely correlate with disease onset and survival, and loss-of-function mutations in EPHA4 were found to be associated with slower disease progression and longer survival. It is tempting to speculate that EphA4 might contribute to the activation of macropinocytosis and uptake of...
protein aggregates, which might be slowed in its absence or inhibition. Identifying such a target that will suppress the neuronal macropinocytosis of protein aggregates but will allow the microglial phagocytosis and degradation of such particles is vital. Blocking all endocytosis of aggregates may be detrimental.

In summary, propagation of protein misfolding and aggregation is implicated in the progressive nature of several neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease and ALS. In order for propagation to proceed aggregates must be able to gain access to the cytosol of naïve neurons and current evidence suggests that stimulated macropinocytosis is responsible for this uptake. The receptor complexes responsible for this are still to be defined, but this information may provide therapeutic targets that could halt or slow the spread of pathology.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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