VPS35 Mechanisms and Pathways that Induce Pathogenesis of Neurodegenerative Diseases

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Retromer dysfunction plays a key role in the pathology of Alzheimer's disease (AD) and Parkinson's disease (PD) along with several other neurodegenerative diseases. Most involved with the pathogenesis of neurodegenerative diseases are mutations like VPS35-D620N or deactivation of VPS35. VPS35-D620N is a specific mutation within the Vascular Protein Sorting 35 (VPS35 gene) which has been known to cause various neurodegenerative diseases due to its ability to interfere with protein trafficking. VPS35 regulates trafficking of plasma membrane proteins like dopamine receptors, with decreased levels of VPS35 leading to lower levels of DRD1, which leads to failure to regulate cell death and synapse activity. Furthermore, low VPS35 levels correlate with higher beta-amyloid precursor protein cleaving enzymes (BACE1), which then leads to aggregation of amyloid-beta (Aβ), a key signifier of AD. Abnormal mitochondrial fragmentation is also observed in VPS35 D620N mutations, which is also commonly observed in PD models. VPS35 deficiency in microglia can also affect AD pathogenesis, as VPS35-deficient microglia struggle to phagocytose Aβ and instead promote neuroinflammation. This paper covers the relationships between VPS35 and the pathogenesis of AD and PD, and the importance of understanding the diverse functions of VPS35 to find novel treatments for neurodegenerative diseases.

Keywords: Parkinson's Disease, Alzheimer's disease, VPS35, VPS35-D620N, DRD1, BACE1, amyloid-beta

Introduction

Neurodegenerative diseases revolve around the death of neurons, which affects neural functions in the central and peripheral nervous systems. Two of the most common forms of neurodegenerative diseases are AD and PD. In the United States alone, there are one million people currently diagnosed with PD, and around 6.2 million AD patients, demonstrating that there is an increasing need for the treatment of neurodegenerative diseases.

AD is a progressive disease that is categorized by memory loss and aberrant changes in mood. The main hallmarks of AD are increased amyloid-beta (Aβ) aggregates and tau tangles within the brain, which are essentially tau proteins entangling with each other to create long fibrillar protein structures. Furthermore, the neurons in AD brains lose connections between each other following excessive synaptic pruning, leading to difficulties in relaying messages or signals. In AD, neurodegeneration tends to begin in the hippocampus, a brain structure that plays a key role in memory formation, before spreading outwards into the cerebral cortex, affecting areas that are key in memory storage, language, reasoning, and social behavior.

PD is another progressive neurodegenerative disease characterized by the onset of uncontrollable movements like shaking and twitching, as well as severe stiffness. One of the most notable symptoms in PD is the lack of dopamine in the basal ganglia, which plays a significant role in controlling movement. As dopaminergic neurons in the basal ganglia die, less dopamine is produced, leading to motor dysfunction in PD patients. The brains of PD patients contain Lewy bodies, which are essentially abnormal clumps of alpha-synuclein.

A major factor of both AD and PD is the alteration of VPS35. VPS35 is a key component of the retromer complex that regulates the trafficking of proteins back to the trans-golgi network. Abnormal polymorphisms of the VPS35 protein can result in the incorrect trafficking of proteins and can affect levels of proteins to affect AD and PD pathology. For example, the decrease of VPS35 levels can cause a subsequent increase in BACE1 levels, which can promote aggregation of amyloid-beta in the brain, thus promoting the pathogenesis of AD.

Different Genetic Mutations in VPS35 in AD/PD

Retromer mutations and dysfunctions are a major contributor to AD and PD[1]. The most notable mutation, VPS35 D620N, is one of the largest genetic risk factors for PD. This missense mutation leads to a reduced expression of VPS35, which leads to alteration of the WASH complex and abnormal intracellular protein transportation[2]. The WASH Complex is a protein complex responsible for the sorting of endosomal protein cargo and is key in vesicle traffic mediation and branched actin polymerization.

