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Arc Expression in Alzheimer's Disease Mouse Model Expected to Increase Amyloid β Intraneuronal Accumulation

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Abstract

Arc expression is significantly disrupted in patients with Alzheimer's disease (AD), especially in the regions with concentrated Amyloid β (Aβ) plaques. Aβ can also accumulate intracellularly, which can lead to cognitive disfunction and is challenging to remove. Arc protein acts as a γ-secretase agonist at presenilin 1 (PS1) site which promotes amyloid precursor protein (APP) cutting, forming Aβ in endosomes. No previous research has linked Arc-promoted APP cutting with intraneuronal Aβ accumulation. I hypothesis that endosomic Aβ produced by Arc protein may accumulate intracellularly. MutantPS1 will be designed to replace wild type (WT) PS1 in both AD mice and AD cell culture to prevent Arc- γ-secretase interaction. Intracellular and extracellular Aβ concentration, and cognitive ability of AD mouse will be measured. If treatment groups with mutant PS1 display lower intercellular-extracellular Aβ ratio and less cognitive impairment, the proposed Arc-PS1 interaction in intracellular Aβ accumulation will be supported. It will further support a potential AD treatment through disrupting AD-PS1 interaction.

Keywords

Alzheimer's Disease; Arc protein; Amyloid β; Amyloid precursor protein; γ-secretase.

1. INTRODUCTION

Arc is commonly known as a virus-like protein that contributes to synaptic plasticity and aids long-term memory formation. In patients with Alzheimer's Disease, Arc expression is significantly disrupted, especially at regions where Amyloid β plaques deposit [1].Such disruption in turn lowers the synaptic Arc protein level, which is harmful to cognitive function [2]. Aβ is formed through the cleavage of amyloid precursor protein by γ-secretase and β-secretase. Arc protein can act as an agonist at presenilin1 site of γ-secretase, and is able to recruit APP and β-secretase into the endosome [3]. Consequently, new Aβ forms inside the endosome.

After formation, Aβ can accumulate intracellularly, or form plaques extracellularly through exocytosis. Both intracellular and extracellular Aβ levels are abnormally high in AD patients, and extracellular Aβ level is inversely related to the intracellular level [4]. Extracellular Aβ deposition is easier to eliminate, but after removal, an increase in intraneuronal Aβ accumulation will cause the extracellular Aβ plaques to reappear [4]. The intracellular Aβ also increases with age [5].

In the CA3 region of AD mouse hippocampus, neurons that produce a high intracellular Aβ level also display an Arc overexpression [6]. However, the causal relationship between intracellular Aβ concentration and Arc expression is unclear, and the specific molecular mechanism that connects these two variables is also ambiguous. Since it is certain that Arc protein increases the cleavage of APP inside an endosome, assuming the endosome does not go

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through exocytosis in living neurons, the activity of Arc could be a reasonable explanation for the Aβ intracellular deposit.

Under this model, suppressing Arc-promoted APP cleavage in endosomes can reduce intracellular Aβ, which could be toxic to neurons when accumulating for a large amount. In other words, reduced Arc-promoted APP cleavage can slow done Aβ-induced cognitive impairment. I hypothesize that in both AD mouse model and AD cell culture, the intervention of Arc-PS1 binding will reduce Amyloid-beta intraneuronal accumulation and cause AD mouse to develop fewer symptoms.

2. METHOD

2.1. Experimental Design

The experiment will be conducted on both AD mice and AD neuron cell culture. Both experiments will consist of multiple replicates (specifics will be described in later sections), and will be assigned of either experimental or control conditions.

To investigate Arc's interaction with PS1 without influencing other Arc regulated pathways, alteration of PS1 sequence will be conducted in the control groups. This treatment will allow Arc to remain its structure and its ability to interact normally with other proteins, while disabling its ability to interact with and promote the function of γ-secretase. In experimental groups, Aβ will still be produced through the cleavage of APP, but the cleavage will happen in a slower fashion due to the absence of Arc-PS1 interaction.

