

SUMMARY OF FINAL REPORT

Development of second generation histone deacetylase inhibitors as therapeutics for Friedreich's ataxia

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Background and aims:

This project concerns the development of a novel class of histone deacetylase (HDAC) inhibitors (pimelic *o*-aminobenzamides) as therapeutics for Friedreich's ataxia (FRDA). Our lab identified pimelic *o*-aminobenzamide HDAC inhibitors as activators of frataxin transcription in cell culture and in a mouse model of the condition. In this project synthetic methods were used to improve on the compounds in terms of pharmacological properties (improved brain penetration and metabolic stability), HDAC enzyme selectivity and efficacy. The effects of the inhibitors on frataxin mRNA and frataxin protein were determined in cell culture using a newly developed human neuronal cell model for FRDA based on patient-derived induced pluripotent stem (iPS) cells.

Here we report that the new HDAC inhibitor molecules have improved penetrability of the brain and are effective in restoring frataxin transcription in early neuronal cells differentiated from patient-derived iPS cells.

Lay summary of Results:

Specific Aim 1: Synthesis and characterization of HDAC inhibitors

To develop our HDACi, two structural features were identified that individually improve brain penetration and metabolic stability. Using a technique called 'click chemistry', eight new compounds were developed. All have a higher brain to plasma ratio in mice than the previously published compound, 109. Particularly, newly developed compound NG1 had a brain to plasma ratio that was 4.5-fold higher than that of compound 109, meaning it has a greater ability to penetrate the brain. The new compounds also showed good stability.

Specific Aim 2: HDAC inhibition assays

The selectivity of the compounds for different classes of HDAC was measured. Compound C1s showed a >200-fold preference for HDAC1 over HDAC3 and compound C3s had a >30-fold preference for HDAC3 compared to HDAC1.

Specific Aim 3: Efficacy of HDAC inhibitors in cell-based assays

Induced pluripotent stem (iPS) cells were made from FRDA patient fibroblasts, using the four transcription factor transduction approach. These cells were then developed into neuronal cells and used to measure the ability of the compounds to increase expression of the frataxin gene.

NG1 increased frataxin gene expression in FRDA early neuronal cells by at least two fold with a minimum effective dose of 5 μ M; similar results to those obtained with compound 109. The other new small molecules were also highly effective at increasing frataxin gene expression in FRDA early neuronal cells, with most of them being of similar or greater potency than the lead compound, 109.

We have previously shown that HDACi that preferentially target HDAC1 and HDAC2 have only a modest effect on frataxin gene transcription and that the target of our inhibitors is HDAC3. However, when we analyzed the effect of compounds C1s (selective for HDAC1 and HDAC2) and C3s (selective for HDAC3), we found that neither is able to fully reverse frataxin gene silencing. The combined treatment of neuronal cells with the two compounds was not effective either. Further studies are necessary to understand how compounds of broader specificity like 109 are far more efficient in reactivating frataxin transcription. This may be linked to acetylation changes to the lysine 9 on histone 3 (H3K9), as this was significantly higher in cells treated with 109 compared to those treated with C1s and C3s.

Summary:

- Using a new synthetic strategy we were able to improve both brain penetration and metabolic stability of our HDACi.
- Compound NG1 is a very promising potential treatment for Friedreich's ataxia, having a good brain to plasma ratio and being at least as effective as 109 in increasing frataxin expression.
- Our HDACi reactivate frataxin gene transcription and increase acetylation of histones on the frataxin gene in early neuronal cells derived from patient-derived iPS cells.
- A compound with >30 fold preference for HDAC3 compared to HDAC1 (C3s) alone or in combination with an HDAC1 specific compound (C1s) does not increase frataxin expression. Understanding how compounds of broader specificity like 109 are far more efficient in reactivating frataxin transcription and what role H3K9 plays in the process will require further investigation.

Lay summary of results

The genetic mutation that causes the neurological condition, Friedreich's ataxia (FRDA), is the expansion of GAA•TTC triplets in the frataxin gene. This mutation causes the gene to shut down and produce too low levels of the essential protein frataxin. We identified promising therapeutics for FRDA, by showing that frataxin silencing is due to modifications of proteins that bind the gene (histones) and that we can restore gene function by inhibiting the enzymes responsible for such modifications (histone deacetylases or HDAC). These small molecules (HDAC inhibitors or HDACi) can increase frataxin activity in cell culture and in a mouse model of the condition.

While these compounds are promising therapeutics for FRDA, they suffer from two limitations, namely less than optimal brain penetration and formation of an inactive

metabolic byproduct in the stomach and serum. Here we report that using a new synthetic strategy, we are able to overcome such limitations by changing two structural features of these molecules and that the new HDACi are effective in restoring frataxin activity in neuronal cells derived from FRDA patients.

The new compounds can penetrate the brain up to five-fold better than our previous molecules and are very stable. These properties are very important in developing promising therapeutics for a neurological condition like Friedreich's ataxia.

Using a methodology developed in the last few years we were able to take skin cells from people with FRDA and produce stem cells. These stem cells were then differentiated into FRDA neuronal cells and used in our assays to determine the efficacy of the next generation HDAC inhibitors in a cell type that is relevant for FRDA. The new compounds increase frataxin gene activity to a therapeutically beneficial level, have a minimum effective dose similar to the old compounds and similar duration of effect. Using the newly developed neuronal cellular model for Friedreich's ataxia we were also able to show that our HDACi can change most histone modifications on the mutated frataxin gene to those of a functioning gene.

To understand the mechanism of action of HDACi in reversing frataxin gene silencing we used small molecules with different specificity. We have previously shown that our HDAC inhibitors preferentially target the histone deacetylase HDAC3 compared to HDAC1 (three-fold preference) and we obtained a new compound that is very specific for HDAC3 (30-fold preference over HDAC1). Surprisingly this molecule failed to increase frataxin expression and further studies are necessary to understand how compounds of broader specificity are far more efficient in restoring frataxin activity.

Conferences/ meetings where this research has been presented:

4th International Friedreich's Ataxia Scientific Conference. Strasbourg, May 5th-7th 2011

Benefits to people with ataxia arisen/likely to arise from this research (also refer to original grant application):

Repligen Corporation will subject the most promising compounds to full preclinical evaluation leading to filing an Investigational New Drug application with the US FDA, and future testing in man.

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