Other mutations in VPS35 that result in alterations of the VPS35 gene, such as L774M, P316S, and R524W, affect VPS35 function but are not linked to PD[3]. In contrast, there is no correlation between AD prevalence and VPS35 polymorphisms, however, dysfunctions within VPS35's signaling pathways do result in AD pathogenesis regardless of VPS35's polymorphisms[3]. Since other mutations of VPS35 are not relevant to PD or AD, in this review paper, the only one being discussed is VPS35 D620N.

Dopaminergic Neurons and VPS35

VPS35 plays an important role in AD and PD, and its prevalence in many PD patients displays a cause of concern because of the correlation between dopaminergic neurodegeneration and VPS35 D620N, a polymorphism of VPS35 that directly causes familial PD. A study of mice with a knock-in VPS35 D620N mutation tend to develop progressive dopaminergic neurodegeneration and reductions in striatal dopamine levels, as well as corresponding motor deficits, which are trademarks of PD. Progressive dopaminergic neurodegeneration due to the VPS35 D620N mutation has several correlations with AIMP2 degradation, which is associated with dopaminergic cell death in PD. VPS35 in its normal functioning form decreases AIMP2, a gene that codes for macromolecule multi-enzyme multi-tRNA synthetase complex (MSC), which leads to less dopaminergic cell death. In the presence of VPS35 mutations like D620N, AIMP2 clearance is inhibited, which leads to greater dopaminergic cell death and thus, exacerbated symptoms of PD.

An experiment – approved by the Institutional Animal Care and Use Committee (IACUC) – was conducted in which mice with VPS35-D620N were compared with WT control mice along three different age groups (6, 10, and 15-16 months) to observe levels of dopaminergic (DA) neurons. It is important to note that all mice were housed under the same standard conditions and were verified to have the same diets via body weight checks. In this experiment, mice aged 15-16 months expressed more pathological similarities to PD compared to younger mice with the same mutation, due to possessing a statistically significant loss in DA neurons ($\sim$12%) (unpaired t-test, p=0.042) relative to WT control mice of the same age, whereas D620N mice of a younger age showed little drops in DA neurons[4]. The results of this experiment demonstrating that dopaminergic degeneration by VPS35 D620N progresses with age, implying that the severity of VPS35 D602N's effects progress with age[4]. A potential change to this experiment would be to determine if VPS35 D620N had more severe effects on VPS35 morphology in different age groups, to ascertain if there is any variance in VPS35 polymorphisms in different ages, though testing in a clinical setting is likely impossible due to ethical standpoints. Within these VPS35-deficient dopaminergic neurons, the mitochondrial morphology is severely altered[1]. Because VPS35-D620N does not mitigate mitochondrial fragmentation like the wildtype form of VPS35, it can be reasonably inferred that the mutation of VPS35 removes its ability to prevent mitochondrial fragmentation, which could potentially lead to pathogenesis of PD, because in the DA neurons of PD-afflicted patients, the mitochondria is often altered or morphed in an abnormal fashion[1]. Thus, we can make a reasonable inference that, as age progresses, dopaminergic degeneration progresses as well, and, at the same time, since the pathological similarities of VPS35 D620N increase with age, mitochondrial fragmentation will also occur at an increased rate, leading to a potential association between age and mitochondrial fragmentation. Therefore, in manners like this, mitochondrial fragmentation in VPS35-D620N could provide further potential explanations for how mitochondrial morphology may be connected to PD pathogenesis.

VPS35 and Microglia

Microglia are macrophages that assist the brain in the immune response, including eliminating foreign microbes, cleaning dead cells, and regulating inflammation in the brain[5]. An experiment conducted on mice determined the importance of the VPS35 deficiency in microglia resulting in AD pathogenesis.

