Identification of Aptamers for Alzheimer's Therapy

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Abstract

This research aims to develop single-stranded DNA aptamers that can disrupt the binding interaction between amyloid β and PrP^c, to be used as a possible therapy for Alzheimer's disease.

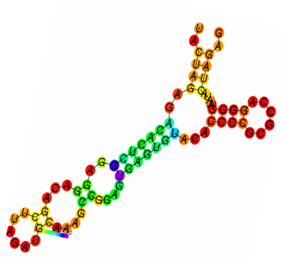


Figure 1a: Secondary Aptamer Structure

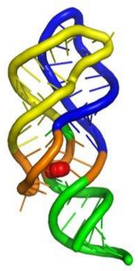


Figure 2: Tertiary Aptamer Structure

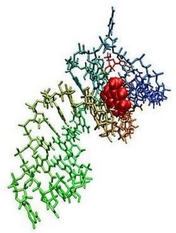


Figure 3: Tertiary Aptamer Structure

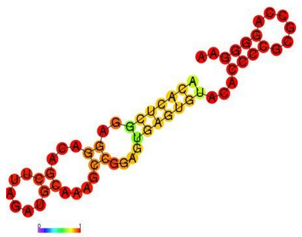


Figure 1b: Secondary Aptamer Structure

Aptamers are small (~20-100) single-stranded DNA sequences that can have a high affinity for certain binding sites. Once screening has identified candidates with desirable binding properties, they can be easily produced on a large scale. Compared to antibody or recombinant protein production, this reduces the costs significantly as they are free from any cell-culture derived contaminates.

Introduction

Alzheimer's disease [AD] is a tragic affliction that is the leading cause of dementia worldwide. One of the reigning hypotheses behind the cause of Alzheimer's disease suggests that the accumulation of the amyloid β protein (Aβ) results in neurotoxicity that ultimately shows itself as dementia. Evidence supporting this has shown that Aβ is the main component of plaques deposited in all AD patients [1], synthetic Aβ peptides are neurotoxic when aggregated [2], and every form of hereditary AD involves mutations directly on the Aβ precursor protein (APP) or the enzymes involved in the generation of Aβ from APP [3]. A recent study has identified the cellular prion protein (PrP^c) as a possible receptor for Aβ protein that transmits the toxicity associated with Aβ [4]. We hypothesize that blocking the binding site on the PrP^c will prevent the neurotoxicity of Aβ.

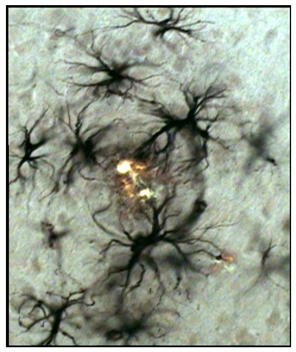


Figure 4

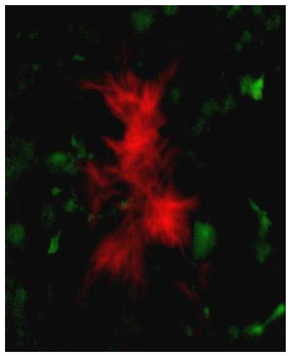


Figure 5

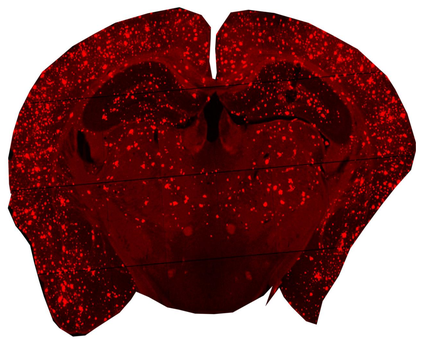


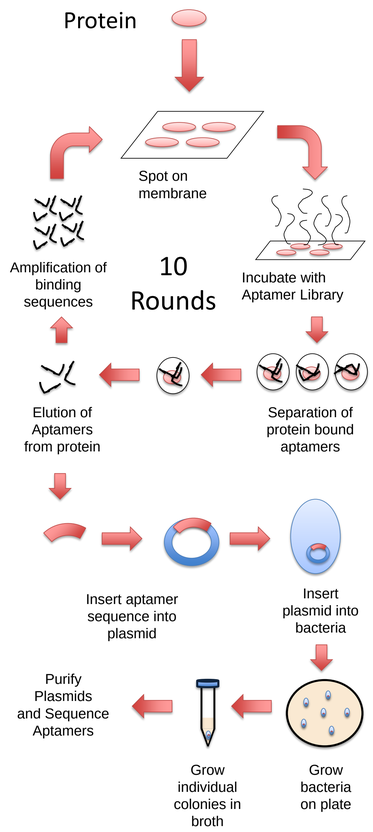
Figure 6

4)Amyloid deposit visualized via congo red staining surrounded by reactive astrocytes visualized by GFAP immunostaining

5) High magnification image 60X taken with a spinning disc confocal microscope, with the amyloid deposits labeled with thiazine red and GFP positive distrophic neurites surrounding the deposit.

6)Distribution of amyloid deposits in transgenic mouse brain visualized by thiazine red stain.

Method



Discussion and Results

Three rounds of SELEX have been completed. After third PCR an 4% Agarose gel showed a band at approximately 60bp for sample #1, but no band for sample #2. The length of the aptamers in the library used is consistent with this finding, showing some binding of the aptamers to the protein-bound membrane. It is unclear whether the aptamers are binding to the protein or the membrane. Further tests will be done to confirm proper binding of the aptamer to the protein. After ten rounds of SELEX are completed the identified sequenced aptamers will be used to test the hypothesis in further research. Aptamers can be tested on primary neuronal cell bodies for binding abilities.

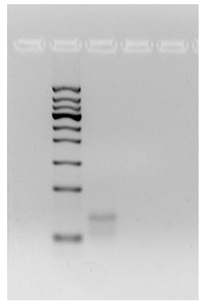


Figure 7

References

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3. Price, D.L. and S.S. Sisodia, *Mutant genes in familial Alzheimer's disease and transgenic models.* Annu Rev Neurosci, 1998. **21**: p. 479-505.
4. Lauren, J., et al., *Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers.* Nature, 2009. **457**(7233): p. 1128-32.

Figure 1a and b, Smaller and larger aptamer structures. Adapted from NYMU-Taipei iGem 2010 Team: Speedy Reporter/Materials and Methods. http://2010.igem.org/Team:NYMU-Taipei/Project/Speedy_Reporter/Material_and_Methods. Accessed November 1, 2011.
Figure2, Aptamer 3D Structure. Adapted from RiNA. RNA Network. Aptamers and Spiegelmers. http://rna-network.com/index.php?id=132&L=1. Accessed November 1, 2011.
Figure 3 Adenine riboswitch aptamer 3D structure. Adapted from Universite de Sherbrooke. Lafontaine lab::Research. http://pages.usherbrooke.ca/dlafontaine/research.html. Accessed November 1, 2011.
Figures 4-6 Pictures adapted from Dr. Aaron Hirko
Figure 7 Picture of agarose gel taken by Jennifer Griffis April 10, 2012.

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