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Herpes Virus Facilitates the Formation of Oligo-Aβp and the Phospholipid Bilayer of Herpes Virus Binds to Oligo-Aβp

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Abstract

Alzheimer's Disease (AD) can induce dementia in elders. The cause of AD is Oligomer Amyloid-β peptide (Oligo-Aβp) fibrilization so that it can decrease the plasticity of synapse and even cause neuron death. Recent findings have shown that Herpes virus such as HSV1 can enhance the possibility of developing AD in human by enhancing the amount of Oligo-Aβp. However, the process of the accelerated formation of Oligo-Aβp after the neuron has been infected by Herpes Virus is not been figured out. Plus, there are another binding site except for glycoprotein on the envelop of HSV1 with Oligo-Aβp. This passage is about a speculation that Herpes virus has the ability to facilitate the formation of Oligo-Aβp by changing gene expression that relates to it and producing signal molecules related to this formation. Furthermore, this work also discusses about the affinity between phospholipid bilayer of Herpes virus and Oligo-Aβp. This work provides assumptions to the mechanism of the facilitation of Oligo-Aβp formation.

Keywords

Oligo-AβP; HSV1; APP; Secretase; Phospholipid bilayer.

1. INTRODUCTION

Alzheimer's Disease (AD) is a ubiquitous disease which can cause dementia in elders [1]. One theory to explain the cause of AD is the Amyloid-β peptide (AβP) fibrilization and deposition hypothesis [2]. It means that AβP can be cut from Amyloid Precursor Protein (APP) by protease in the endosomal-lysosomal pass way. There are three kinds of secretase(α-secretase, β-secretase and γ-secretase) that relate to the formation of monomer AβP. β-secretase and γ-secretase can cut the monomer AβP from APP while α-secretase can disrupt monomer AβP by cutting it into two short pieces. Then the monomer AβP can form into oligomer-AβP(Oligo-AβP), which can bind to some receptors on the neuron membrane such as PirB in mice or LilrB2 in humans. Therefore, the combination of oligo-AβP and such receptors can activate some kinds of protein that are detrimental to neuron and memory formation [3].

What this work is interested in is the formation of oligo-AβP. Now more researches are focus on the induction of AD by the pathogen, especially Herpesviridae [4]. Those researches mainly explain that oligo-AβP is formed to protect the neuron from being infected by the virus. The fact that monomer AβP can be built into oligo-AβP and it can identify and bind to the herpes virus is responsible for the conclusion that a fibrillar network links virus particles, and their ability of infection is attenuated [4]. However, AD is more likely to happen after the oligo-AβP has been accumulated [3].

Based on these findings, this work is about a speculation about the mechanism that Herpes virus can facilitate the formation of oligo-AβP. In fact, AβP is cut from APP and three kinds of

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secretase are related to this process. Therefore, there might be a relationship between the Herpes virus infection and the expression of APP and three kinds of secretase. So this work intend to test if the gene expression and the amount of APP and three kinds of secretase. There is a possibility that the gene expression of APP, β-secretase and γ-secretase are facilitated or α-secretase are decreased after the neuron has been infected by Herpes virus, and this might be a probable mechanism of how the Herpes virus can facilitate the formation of oligo-AβP. As signaling is common in multicellular organism, there is another assumption that after the neuron has been infected, it releases signal molecules which play a role in the function of facilitating the amount of oligo-AβP.

The oligo-AβP is also considered as a type of Antimicrobial peptide (AMP) for it can defense the infection of Herpes virus[4]. It has been proved that oligo-AβP can bind to glycoprotein (α-gB, α-gC, α-gD, α-gE, α-gH, α-gG) on the envelop on HSV1, a type of Herpes virus and the research also points out that there might be another sites on the envelop can bind to oligo-AβP. There is a phenomenon that most AMP can bind to phospholipid bilayer on bacteria. We hypothesize that oligo-AβP can also bind to the phospholipid bilayer on the envelop on HSV1. Therefore, more ideas for the further medical studies of HSV1 or even the herpes virus are provided by this work.

2. METHODS

2.1. Materials

Wild type HSV1, monkey brain tissue, culture dish, transgenic HSV1 that removes the glycoprotein (α-gB, α-gC, α-gD, α-gE, α-gH, α-gG) on the envelop, different kinds of phospholipid (depends on the consequence of thin layer chromatography), drug that can inhibit the viral DNA replication, oligo-AβP antibody.

Deoxynucleotide marked by radioactive elements is added in the culture dish so the virus is able to gain them by infecting the cell that has marked deoxynucleotides and therefore the viral DNA is marked.

2.2. Radiation Survey Meter

Radiation Survey Meter is utilized to analyze the radioactivity of experimental tissue.

2.3. RT-qPCR

Gene expression of APP and three kinds of secretase (α-secretase, β-secretase and γ-secretase) can be measured by RT-qPCR. Firstly, genes of APP and three kinds of secretase should be found in Genebank and primers and reporter probe of them should be designed.