In the experiment, mice were split up into categories, with two main ones being VPS35:5xFAD and CR3CR1/ VPS35. Five mice were placed in each cage, and the experiment was conducted in accordance with the ethical guidelines of the Institutional Animal Care and Use Committee at Case Western Reserve University, who approved this experiment. The mice were all provided with the exact same light/dark cycles and the standard rodent chow diet, eliminating any possibility of their environmental conditions causing a potential difference in the results of collected data. Furthermore, the mice were all tracked for $>$6 generations to ensure that a large sample size was used and that there were no errors due to small sample biases and skews. All data were presented with a standard deviation of $\pm$0.5 and was considered significant when $p<0.05$. In this model, neurons displayed dystrophic neurites, a feature associated with AD. In 5xfAD mice with VPS35 CR3CR1-Ce or Microglial VSP35 deficient 5xfAD mice, Aβ plaques are increased in cortex, hippocampus, subiculum and thalamus. This experiment demonstrates that VPS35 deficiency in microglia can contribute to a higher likelihood of expressing AD symptoms like dystrophic neurites and elevated levels of Aβ.[6] However, it is important to note that there was not any clinical application of these experiments, so there is still a possible disconnection between the data observed in this study and the usability of it when applying to human subjects via clinical trials. Therefore, the investigation of this in clinical trials would also provide insight to how VPS35 mutations impact the microglia in human cells, allowing for a more reliable result to be applied in the clinical field as well.

Microglia-specific knockout VPS35 -/- mice die before birth, while VPS35 +/- (which is where there is a mutation in VPS35 rather than a full knockout) mice are viable and can survive into adulthood. Microglia, which normally prevents pathogenesis of AD by phagocytosing Aβ, in AD, would instead promote inflammatory factors and engulf neuronal synapses. Although the causal role of inflammation in Aβ accumulation and pathogenesis has yet to be confirmed, VPS35-deficient microglia do promote higher levels of Aβ, which in turn leads to neurodegeneration and neuroinflammation, one of the major features of AD[6].

Microglia interact with various other immune cells in the nervous system, with the most notable being astrocytes. Both microglia and astrocytes are necessary in regulating pathogenic processes in the brain, so it is inevitable that the two would have shared pathways, especially in dealing with neurodegeneration and neuroinflammation. When homeostatic microglia are activated, they release cytokines like IL-1β and TNFα, which activate other microglia and astrocytes via NF-κB activation – proteins commonly functioning as structural transcription factors. Activated astrocytes release these same cytokines to initiate a neuroinflammatory response[7]. Therefore, alteration of microglial functions by VPS35-deficiency could result in interactions between astrocytes and microglia propagating excessive neuroinflammation, which is a key sign of AD.

Microglia also demonstrates many similarities in roles with monocytes in the maintenance of Aβ and the prevention of AD pathogenesis. Blood monocytes monitor Aβ presence in the peripheral parts of the body, as 50-62% of Aβ diffuses into blood[8]. Furthermore, monocytes participate in inflammatory processes that are propagated in AD, as is noted by decreases of classical monocyte populations and an increase in non-classical monocyte populations as the disease progresses, further implying the role of monocytes in AD. During inflammation of the brain, the blood-brain barrier (BBB) tends to be damaged and thus allows inflammatory monocytes, which would normally be prevented from entering, to infiltrate into the brain. These infiltrating monocyte-derived cells, like monocyte-derived macrophages (MDMs) and monocyte-derived dendritic cells (moDC), assume a microglia-like phenotype when they enter, and from there, promote neuroinflammation. If the BBB is still intact, then monocytes traffic through the semipermeable parts of the brain, like the choroid plexus, and thus canpha be recruited from the blood[8]. Therefore, peripheral immune cells and inflammation could also influence neuroinflammation and the onset of AD, and vice versa.

