"THERAPEUTIC TARGETING OF MIRNAS AS POTENTIAL THERAPY FOR ALZHEIMER'S DISEASE"

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Abstract- Alzheimer's disease has always been an unpleasant burden for many people aged over 85 or even less and it has been supposedly harder to encounter for the families of the patients and those around them. Considering both the agony and the costs dedicated for this disease, it has imposed a high morbidity rate to all societies. Alzheimer's Disease is mainly caused by amyloid aggregation in the brain, mostly in the temporal and parietal lobes; and formation of neurofibrillary tangles which all lead to cortex atrophy and presentation of memory loss and failure in cognitive acts. The specific type of amyloid protein responsible for Alzheimer's Disease progression is amyloid-beta, produced by the enzyme beta secretase. While the physiological role of this enzyme is still under debate, a decrease in its production may lead to a relative decrease in the severity of Alzheimer's Diseasepresentations in a patient. MicroRNAs, on the other hand, are well-known types of RNAs which moderate gene expression. They may also be used as a therapeutic target to inhibit the expression of the gene responsible for beta secretase enzyme production and consequently decrease the unpleasant symptoms of Alzheimer's Disease. In this review, possible therapeutic acts and aspects of Alzheimer's Diseaseare explained, which may be considered for further research activities.

I. AN INTRODUCTION TO ALZHEIMER'S DISEASE

Alzheimer's disease is a progressive disease affecting the brain usually in the elderly ages. It mainly starts affecting the memory and cognitive acts and as it progresses, motor and sensory cortex may get involved as well. It usually takes about 10 to 13 years until the patient dies because of dysfunction of the respiratory muscles and reflexes. Even before serious involvement of muscles and movements, the loss of memory is an intolerable symptom which plays the most unpleasant role in the course of the disease. In some cases, the patient is aware of the memory loss and in some others, it's family or friends who notice abnormalities and ongoing changes in the behavior of the patients. Alzheimer's disease contributes to 60-70 percent of dementias which is a considerable amount in prevalence and incidence of the disease.

The pathophysiology of the disease was not fully understood until recent years and it's still being understood better and better. The most accurate and acceptable theory which has also been proven by Para clinical tests, focuses on two main pathologies causing defects in the function of brain. The first pathologyis the formation of amyloid beta deposits in the brain and the second one is the formation of neurofibrillary tangles caused by hyperphosphorylated tau protein accumulation inside neurons. These two suggested pathologies cause the brain to degenerate over the course of time and depending on how fast it progresses, it can despair the function and eventually the life of the patients.

II. PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

The first data discovered about the physical aspect of Alzheimer's Disease, was observing the shrinkage of the brain and atrophy mainly in the temporal and parietal lobes in MRI and other imaging techniques. Concentrating more on these affected areas of the brain, scientists conducted autopsies on cadavers who had previously endured Alzheimer's Disease. Microscopy of the neurons in atrophic parts of the brain showed amyloid plaques and neurofibrillary tangles. These findings lead to the most important theory in explanation of Alzheimer's Diseasepathophysiology. Several models have been suggested ever since.

Amyloid Beta protein is a fragment of the larger amyloid precursor protein which itself is a membranous protein in neurons. We are going to focus on details shortly in the APP section. This process of overproducing amyloid beta proteins and its accumulation in the neurons is called the proteopathy theory.

On the other hand, tauopathy theory was introduced. Tau protein plays a fundamental role stabilizing the microtubules and therefore the cell skeleton. When phosphorylated, it keeps the cell skeleton particles together and prevents the neurons from falling apart. However, in AD, some chemical changes lead to hyperphosphorylation of Tau protein. These abnormally formed proteins begin to pair with other threads and gradually form neurofibrillary tangles which in turn, causes disintegration in the neuron's transport system and leads to neuronal death.