2.4. Western Blot (WB)

The amount of APP and three kinds of secretase (α-secretase, β-secretase and γ-secretase) is measured by WB after the brain tissue is dissociated. Firstly, we need to design Primary antibodies of APP and three kinds of secretase in order to target these kinds of protein on the SDS-PAGE gel. Then we design Second antibodies which have fluorescent dye on them that recognize Primary antibody to detect protein we are interested in. By measuring the brightness of the sample, we can measure the quantity of APP and three kinds of secretase.[5]

2.5. Centrifuge

Centrifuge is used to make the cell and virus into sediments by designing rotate speed that can exactly deposit the viral molecule.

2.6. Thin Layer Chromatography

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This method is used for analyzing the composition of the lipid in the viral envelop.

2.7. Enzyme-linked Immunosorbent Assay (ELISA)

ELISA is used to analyze the affinity between different kinds of phospholipid and oligo-AβP. We put the antibody of oligo-AβP on the bottom of the plate and add oligo-AβP on it, after washing, we add the radioactive phospholipid and washing again and measure the radioactivity on the plate.

3. RESULTS

3.1. The Infection of HSV1 Changes Gene Expression of APP and Three Kinds of Secretase Thus Increases the Amount of Oligo-AβP

In order to test the amount of oligo-AβP is enhancing and figure out the mechanism of this process, gene expressions of APP and three kinds of secretase are analyzed by RT-qPCR and WB after the brain tissue has been infected by HSV1.Brain tissue that use phosphate buffer(PBS) with no virus is used as a control and the control group shows no changes in the gene expression of APP and three kinds of secretase and the amount of oligo-AβP. Here are four possible results that might happen.

Possible Result 1: After the infection of HSV1, gene expression of APP is enhancing while there is no change in three kinds of secretase. And the amount of APP, oligo-AβP is also enhancing.

Possible Result 2: After the infection of HSV1, gene expression of APP is not enhancing while gene expression of β-secretase and γ-secretase is enhancing while α-secretase is decreasing. And the amount of oligo-AβP, β-secretase and γ-secretase is enhancing while the amount of α-secretase is decreasing.

Possible Result 3: After the infection of HSV1, gene expression of APP, β-secretase and γ-secretase are enhancing while α-secretase is decreasing. And the amount of APP, oligo-AβP, β-secretase and γ-secretase is enhancing while the amount of α-secretase is decreasing.

Possible Result 4: After the infection of HSV1, there is no change in gene expression of APP and three kinds of secretase but the amount of oligo-AβP is enhancing.

The Possible Result 1, 2 and 3 are able to prove the hypothesis that HSV1 can facilitate the formation of oligo-AβP by changing the gene expression that relates to this process. Either higher gene expression of APP or higher gene expression of β-secretase and γ-secretase or lower gene expression of α-secretase can facilitate the formation of oligo-AβP. Nevertheless, the Possible Result 4 shows that there is no gene expression changes in APP and three kinds of secretase but other changes because the amount of oligo-AβP is increasing. The assumption that β-secretase and γ-secretase might be activated or α-secretase is suppressed is responsible for Possible Result 4.

Therefore, based on the hypothesis 1, the mechanism of the facilitation of oligo-AβP about changes in gene expression of APP and three kinds of secretase and there is signal that facilitate the formation of oligo-AβP is discovered.

3.2. Signal Molecules Which Can Facilitate the Formation of Oligo-ABP Are Released by Neuron Infected by HSV1

Then the hypothesis that neuron infected by HSV1 can release signal molecules which plays a role in the function of facilitating the formation of oligo-AβP after the HSV1 infection is remained to be testified. This work is done by adding drug that can inhibit the replication of viral DNA to the culture dish and add HSV1. After a period of time we put the solution in the culture dish to the centrifuge and separate the virus molecule and the supernate. Then the

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supernate is transferred in another culture dish with no HSV and measure the amount of oligo-AβP by WB. The supernate with no virus infection is negative control group and culture dish with HSV1 is positive control group. The negative control group shows no enhancing of the amount of oligo-AβP and the positive control group shows significant enhancing of the amount of oligo-AβP. Here are two possible results of this experiment.

Possible Result 5: The supernate that removes from the HSV1 infected culture dish can enhancing the amount of oligo-AβP.

Possible Result 6: The supernate that removes from the HSV1 infected culture dish cannot enhancing the amount of oligo-AβP.

The Possible Result 5 shows that there is signal molecule that can be released from the neuron after it has been infected by HSV1 while the Possible Result 6 shows that there is no signal molecule that can be released from the neuron after it has been infected by HSV1.