VPS35 Signaling Pathways

The VPS35 protein complex is a vital component of the retromer complex which plays an important role in plasma membrane transport from endosomes to the trans-Golgi network and the recycling of protein cargo that has crossed the membrane[9]. According to Figure 1, VPS35 regulates trafficking of plasma membrane proteins such as DRD1, a dopamine receptor, with VPS35 depletion leading to decreased DRD1 levels[10]. DRD1 plays an important role in phosphorylating cAMP-response element binding protein (CREB) and extracellular signal-regulated kinase (ERK), which play a major role in regulating cell death and synaptic activity, by transcribing brain-derived neurotrophic factor (BDNF) which is a protein important in regulating both[10]. Therefore, a decrease in VPS35 would result in a decrease of DRD1 expression, leading to a failure to regulate cell death and synapse activity. VPS35 levels heavily influence levels of beta-site amyloid precursor protein cleaving enzyme (BACE1), which is required in the creation and aggregation of all forms of amyloid-beta (Aβ). In a test comparing BACE1 presence in mice treated with scrambled miRNA or VPS35-targeting shRNA, there was an increased amount of BACE1 in non-lymphoid tissues (NLT) and hippocampal neurons in the mutated variant[11–13]. Aβ is a known biomarker of Alzheimer's Disease (AD), and is observed at high levels in AD patients, along with BACE1,[14] demonstrating a correlation between levels of BACE1 and Aβ. BACE1 normally, under functioning WT-VPS35, is localized in denser levels near the Golgi Apparatus, but in non-functioning VPS35 or lower levels of VPS35, BACE1 becomes more condensed in endosomes or lysosomes, thereby allowing it to increase activity. When BACE1 is in this form, BACE1-mediated cleavage of amyloid precursor protein (APP) occurs, resulting in an increase of Aβ, thus indicating the mechanisms that VPS35 uses to regulate BACE1 and its subsequent cleavage of APP to promote AD pathogenesis[12].

Discussion

VPS35 is an important protein of the retromer complex and is key in the creation of mitochondria-derived vesicles and is important in the transportation of cargo proteins. VPS35 mutations are implicated in the pathogenesis of AD and PD and can contribute to the onset of neurodegeneration and neurodegenerative diseases. The most notable VPS35 mutations include VPS35 D620N, a missense mutation that, when present, can initiate the onset of AD or PD. VPS35 is very important in certain pathways that preserve proper brain function and regulate damage to the brain. One of the major pathways VPS35 influences is the ERK pathway, which it regulates via DRD1, a dopamine receptor. The ERK pathway regulates cell death and synaptic activity. When VPS35 is not present or is altered via a mutation, phosphorylation of CREB and ERK is diminished, which leads to an inability to regulate cell death and synaptic activity, inevitably leading to a large loss of neurons and a detriment to proper synapse functions. VPS35 promotes lysosomal degradation of AIMP2, a protein that is heavily associated with dopaminergic cell death in PD. However, in mutations of VPS35 like D620N, AIMP2

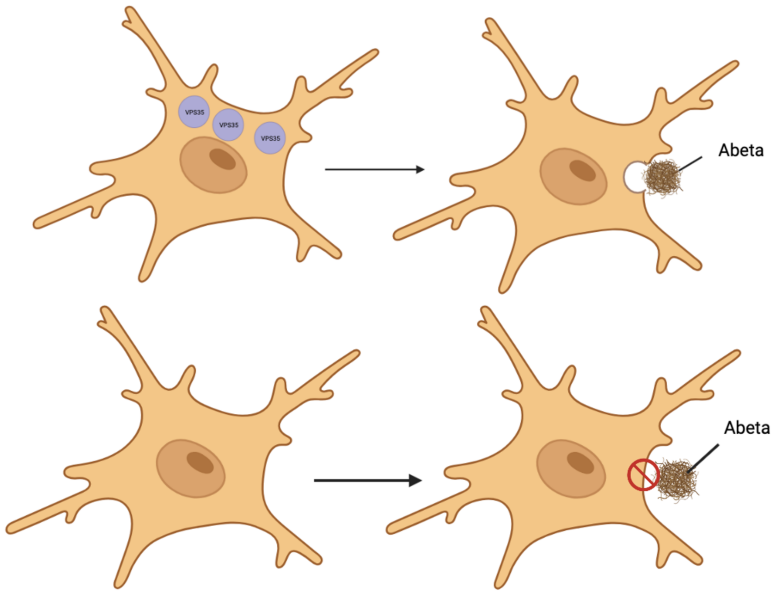

Fig. 1 Microglia with VPS35 can successfully phagocytose amyloid-beta, while, VPS35-deficient microglia, in contrast are unable to do so, and thus lead to pathogenesis of AD.[6]

