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Protein aggregation and Arfaptin2: A novel therapeutic target against neurodegenerative diseases

Aida M. Mohammedeid, Vera Lukashchuk, Ke Ning *

Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, 385A Glossop Road, Sheffield S10 2HQ, UK

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ABSTRACT

Therapeutic targets for neurodegenerative conditions are constantly emerging. Diseases such as amyotrophic lateral sclerosis and Huntington's disease are multifactorial and involve dysfunction of various cellular pathways. Protein aggregate formation is one of the crucial pathological signs of cellular dysfunction, and is characteristic of many neurodegenerative conditions. Proteins recruited to these aggregates are thought to play a role in formation of the pathogenic inclusions. This review aims at exploring the current evidence for protein aggregation and the role for Arfaptin2, as a candidate factor contributing to the formation of aggresomes and a potential therapeutic target in motor neuron disease.

Focal points:

- Bedside
  Understanding the multifactorial nature of the pathogenesis of neurodegenerative diseases will contribute to the research into the therapeutic targets of the disease, allowing more factors to be discovered in patients affected by the debilitating disorders of the nervous system.
- Benchside
  Collaborative efforts in investigating the causes and pathways of neurodegeneration are likely to increase the chance of discovering novel therapy approaches that may be utilised in more than one type of neurodegenerative disorders.
- Industry
  The application of the novel therapeutic target such as Arfaptin, and other proteins associated with protein aggregates, to the development of therapy approaches may open new avenues in drug discovery for neurodegenerative diseases.
- Community
  Diseases of central nervous system bear a great impact on the quality of life of the patients and their carers. Promoting the awareness through communicating the current state of the research provides a form of a mental support to those affected by these conditions.
- Regulatory agencies
  The need for funding the research into the basic understanding of the mechanisms involved in the pathogenesis of the neurodegenerative conditions must not be overlooked. The research into the cellular defects provides with invaluable findings about the healthy and diseased cell functioning.

1. Introduction

Neurodegenerative diseases constitute a class of disorders of the central nervous system characterised by progressive loss of neurons, which subsequently leads to death as a result of loss of vital physiological functions. Neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's (AD), Huntington's (HD) and Parkinson's (PD) diseases show some similarities in their pathogenesis. One of the common mechanisms causing neurodegeneration in these conditions is protein aggregation. Protein aggregates are a significant

* Corresponding author. Tel.: +44 114 2222245; fax: +44 114 2222290.
E-mail address: k.ning@sheffield.ac.uk (K. Ning).

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pathological hallmark of many, if not all, neurodegenerative diseases. Protein aggregation is manifested in the form of protein inclusions, also known as aggresomes. These are non-membranous, stable, detergent-insoluble, β-sheet enriched, poly-ubiquitinated protein aggregates with high molecular weight, which are formed due to either overexpression of a protein which exceeds the degradation capacity or defective proteolytic pathways [12,25]. It is yet to be established what precise molecular mechanisms precede the formation of protein aggregates; however, ubiquitin-proteasome pathway plays one of the key roles. Therefore, components associated with the ubiquitin-proteasome machinery may be important players in the establishment of the disease pathogenesis. This review focuses on the general characteristics of protein aggregates in neurodegenerative diseases and their interference with proteasome-mediated degradation and presents Arfaptin2 as a modifier of the this pathway and a potential therapeutic target.

2. Types of protein aggregates

There are three common hypotheses regarding the role of protein aggregates in the pathology of neurodegenerative diseases. Firstly, protein aggregates have toxic effect on neurons and induce their death. Secondly, aggregates are formed as a defensive response to protect the cells against toxic abnormal proteins. Finally, the aggregates are formed as a result of other toxic effects [2].

Protein inclusions vary in structure, and may be characteristic of different neurodegenerative diseases. Four inclusion structures have been reported in neurodegenerative diseases which are skein-like inclusions, Lewy bodies, Bunina bodies and hyaline bodies. Skein-like inclusions are the most common in and specific to ALS [29]. The protein composition of these aggresomes can be detected by immunostaining, and may vary depending on the cause of the disease. Their appearance can range from small dot-like once they begin to form, to filament-like aggregates that increase in size by fusing with other aggregates [14]. The causative factors that may lead to protein aggresome formation and contribute to the pathology observed in neurodegenerative conditions may be speculated given the current evidence, which is discussed further onwards.

