Calvin Tran Week 7 Journal Recitation

A novel gene editing system to treat both Tay–Sachs and Sandhoff diseases

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Overall: Demonstrate the potential of developing in vivo genome editing for Tay–Sachs and Sandhoff patients.

- Putting results of previous studies together
    - Use fewer vectors = study with single subunit
    - Localize to liver with specific plasmid
    - Crossing the blood brain barrier

Tay-Sachs/Sandhoff are genetic disorders due to genetic errors in regions encoding the subunits of lysosomal enzyme β-hexosaminidase A resulting in deficiency in this enzyme and then accumulation of GM2 gangliosides

- In the past, other studies have shown a modified subunit can treat both diseases by degrading GM2 gangliosides.
    - Putting this subunit into a gene vector and into mice is the focus of this study
- Apply this modified subunit to their gene editing system
- Tested on mice with Sandhoff disease.
- Effective at reducing GM2 nucleosides in multiple areas to normal levels except in the brain.
    - Improved results in liver
    - Coordination and motor memory improved

In 2020: *"However, there is no effective treatment for GM2-gangliosidoses now, with palliative measures being the current standard of care."*

- Stem cell transplantation achieved increased enzyme activities but could not prevent the disease progression and demise
- Gene therapy is being investigated in animal models

- Outlined 3 major obstacles = "*critical need to develop an innovative gene therapy protocol which surmounts these problems for treating GM2-gangliosidoses*"

1. Continuous delivery (vs pulsatile)
2. Sufficient transgene product to the brain
3. Minimizing vector-associated risk

The Study (Methods)

- Animals and injections: Sandhoff mice and control mice.
- Vectors: Adeno-associated virus vector (AAV) = a typical / common method of gene delivery.
    - Plasmids contained SaCas9 (insertion) and HEXB cDNA (codes HEXB subunit) donor
    - Insertion sites determined and vectors produced, inserted into Mouse Embryonic Fibroblast (MEF) cells
    - "CRISPR-mediated genome editing" as their novel technique
        - "*AAV8 vectors are liver-tropic, and SaCas9 is under control of a liver-specific promoter. By virtue of this, genome editing and transgene expression can be limited to hepatocytes*."
        - Insert promoter-less cDNA
- Ganglioside Quantification: Measured tissue samples of brain, liver, spleen
    - Histology
- Two behavioral tests = pole test, fear conditioning
- Statistics: two sided t-test, vector group vs control

Results

Delivery of gene editing system

- In mice treated with the donor alone (**the control mice**) , the MUGS and MUG activities did not significantly increase (data not shown), indicating that there was no episomal transgene expression from the promoterless donor
- After 4 months, all mice were euthanized and tissues were harvested for further analyses. **MUGS (enzyme) activities in the liver, heart, and spleen increased to 25, 3, and 2 fold of wild-type levels, respectively** ($p < 0.0001$, Fig. 3c)

Behavioral Tests

- Pole test that assesses fear conditioning (learning and memory) = no observed difference
- Rotarod test assesses coordination, motor function and motor memory, a significant difference between untreated Sandhoff and normal mice was observed.

Histology:

- Cellular vacuolation = when lysosomes are engorged with storage materials that they cannot break down
- histological analysis of the brain and liver was performed
    - Liver = treated mice reduced
    - Brain = only 1 in 3 treated mice were reduced.

Measured GM2 gangliosides in tissue

- GM2 gangliosides were **significantly reduced in the liver, heart, and spleen** of treated mice ($p < 0.0001$).
- However, GM2 **gangliosides in the brain of treated mice were not significantly reduced**
    - "*It is unlikely that gene editing occurred in the brain because Cas9 is under the control of a liver-specific promoter.*"
        - Were depending on being able to cross the blood-brain barrier

Discussion

One challenge for PS813 to treat a neurological disease is to deliver the enzyme to the brain

- Previous studies using high dose enzyme replacement therapy have achieved significant neurological benefits
- These results indicated that when there is a constant high enzyme level in the bloodstream, a small amount may be able to cross the BBB.

Vector includes only beta subunit which is enough to treat mice, but enough for humans due to different mechanisms.

- "*making translation of this strategy into clinical practice difficult*"
- "However, it is difficult to package both HEXA and HEXB cDNA into one AAV vector, while the use of two vectors brings about higher manufacturing cost and vector-associated risk"

Still no current effective therapy, only palliative measures --- limited therapeutic benefic

- Gene therapy holds promise of potential permanent single-dose treatment
- Previous methods has issues with randomly integrating into the genome, or to not provide permanent results = decline in therapeutic effects.
- **The feasibility of this liver-targeting gene editing approach to treat a neurological disease is supported by multiple preclinical studies** with high doses of ERT that are relatively high compared with usual doses of ERT used to treat patients with (Table 1). **These studies showed that a high level of enzyme in circulation could facilitate entry of enzyme into the brain.**
- Moreover, in a parallel study in MPS I mice (unpublished data), when a tenfold-dose of this gene editing system was used, no vector-associated toxicity

or microscopic findings were observed in the 11-month follow-up. **These results further support the feasibility of increasing the dose to improve the efficacy.**