Since the treatment changes the sequence of a binding site on an important enzyme, it is crucial to ensure that this alteration does not influence the function of γ-secretase. Before testing the treated enzyme in the experimental group, it is necessary to assume that the function of γ-secretase is not changed. Additionally, it is also assumed that the sequence designed to replace PS1 will not cause the binding of any other proteins that can alter the enzyme's function.

2.2. Treatment

The treatment of altered ps1 sequence will be applied to selected neurons in CA3 region of the mouse hippocampus, or selected neurons in the AD cell culture. This selection will ensure that the alteration has minimal effect on the brain or cell culture environment. The sequence of PS1 will be retrieved from NCBI database according to the mouse strain available. An unfunctional mutant PS1 sequence will be designed by replacing several crucial amino acid cites with different codons. To ensure this alteration creates minimal Arc-PS1 binding, the crystal structures of the two proteins will be consulted while designing the mutant Arc sequence.

To replace the wild type PS1 sequence with the mutant sequence, Cre/loxP-mediated conditional knockout will be utilized. For both control and experimental groups, promoter sequence will be inserted upstream of the wild type ps1sequence. An inverted mutant sequence will be inserted after the WT sequence. As a result, only WT will be expressed in cell. After applying conditional knockout in the experimental group, the WT-mutant sequence will be inverted. As a result, only mutant will be expressed in cells. To label the treated cells, an inverted GFP sequence will also be incorporated into the genome. As a result, GFP will be expressed and display florescence if WT PS1 is replaced by mutant through the addition of recombinase.

Immuno-precipitation will be used to examine the difference between Arc-WT binding and Arc-mutant binding. The cells are broken open after sufficient growing time, and Arc antibodies will be added to the system. The treatment will be deemed successful if the major stains for treatment and control groups are about 230 kDa apart.

Western blot will be used to test if the knockout has induced undesirable changes in the system. Important γ-secretase-dependent proteins will be blotted for both control (wild type)

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cells and treatment (mutant) cells. If immune staining displays a significant decrease for the treatment group, the mutant sequence will be redesigned and retested.

2.3. AD Mice Model Experiment

The purpose of experiment A is to investigate Arc's necessity in Aβ intracellular accumulation in live mouse model. The AD mice will be used are asymptomatic before two months old, and will develop Alzheimer's disease at around ten month. Both experimental group and control group will be pretreated to insert mutant PS1 sequence in hippocampal CA3 neurons.

The treatment will be applied to a selection of CA3 neurons of experimental group at two-month age by the addition of Cre recombinase. This procedure allows an interference with Arc's participation in APP cleavage at an early time into the development of AD.

Both experimental groups and control groups consist of 30 mice, and each group of ten will be placed in three different conditions. 1) Dark box with no light exposure; 2) Normal laboratory environment; 3) Extensive light exposure. This design will induce a different Arc expression level, which may lead to different increases in APP cleavage in the experimental group. The same three conditions will also be applied to the control groups. Mice will be placed in the three environments when they reached three months old.

At ten-months old, all mice will be tested for cognitive function and measured for Aβ deposition. Cognitive function will be measured through Morris water maze. Each mouse will be placed inside the maze as soon as it is taken out of its cage, and the time they take to succeed will be recorded. After the tasks, mice will be immediately sacrificed. Specimens of CA3 region will be collected, and Aβ intracellular deposition amount in labeled neurons will be marked through immunostaining, and observed under confocal microscopy.

2.4. AD Cell Culture Experiment

The second experiment will be conducted in cell cultures. Both experimental and control groups will consist of 20 cultures. Cells were previously selected from the CA3 region of AD mice, and mutant PS1 sequence will be inserted inside the genome through the same method discussed in experiment A. Recombinase will immediately be added into control group. Cells will then grow into mature cultures under laboratory environment.

After a month, cells will be separated from the surroundings through centrifugation. For each replicate, western blot will be applied to measure Aβ content for both intracellular and extracellular environments, and a quantitative ratio of inner and outer Aβ content will be determined. Averages will be taken among the twenty replicates for both groups with error bars of +/- 1SE.

