Gene therapy in treating Alzheimer’s disease

Zhewei Wang @PBI05500
Causes of Alzheimer’s disease

1. Accumulation of Tau protein within the brain neurons
2. Plaques between the dying cells in the brain
3. Progressively fewer nerve cells and connections
4. Hippocampal shrinkage

Therapeutic strategies for AD

1. Acting directly on amyloid precursor protein (APP) metabolism
   a. APP is the primal components of amyloid plaques.
   b. Inhibiting secretase activity to decrease amyloid pathway, increasing amyloid degrading enzymes, delivering anti-amyloid antibodies, ...

2. Increase neuroprotection
   a. Increase nerve growth factor level, overexpress brain-derived neurotrophic factor, overexpress Glial cell-derived neurotrophic factor, ...

3. Boosting autophagy-mediated pathways

4. Targeting inflammatory pathway
Glial cell-derived neurotrophic factor (GDNF)

- Glial cell-derived neurotrophic factor (GDNF) is a protein that, in humans, is encoded by the GDNF gene. GDNF is a small protein that potently promotes the survival of many types of neurons. It signals through GFRα receptors, particularly GFRα1. [https://en.wikipedia.org/wiki/Glial_cell_line-derived_neurotrophic_factor](https://en.wikipedia.org/wiki/Glial_cell_line-derived_neurotrophic_factor)

- Two papers
  - Overexpress the GDNF gene in hippocampus
  - Deficiency of GDNF Receptor GFRα1 in Alzheimer's Neurons Results in Neuronal Death
Overexpress the GDNF gene in hippocampus

- Recombinant lentiviral vectors with Human cytomegalovirus promoter (CMV) were used to overexpress GDNF gene in hippocampal astrocytes of 3xTg-AD mice in vivo.

- Pseudotype
  - Pseudotyping is the process of producing viruses or viral vectors in combination with foreign viral envelope proteins. The result is a pseudotyped virus particle. With this method, the foreign viral envelope proteins can be used to alter host tropism or an increased/decreased stability of the virus particles. [https://en.wikipedia.org/wiki/Pseudotyping]
  - Lyssavirus Mokola glycoprotein (Mokola-G) pseudotype, ...highly stable and confers an exceptionally wide host range. [https://www.med.upenn.edu/gtp/vectorcore/production.shtml]
Overexpress the GDNF gene in hippocampus

- Human cytomegalovirus promoter (CMV)
  - While restriction of gene expression to specific cell populations is of particular importance, highly efficient cell-type-specific gene expression after viral gene transfer so far has been hampered by low levels of transgene expression. [Gruh, I., Wunderlich, S., Winkler, M., Schwanke, K., Heinke, J., Blömer, U., ... & Martin, U. (2008). Human CMV immediate-early enhancer: a useful tool to enhance cell-type-specific expression from lentiviral vectors. The journal of gene medicine, 10(1), 21-32.]

- Intrahippocampal injection

- The viral titers were obtained using a real-time quantitative PCR (qPCR)-based method.

- Morris water maze test
Overexpress the GDNF gene in hippocampus
Overexpress the GDNF gene in hippocampus

- Human cytomegalovirus promoter (CMV)
  - While restriction of gene expression to specific cell populations is of particular importance, highly efficient cell-type-specific gene expression after viral gene transfer so far has been hampered by low levels of transgene expression. [Gruh, I., Wunderlich, S., Winkler, M., Schwanke, K., Heinke, J., Blömer, U., ... & Martin, U. (2008). Human CMV immediate-early enhancer: a useful tool to enhance cell-type-specific expression from lentiviral vectors. The journal of gene medicine, 10(1), 21-32.]

- Intrahippocampal injection

- The viral titers were obtained using a real-time quantitative PCR (qPCR)-based method.
- Morris water maze test
Overexpress the GDNF gene in hippocampus

- Recombinant lentiviral vectors with Human cytomegalovirus promoter (CMV) were used to overexpress GDNF gene in hippocampal astrocytes of 3xTg-AD mice in vivo.
Deficiency of GDNF Receptor GFRα1 in Alzheimer's Neurons Results in Neuronal Death

- NC and AD brain tissues were obtained from the Sun Health Research Institute Brain and Body Donation Program.
- In the presence of GDNF, there was no obvious axonal growth of AD neurons, while NC neurons were able to grow neurites.
- Glial cell-derived neurotrophic factor (GDNF) is a protein that, in humans, is encoded by the GDNF gene. GDNF is a small protein that potently promotes the survival of many types of neurons. It signals through GFRα receptors, particularly GFRα1. 
  

  - Determination of mRNA levels of GFRα1–4 by RT-PCR analyses.
Deficiency of GDNF Receptor GFRα1 in Alzheimer's Neurons Results in Neuronal Death

- GFRα1 expression was significantly reduced in AD neurons compared with NC neurons.

- Western blot analysis to determine the protein levels of GFRα1–4.
  - Western blot is similar to ELISA.
  - GDNF enhances the expression of GFRα1 in NC but not in AD neurons
Deficiency of GDNF Receptor GFRα1 in Alzheimer's Neurons Results in Neuronal Death

- Used antisense oligonucleotides (ASOs) against GFRα1 in the presence of GDNF for 5 days.
  - GFRα1 ASOs inhibited GFRα1 mRNA and protein expression in NC neurons in a dose-dependent manner.

- Introduce cDNA for GFRα1 and promoter to AD neurons
  - Survival and neurite outgrowth improve
Deficiency of GDNF Receptor GFRα1 in Alzheimer's Neurons Results in Neuronal Death

- It is generally accepted that glutamate receptors are important for the expression and function of NTFs and their receptors.
  - Neurons were incubated with 20 ng/ml GDNF in the presence of either the AMPA receptor blocker CNQX (10 M) or the NMDA receptor blocker AP-5 (50 M; Sigma-Aldrich) for 7 d.
  - In NC neurons, CNQX and AP-5 were found to completely abolish GDNF-enhanced GFRα1 protein expression.
  - In AD neurons, on the other hand, CNQX and AP-5 significantly enhanced GFRα1 protein expression in the presence of GDNF.
Deficiency of GDNF Receptor GFRα1 in Alzheimer's Neurons Results in Neuronal Death

● Conclusion

○ In NC neurons, the presence of glutamate receptors is necessary for GDNF-linked GFRα1 expression, while in AD neurons the absence of glutamate receptors is absolutely required for GFRα1 expression by GDNF stimulation.

○ The combination of NTFs such as GDNF with glutamate receptor antagonists may be necessary to rescue neuronal damage in AD neurodegeneration. The administration of GDNF alone is also unlikely to show some beneficial effects on damaged AD neurons.
Deficiency of GDNF Receptor GFRα1 in Alzheimer's Neurons Results in Neuronal Death

- In the presence of GDNF, there was no obvious axonal growth of AD neurons, while NC neurons were able to grow neurites.