Beside the proteopathy theory and tauopathy theory, several other hypotheses have been introduced to explain the physiopathology of Alzheimer's Diseaseand consequently to find treatments and perhaps even cure for this disease. In the next parts, I'm going to focus on the proteopathy theory and amyloid formation and suggest possible treatments based on this element of the disease.

III. MICRORNA ROLE AND MODERATING GENE EXPRESSION

microRNA is a small protein and a non-coding type of RNA. Its main role is to moderate gene expression. As we all know, gene expression is a process, starting in the DNA with transcription of genes and production of mRNAs. mRNA is involved in the next phase called translation in which other types of RNAs (tRNA and rRNA) are involved as well. In this phase, peptide chains are produced based on the sequence of nucleotide bases encoded in mRNA. This whole process leads to production of essential proteins in the cells.

microRNA has a post-transcriptional role in regulating gene expression. It's commonly said to be a RNA silencer because it acts in different ways and prevents the production of effective proteins from mRNA. What are these ways? In general, microRNA functions via base-pairing with complimentary sequences within mRNA. As a result, mRNA is silenced (i.e. it cannot be translated to form efficient proteins).

In details, miRNA can cause the mRNA strand to be cleaved in two pieces and shorten the polyA tail of mRNA so that efficient translation cannot be performed by ribosomes.

Consideration of miRNA molecule as a therapeutic target for Alzheimer's Diseaseis quite a recent study which has its own advantages and disadvantages. We are going to investigate how it can be used to treat AD in a while.

Beta-secretase enzyme and amyloid production

As I mentioned in the pathophysiology section, beta amyloid protein plays an important role in causing the neurons to be degenerated. But how are these proteins produced in brain cells?

Amyloid beta precursor protein (APP) is a membrane protein not only present in neurons, but also in several tissues of the body. Its primary function is yet to be known, however recent theories have suggested it as a synapse formation regulator in the brain.

APP can be cleaved by beta secretase enzyme 1 and consequently by gamma secretase enzyme, both of which are membrane enzyme. Beta Secretase Enzyme is said to be involved in the formation of myelin sheaths in peripheral nerve cells. It's encoded by the BACE1 gene in humans. The transmembrane protein contains two active site aspartate residues in its extracellular protein domain and may function as a dimer.

The physiologic function of this enzyme in brain is still vague to many scientists. On the other hand, its pathologic role in AD has been clearly proven. Its function in the cleavage of APP protein in neuronal membranes, leads to overproduction of beta amyloid protein and consequently its accumulation in the brain and neuronal death which is a crucial part of AD.

Inhibition of beta secretase enzyme has been among the most popular approaches to find a treatment for AD. However, since its physiological function is not fully understood, scientists tend to act cautiously in their methods and theories in inhibition of this enzyme.

BACE inhibitors were suggested for AD treatment and underwent early stages of testing in 2008. Verubecestat was introduced in 2012 and began its trial which is supposedly going to be completed in 2019.

Several other drugs were introduced as well. Lanabecestat was introduced in 2014, shortly before its trial was halted due to reported poor results.

The effort to find a proper solution based on this physiopathology is still a running issue.

In the next section, I will investigate how miRNA may be used to prevent beta amyloid proteins formation.

Possible AD therapeutic targets focusing on microRNA

As we saw earlier, miRNA acts as a gene silencer by disrupting the translation process.

BACE1 is a gene through which RNA polymerase II enzyme produces mRNA via transcription. The mRNA containing the genetic contents of BACE1 gene is transported to the cytoplasm and translated to beta secretase 1 protein. This last step is exactly where miRNA can play its role and inhibit the production of the enzyme. miRNA can cleave the mRNA which contains the genetic codes of BACE1 gene in two pieces and is also capable of shortening the polyA tail of the mentioned mRNA and therefore disrupting the beta secretase enzyme production.

Studies have shown that the specific type of miRNA involved in this regulation is miR-384. It has been indicated that it can potentially target the 3' untranslated regions (3'-UTRs) of both APP and BACE-1 and that the overexpression of miR-384 suppressed the mRNA and protein expression of both APP and BACE-1. A parallel study showed that The miR-384 inhibitor oligonucleotide induced the upregulation of APP and BACE-1.