3. Protein degradation impairment in neurodegenerative diseases

There is mounting evidence showing that proteolytic machineries, such as ubiquitin-proteasomal system (UPS) and/or autophagy-lysosomal degradation, are impaired in neurodegenerative diseases, which causes cell toxicity and death [5,16,21,26,27]. For example, the inhibition of proteasome degradation pathways in primary motor neurons causes redistribution of the transactive response DNA-binding protein 43 kDa (TDP-43), which is involved in ALS pathogenesis, to the cytoplasm and aggregation, while other cellular stressors had no effect on its distribution. This redistribution was accompanied by increased insolubility, molecular weight (~50 kDa), ubiquitination and phosphorylation. Reduction in TDP-43 levels makes the neurons vulnerable, and knocking down TDP-43 increases their death rate. Therefore, it seems that TDP-43 distribution is controlled, at least partly, by the proteasome system and that a first hit, such as TDP-43 mutation, increases the cell vulnerability and a second hit, such as proteasome dysfunction, induces neurodegeneration and *vice versa* [27].

In addition, impaired ubiquitin proteasome activity plays role in tauopathies such as those representative of AD. Tau is a protein that is involved in microtubule formation, which has a direct impact on axonal transport. It has been shown that a phosphorylated form of a pro-survival kinase Akt phosphorylates tau at Ser214, thereby protecting it from aggregation. However, proteasome inhibition decreases phosphorylation of Akt leading to its decreased activity. This causes tau de-phosphorylation at normal site (Ser214) while inducing phosphorylation at other abnormal sites by Akt downstream effector protein glycogen synthase kinase-3β (GSK-3β) forming aggregate-prone protein, resulting in accumulation and aggregation of abnormal misfolded proteins [30].

4. Impaired endosomal trafficking and axonal transport

Given the highly polarised structure of neurons, functional endosomal trafficking and axonal transport are essential for neuron survival. Impairment of these functions is one of the pathological characteristics of ALS neurons, manifested by dysregulated protein and organelle transport between the dendrites, cell body and axon, and turnover of the membrane proteins [9]. Some of the evidence supporting this statement is discussed further. In one study, pre-symptomatic ALS-SOD1 (superoxide dismutase 1) mouse models showed impaired axonal transport signs such as decrease in speed and frequency of retrograde movement of endocytic carriers [3,28]. Alsin, a protein encoded by *ALS2* gene, which is involved in nuclear import and export, vesicle transport and endosomal trafficking, is a representative factor. ALS-related mutations of *ALS2* gene have been found in juvenile ALS cases causing loss of function of this protein (Yang et al., 2001). Dysfunction in axonal transport may therefore result in aggregation of proteins and contribute to the pathogenesis of ALS.

5. Arfaptin2 protein structure

ADP-ribosylation factor-interacting protein 2 (Arfaptin2), also known as partner of Rac1 (POR1), is a protein consisting of 341amino acid (a.a) with a molecular weight of ~38.6 kDa that is ubiquitously expressed in different types of cells. It is expressed as a cytoplasmic protein that predominantly localises to the perinuclear region and is associated with microtubules-organising centre, and colocalises with the trans-Golgi marker TGN46 [15,17–19]. It shares 81% sequence homology with Arfaptin1. Though the exact function of Arfaptin2 is still unknown, its protein composition gives some clue of the possible cellular processes that it might be involved in. Arfaptin2 contains a leucine zipper which gives it a high positive charge that might have a function in DNA binding. The C-terminus contains the Bin/amphiphysin/Rvs (BAR) domain which is present in different proteins that are involved in membrane curvature and it is responsible for dimerisation, membrane binding and curvature sensing [13,17]. Arfaptin2 also contains a highly conserved amphipathic helix (AH) region (a.a 92–112) (Fig. 1). Both, BAR and AH, are believed to be essential for Arfaptin2 binding to the trans-Golgi network. This binding occurs via small GTPase and phoshatidylinositol 4-phosphatase (PI(4)P) binding [6].