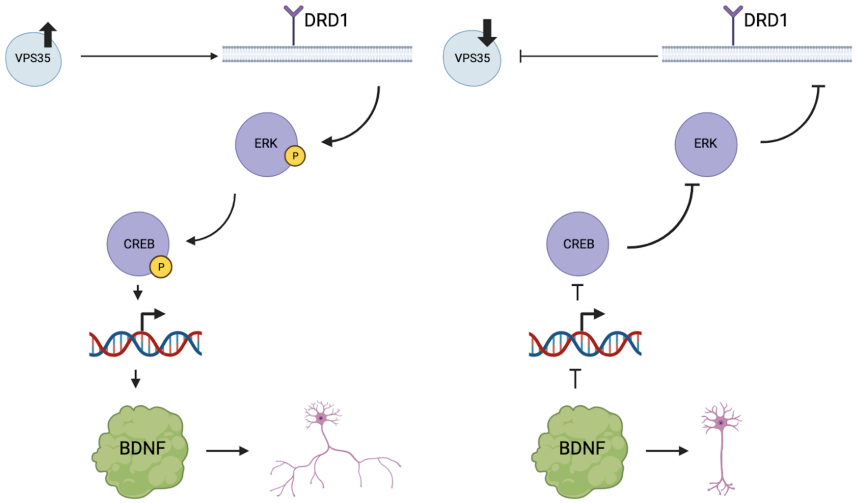

Fig. 2 Expression of VPS35 results in trafficking of DRD1 which results in phosphorylation of ERK and CREB, thus transcribing BDNF and promoting synaptic differentiation and neuronal growth. However, downregulating of VPS35 results in a decrease of cell-surface level DRD1 which causes less phosphorylating of ERK and CREB which means that BDNF is not transcribed, and neuronal growth and differentiating does not occur[10].

clearance is inhibited, which leads to a buildup of AIMP2 and increased dopaminergic cell death, contributing to PD pathogenesis. Furthermore, D620N mutations are shown to induce mitochondrial fragmentation, which is commonly observed in PD and can contribute to increased levels of apoptosis. This is because mitochondrial fragmentation is caused by high levels of fission, which is necessary to create new mitochondria, but promotes apoptosis under high cellular stress. VPS35 in its functioning state regulates and prevents mitochondrial fragmentation, but the D620N damages or prevents this role and thus leads to an altered mitochondrial morphology. By investigating these altered morphologies of the mitochondria, we could inves-

tigate mitochondria-specific therapies to treat symptoms of PD. VPS35-deficient microglia also appear to play a major role in the progression or pathogenesis of AD, because these mutated microglia do not possess the ability to phagocytose Aβ, thus contributing to Aβ plaque accumulation and AD pathogenesis.

In the future, VPS35 research could explore what would occur if astrocytes or other cells were deprived of VPS35, which would help us understand cell-specific functions of VPS35 and find ways to target specific cells to treat neurodegeneration. As VPS35 depletion contributes to AD and PD pathogenesis, we may be able to activate VPS35 as a potential treatment of the synaptic damage that occurs in neurodegenerative diseases. VPS35 activation would lead to more robust phosphorylation of CREB and ERK by DRD1, which would then in turn regulate synaptic activity in AD or PD patients. If VPS35 activity could be regulated or even initiated by therapeutics, we could promote microglial phagocytic activity to allow for robust clearance of Aβ plaques to prevent or treat AD. However, currently, there are no preclinical studies that identify a drug or compound that is able to sufficiently promote VPS35 production or activation, and so, if earlier questions are to be answered, a compound that can activate or supplement VPS35 must be developed. Research dedicated to developing VPS35-promoting compounds or drugs would allow for many more potential possibilities regarding further research into regulating AD or PD symptoms via VPS35 promotion.

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