This technique could be a huge revolution in the treatment of AD. Trials and testing have been conducted to ascertain the efficiency of this theory and its accuracy. A study in 2014 on mice showed that patients with mild cognitive impairment and dementia of Alzheimer's disease had lower blood miR-384 levels compared with the controls. In addition, patients with dementia of Alzheimer's disease had lower blood miR-384 levels in blood compared with the mild cognitive impairment group.

Another study indicated the role of miRNA-339-5p. In this study, Co-transfection of miR-339-5p with a BACE1 3'-UTR reporter construct resulted in significant reduction in reporter expression. Delivery

of the miR-339-5p mimic also significantly inhibited expression of BACE1 protein in human glioblastoma cells and human primary brain cultures. Another prominent finding in this study was the significantly reduced levels of miRNA-339-5p in Alzheimer's disease compared to age-matched controls.Therefore, miR-339-5p regulates BACE1 expression in human brain cells and is most likely dysregulated in at least a subset of AD patients making this miRNA a novel drug target.

These two miRNAs have not been the only ones studied for finding therapeutic targets in AD.

A study on MicroRNA-455-3p showed exactly the opposite finding. It appears that this miRNA acts as a provoker to the translation of BACE1 gene and the production of beta secretase enzyme. Surprisingly, the level of MicroRNA-455-3p was found to be higher in AD patients which can support the fact that MicroRNA-455-3p can be a potential peripheral biomarker for Alzheimer's disease.

Conclusion:

Alzheimer's disease has always been a morbid disease. The overexpression of miRNA-384 and miR-339-5pcan lead to a decrease in the translation of BACE1 gene and production of beta secretase protein. Other types of miRNA (MicroRNA-455-3p for instance) on the other hand, have been found as a provoker for the production of this enzyme and its level is higher in AD patients. Lower levels of beta secretase enzyme in the blood results in lower amount of cleavage of Amyloid precursor protein in neuronal membranes and less formation of beta amyloid protein and as a result a we will have a reduction in its accumulation in the brain cells which is one of the two main ways of pathogenesis of Alzheimer's disease. As a result, neuronal death is going to be decreased in temporal and parietal lobes which are affected more in the course of AD and these parts of the brain will relatively stay intact and therefore the patient can experience less severe symptoms and maybe even a better prognosis in his/her disease.

REFERENCES

[1] Rev Neurosci. 2018 Feb 23;29(2):161-182. doi: 10.1515/revneuro-2017-0042.

[2] Rev Neurosci. 2018 May 28. pii: /j/revneuro.ahead-of-print/revneuro-2018-0008/revneuro-2018-0008.xml. doi: 10.1515/revneuro-2018-0008.

[3] -Circulating microRNAs as novel biomarkers of Alzheimer's disease, ClinChimActa. 2018 Sep;484:99-104. doi: 10.1016/j.cca.2018.05.039. Epub 2018 May 23.

[4] -MicroRNA-455-3p as a Potential Biomarker for Alzheimer's Disease: An Update, Front Aging Neurosci. 2018 Feb 23;10:41. doi: 10.3389/fnagi.2018.00041. eCollection 2018.

[5] -Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression, Proc Natl AcadSci U S A. 2008 Apr 29;105(17):6415-20. doi: 10.1073/pnas.0710263105. Epub 2008 Apr 23

[6] -MicroRNA-339-5p Down-regulates Protein Expression of β-Site Amyloid Precursor Protein-Cleaving Enzyme 1 (BACE1) in Human Primary Brain Cultures and Is Reduced in Brain Tissue Specimens of Alzheimer Disease Subjects' J Biol Chem. 2014 Feb 21; 289(8): 5184–5198.Published online 2013 Dec 18. doi: 10.1074/jbc.M113.518241

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