It interacts with the ADP-ribosylation factor (ARF) family proteins, which are GTP-binding proteins that are involved in intracellular vesicular transport including formation of coated vesicle and cytoskeletal reorganisation [7,10,15,23] and transportation between the endoplasmic reticulum and Golgi [1,13] (Table 1). It also binds to

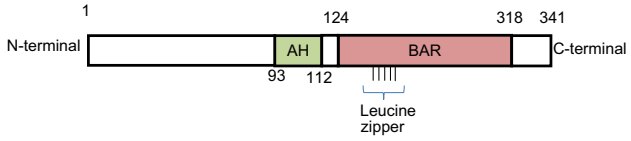

Fig. 1. Schematic presentation of known important Arfaptin2 domains. AH, amphipathic helix; BAR, Bin/amphiphysin/Rvs domain.

Table 1
List of molecules interacting with arfaptin2 and their functions.

Protein	Function	References
Rac1	Membrane ruffling and actin cytoskeleton rearrangement	[20]
Arf1–6	Membrane trafficking, recruit effectors to the Golgi apparatus, and cytoskeleton organisation	[7,10,15,23]
Arl-1	Formation of endosomes, endosome-to-Golgi trafficking, and trans-Golgi network protein sorting	[4]
PI(3)P	Endosome-derived transport vesicles, autophagy, trans-Golgi network protein sorting, and cytokinesis	[6,22]
PI(4)P	Vesicular trafficking, endoplasmic reticulum (ER) export, autophagy, cytokinesis, and actin cytoskeleton rearrangement	[6,8]
PI(5)P	Induce apoptosis, actin cytoskeleton rearrangement, vesicular trafficking, and glucose maintenance	[6,24]

Rac1, which is also a small GTP-binding protein that is involved in membrane ruffling, actin cytoskeleton rearrangement [20], generation of superoxide, transformation of oncogenic cells and activation of transcription factors in a GTP-dependent manner through its C-terminal.

6. Arfaptin2 role in Huntington's disease

A striking evidence for the involvement of Arfaptin2 in protein aggregation in neurodegenerative diseases came from a study of Huntington's disease. HD is a neurodegenerative disease, which is caused by a pathologic poly-glutamine (polyQ) repeat expansion in *huntingtin* gene giving rise to a protein product polyQ-huntingtin. The functions of huntingtin protein include cellular processes such as transcription, vesicular trafficking and mitosis. The polyQ-huntingtin pathogenic effect is through disruption of proteasomal activity [11]. Co-localisation of Arfaptin2 with huntingtin aggregates in HD cell models was suggestive of its potential role in aggresome formation. Evidence showed that both full length and the N-terminus of Arfaptin2 alone are able to inhibit proteasome activity and subsequently induce polyQ-huntingtin aggregation in neuronal cells. On the other hand, expression of the C-terminus of Arfaptin2 (HC-AIP2) showed extensively decreased aggregate formation. This aggregate formation inhibition was found to be through the proteasome pathway, as inhibition of proteasome pathway by lactacystin reversed the inhibitory effect of HC-AIP2 [18].

Consistent with the discussion in the earlier section, phosphorylation pattern of Arfaptin2 is another factor which may be a key determinant in protein aggregation process. It has been reported that phosphorylated Arfaptin2 at serine residue 260 (Ser260) can reduce huntingtin aggregation [19]. Insulin-like growth factor 1 decreases the PolyQ-huntingtin neurotoxic effect through phosphorylating Akt. The phosphorylated Akt in turn phosphorylates the polyQ-huntingtin. However, Akt has been shown to have another pathway for inhibiting the polyQ-huntingtin neurotoxic effect that is huntingtin phosphorylation-independent. Neuronal cells transfected with *huntingtin* that contain the pathologic poly-glutamine expansion but lacks the phosphorylation site showed significantly induced survival and decreased intracellular inclusion formation after treatment with Akt. It has been shown that Akt phosphorylates the full-length Arfaptin2 at Ser260. The dephosphorylation of Arfaptin2 by mutating Ser260 causes its redistribution from the perinuclear region to form a network structure throughout the cytoplasm in a microtubule-dependent manner. In addition, primary striatal neuron cultures from HD embryonic rats were double transfected with Arfaptin2 and an active form of Akt showed that Arfaptin2 was phosphorylated by Akt leading to significant increase in survival and decreased protein inclusion formation. This survival was decreased when cells were transfected with a mutant dephosphorylated form of Arfaptin2 [19].

