

SUMMARY OF FINAL REPORT

Development of histone deacetylase inhibitors as treatment for Friedreich's ataxia

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Background and aims:

Friedreich's ataxia (FRDA) is caused by the *frataxin* gene, which encodes the essential mitochondrial protein frataxin, being abnormally 'switched off'. This is due to a mutation in the gene that consists of an increased number of 'GAA•TTC' repeats in the DNA; this is called a triplet-repeat expansion. The mutation changes the structure of the DNA and this leads to 'switching off,' or repression, of the gene. HDAC inhibitors (HDACi) work by switching the frataxin gene back on and this project was aimed at developing HDACi as an effective treatment for FRDA.

Specifically, the aims of the project were 1) to analyse the effect of HDACi on the frataxin gene "environment" (chromatin composition) and expression in white blood cells (lymphocytes) taken from people with FRDA, 2) to screen new inhibitors that specifically inhibit class 1 HDAC enzymes and 3) look at the ability of HDACi to turn the frataxin gene back on in neuronal Friedreich's ataxia cells.

Lay summary of Results:

1) Analysing the effects of HDACi on the frataxin protein expression in lymphocytes taken from people with FRDA

To help the development of HDACi for FRDA, a suitable dose regimen needs to be established for clinical trials. To this end, we took white blood cells (lymphocytes) from people with FRDA, treated them with HDACi and analyzed the timing of frataxin protein expression in the cells.

We have previously demonstrated that a 10-hour treatment with our lead molecule HDACi **109** is necessary to achieve a therapeutically significant increase in frataxin expression. An increase in frataxin protein is first detectable 24 hours after the start of the treatment, and continues for up to 72 hours. After the treatment finishes, therapeutically significant levels of frataxin protein are still detectable for 24 hours, indicating that the frataxin protein is very stable. These results show that HDACi **109** has a long lasting effect, making it an optimal candidate for clinical trials.

2) Screening new inhibitors that specifically inhibit class 1 HDAC enzymes

We wanted to develop new HDACi that were more effective and showed better penetration of the brain. Using a method called 'click' chemistry which involves joining different parts of different molecules together to get the most effective compound, a new group of HDACi was synthesized in our laboratory. Very encouraging preliminary results were obtained with two of these compounds; click1 and click2.

When these compounds were compared to our lead compound, HDACi **109**, for their ability to increase expression of the frataxin gene in cells from people with FRDA, click1 was found to be as effective, or more effective than compound **109**. In blood samples from people with FRDA that did not respond to treatment with compound **109**, Click2 was able to increase frataxin considerably (by a factor of 2.5). Moreover, click1 displayed excellent penetration of the blood-brain barrier in rats, meaning that it is readily available to the brain. Taken together, the above results show that although further characterization is necessary, the click compounds could be new improved therapeutics for FRDA.

3) Looking at the ability of HDACi to turn the frataxin gene back on in neuronal Friedreich's ataxia

Research into Friedreich's ataxia lacks an appropriate cellular model for studying the condition and screening potential therapeutic compounds. The currently available cell models differ greatly from the cell types primarily affected in FRDA, ie heart cells and brain cells (neurons). They also rely on the availability of people with FRDA to obtain blood donations and skin biopsies from which to source the cells. Very recently it has been shown that skin cells can be reprogrammed into stem cell-like cells, called induced pluripotent stem (iPS) cells, and these can be developed (differentiated) into different cell types, eg neurons and heart cells.

Following a previously published protocol, we obtained FRDA iPS cells. Our FRDA iPS cells exhibit the same abnormalities as FRDA cells ie switching off of the frataxin gene, and other features of FRDA. Hence these cells could be used to study the development of FRDA and possibly also to screen for new potential therapeutics.

To establish whether the FRDA iPS cells could be used to screen for new therapeutics, we wished to determine whether treatment with HDACi could restore frataxin gene expression. Encouragingly, treatment with compound **109** improved the expression of the frataxin gene and increased frataxin protein levels in the cells.

Obtaining neuronal cells from our iPS cells is the more challenging goal. Using a protocol previously published, we were able to generate neuronal-type cells from the iPS cells. Many different types of neuronal cells exist and it is likely that our population of cells is a mixture of these. Therefore our next effort will focus on improving the development (differentiation) of these cells and devising a method to select or separate the different types of neuronal cells from their neighbouring cells. After this, we will use the neuronal cells to study the mechanism of FRDA and test our HDACi.

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