3.3. Phospholipid on the Envelop of Virus Is Also Binding Site with Oligo-AβP

There is an assumption that viral glycoprotein is not the only pathway that mediate the binding of HSV1, so this work is about using mutant HSV1 that removes the glycoprotein (α-gB, α-gC, α-gD, α-gE, α-gH, α-gG) on the envelop of HSV1 to target oligo-AβP. By marking the viral DNA by radioactive elements, the affinity between virus and oligo-AβP can be measured by measuring radiation intensity. Wild type HSV1 is used as positive control group and HSV that remove the envelop by mild detergent is used as negative control group. The positive control shows significant radioactivity while the negative control group shows little radioactivity. Here are two possible results.

Possible Result 7: Mutant HSV1 shows significant higher radioactivity than negative control group and lower radioactivity than positive control group.

Possible Result 8: Mutant HSV1 shows little radioactivity that similar to negative control group.

The Possible Result 7 shows that there is other sites on the mutant HSV1 envelop that can bind to oligo-AβP while the Possible Result 8 shows that there is no site on the mutant HSV1 envelop that can bind to oligo-AβP so it is certain that the glycoprotein on the HSV1 envelop is the site that binds to oligo-AβP.

After the hypothesis that mutant HSV1 can also bind to oligo-AβP has been proven, the lipid composition of the HSV1 envelop should be tested by thin layer chromatography in order to figure out the major phospholipid composition of the viral envelop. After the phospholipid composition of the viral envelop is clear, different kinds of phospholipid are used in ELISA experiment to test the affinity between them and oligo-AβP. In this experiment, phosphatidylcholine(PC) is utilized as control group because it is the major phospholipid in mammal cell membrane. The phospholipid are marked by 32P so we can measure the affinity by testing radioactivity. Here are three possible results.

Possible Result 9: A type of phospholipid on the HSV1 envelop shows significant radioactivity that binds to oligo-AβP.

Possible Result 10: Several types of phospholipid show different radioactivity that binds to oligo-AβP.

Possible Result 11: No phospholipid show radioactivity that binds to oligo-AβP.

Possible Result 9 shows that a kind of phospholipid on the envelop can bind to oligo-AβP while the Possible Result 10 shows that several types of phospholipid can also bind to Several types of phospholipid show different radioactivity that binds to oligo-AβP in different degree. Possible Result 11 shows that no phospholipid on the HSV1 envelop can bind to oligo-AβP.

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After the assumption that the specific phospholipid can bind to oligo-AβP has been proven, the experiment in vivo should be done. That adding the specific phospholipid into the HSV1 envelop by fusion so the lipid composition of HSV1 envelop is altered. The viral DNA is marked by radioactive elements. Then this type of HSV1 is used to target oligo-AβP and the affinity between virus and oligo-AβP is evaluated by measuring radiation intensity. Wild type HSV is used as control group. Here is a possible result.

Possible Result 12: HSV1 that adds the phospholipid which shows higher affinity with oligo-AβP on its envelop shows significant higher radioactivity than control group.

Possible Result 12 shows that the HSV1 that adds the phospholipid on the envelop has higher affinity with oligo-AβP, which also proves the hypothesis that the phospholipid on the viral envelop can bind to oligo-AβP.

4. CONCLUSION

This study explores the specific effect that the HSV1 would have on the forming of oligo-AβP, in a broad view, by studying which protein on the viral envelope is oligo-AβP binding to has a beneficial progress toward clinical trial. Mutating or Changing the gene expression of HSV and then disable the protein on the surface which is binding to the oligo-AβP would completely cut the relationship between the herpes virus and oligo-AβP. If cutting the binding site between HSV and oligo-AβP is not feasible, finding out which step is HSV1 participating in during the formation of oligo-AβP would also be a way to reduce the effect that HSV has on forming oligo-AβP, also on the Alzheimer's Disease. The experiment on signal molecules would verify the function or HSV1 toward brain cells even though both of experiment has negative results. By confirming the existence of the signal, even though the HSV won't increase the quantity of β-secretase, the signal is what really speeding up the process. Then developing a drug or a technique that would block the signals would terminate the effect that the virus has toward the neuron damages. All the experiments are aiming to find out the specific step or particular place that the HSV is affecting the process in order for the scientists to get a thorough understanding of how to cease the influence that HSV has to AD, even curing it.

There are also problems that remain to be figured out. Firstly, if the formation of oligo-AβP is facilitated by HSV1 infection, there is also a possibility that β-secretase or γ-secretase is activated or α-secretase is suppressed. But the way how the secretase is activated or suppressed is uncertain. So the activity of three kinds of secretase can be evaluated by some experiments and the hypothesis that whether the structure of them is altered or some sites of them are modified should be testified. Furthermore, phospholipid bilayer of HSV1 envelop might not the only pass way that mediate the binding of oligo-AβP and HSV1. More types of protein which can bind to oligo-AβP on the HSV1 envelop remain to be discovered [4]. Besides, this study has shown that there is signal that can mediate the facilitation of oligo-AβP after the neuron has been infected by HSV1, but which type of signal molecule is still unknown. So the supernate is needed to be examine and the difference it has in comparison with negative control group should be figured out. And the mechanism of how the signal molecule is produced and released from the infected neuron remains to be discovered.

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