Also, Arfaptin2 expression was found to be increased in brain of HD mouse [18] and patients [19]. Arfaptin2 has been found to disrupt the proteasome function in HD cell models [18,19].

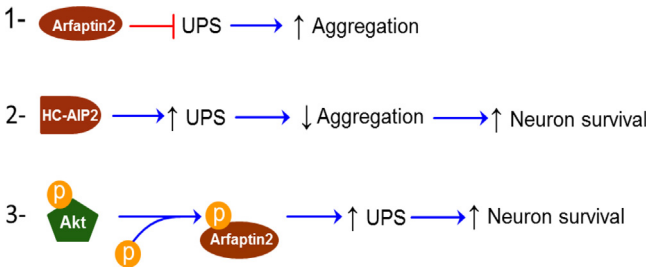

Fig. 2. Schematic presentation of suggested pathways of Arfaptin2 involvement in neuronal survival in Huntington's disease.

Therefore, two hypotheses were raised. Firstly, Arfaptin2 is involved in the pathogenesis of HD by inhibiting the proteasome degradation pathway, whereas its truncated HC-AIP2 works as a negative modulator for Arfaptin2 [18]. Secondly, Arfaptin2 is a defence protein that, when phosphorylated, retains the proteasome function and inhibits protein aggregation [19] (Fig. 2).

7. Future perspectives for Arfaptin2 as a therapeutic target

Whether a similar Arfaptin2-induced pathogenesis in degenerating neurons applies to other disorders such as ALS and AD is still to be investigated. Though the exact function of Arfaptin2 is not fully understood, knowing its binding partners gave some insight of its function. Arfaptin2 involvement in Akt pathway, which inhibits apoptosis, suggests its importance in cell survival. In addition, it binds to proteins involved in endosomal trafficking and protein degradation [6,7,13]. Evidences also showed that modulation of Arfaptin2 decreases motor neuron death in Huntington's disease by rescuing the proteasome activity [18,19]. As proteasome activities are impaired in neurodegenerative disorders and dephosphorylated Arfaptin2 was shown to inhibit proteasome activities, Arfaptin2 protein can provide a novel target for therapeutic interventions against neurodegenerative diseases.

Executive summary

- One of the cellular pathological hallmarks of neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and motor neuron disease, is accumulation of proteins into aggregates.
- It is thought that interference with key cellular pathways such as ubiquitin proteasome degradation and endosomal trafficking contributes significantly to the formation of protein aggregates (aggresomes), in which some of the proteins become sequestered and hence lose its normal functions.
- Research into one of the neurodegenerative diseases, Huntington's disease, has led to a discovery of a ribosylation factor-interacting protein Afraptin2 association with these protein inclusions.

- Given the commonalities between the cellular neuronal degeneration pathways in other neurodegenerative conditions, it is proposed that Arfaptin2 may also be involved in protein aggregate formation in motor neuron disease.
- Structural insights into Arfaptin2 protein sequence suggest its involvement in multiple pathways, including DNA binding, proteasome and Akt pathways, as well as association with trans-Golgi network.
- Further analyses into the mechanisms leading to Arfaptin2 directly or indirectly triggering aggregate formation are necessary; however, currently there are significant clues suggesting its inhibition may lead to rescuing the protein aggregation formation. This will further enhance the research into Arfaptin2 being a potential target against neurodegenerative conditions.

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