3. EXPECTED RESULTS

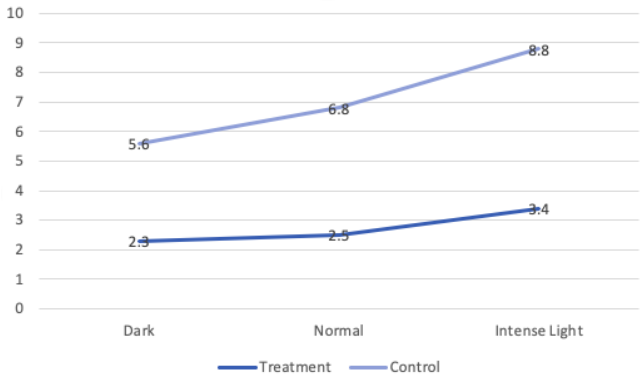

Figure 1. Predicted intracellular Aβ deposit on a scale of ten

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In AD mice, intracellular Aβ content (scaled from 1 to 10) is expected to increase with the increase of light exposure for both experimental and control groups (Figure.1). Treatment group is expected to display less Aβ intracellular deposit in each type of environment, and increase less under intense light exposure. A similar trend is expected for time recorded in the Morris water maze cognitive test.

In cell cultures, the ratio between intracellular and extracellular Aβ content for the treatment group is expected to be significantly lower than the control group (Figure.2).

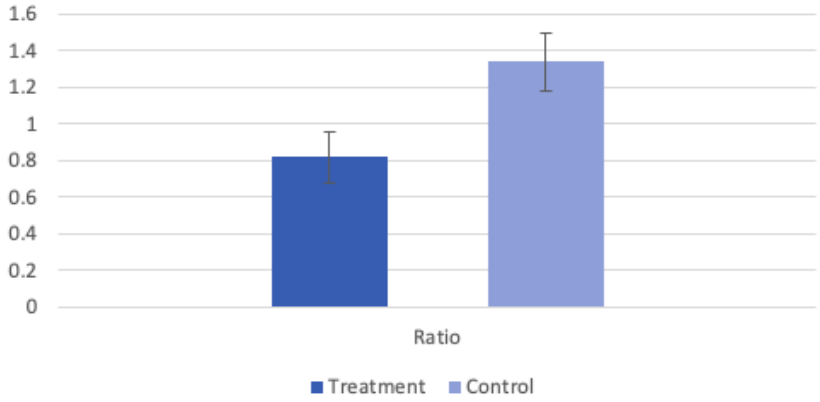

Figure 2. Predicted intracellular and extracellular Aβ content ratio

4. IMPLICATION

It is hypothesized that interfering the binding between Arc and PS1 in γ-secretase will reduce the intracellular Aβ deposition in both AD mice and cell cultures, and improve AD mice's cognitive function. If the experimental result aligns with expectation, it may be reasonable to conclude that Arc is an effective agonist of γ-secretase at its PS1 site. The result may also implicate that the endosome where Arc-mediated APP cleavage takes place does not got through exocytosis, and accumulate inside neurons. An improvement in mice' Morris water maze performance will imply that intracellular Arc accumulation is detrimental to one's cognitive function.

The experimental result may also go against expectation for several reasons. If the treatment reduced overall Aβ content, but no significant difference in intracellular and intercellular Aβ content is observed, it could be explained by the exocytosis of the cleaving endosome, or the continuing exposure of inner Aβ after increasing deaths of disturbed neurons. The PS1 mutant sequence designed may also influence the cellular system in unexpected ways.

5. CONCLUSION

Intracellular Aβ accumulation may be as detrimental to cognitive function as extracellular Aβ plaques. The difficulty in targeting intracellular Aβ can contribute to the reason why merely targeting Aβ plaques is not ideal in treating Alzheimer's disease. If the experimental result meets expectations, it can serve as a good support that Arc plays an important roll in this process. Disrupting Arc-PS1 binding could be an efficient treatment method in reducing intracellular Aβ accumulation. It will further confirm that Arc-promoted APP cleavage inside endosomes is an important pathway in this process. The potential new direction in medical treatment of AD is still yet to be determined through further research and observations on AD mice and human patients.

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