Testing compounds for encouraging mitochondrial biogenesis and developing an effective model to check toxicity and efficacy

James Bryson* BSc Biomedical Genetics (j.bryson@newcastle.ac.uk), Oliver Russell, Professor Doug Turnbull (Wellcome Trust Institute for Mitochondrial Research)

Newcastle University

Introduction

Many debilitating metabolic diseases are caused by mutations in mitochondrial DNA. These mutations can affect the 13 genes involved in oxidative phosphorylation. This means these organelles can no longer produce enough ATP (through oxidative phosphorylation) for healthy cell function. As a result prognosis for many children with these mitochondrial mutations is very poor. One potential therapy for these conditions is to up-regulate mitochondrial biogenesis to increase the number of mitochondria within the cell, thereby increasing ATP production and alleviating symptoms (this has proven to be an effective remedy in exercise studies (Ref 1)).

This project focused on the analysis of growth rates of mitochondrial disease patient cell lines to develop a system for analysing changes in mitochondrial biogenesis. Changes in growth rates of defective cell lines after treatment with selected mitochondrial acting compounds could indicate an alleviation of the biochemical defect.

Aims

- Determine glucose/galactose growth rates in mutant cell lines
- Assess which cell lines are appropriate for large scale experiments

Methods

Wild-type Cell Model

1. Cultivated wild-type fibroblast cells until confluence was high enough to be split into two 75ml flasks containing glucose media
2. Incubated for a day to allow cells to adhere to bottom of flasks (requires glucose in media)
3. Removed media from one flask and replaced the glucose media with galactose media (Figure 1)
4. Incubated cells for a day
5. Performed a cell count for both flasks using Haematocytometer
6. These counts were used to approximate the volume of media required for transferring 10,000 cells each to three separate 25ml flasks for both of the original flasks (6 total)
7. A cell count was performed for one of the 25ml flasks from each of the two media types on day 4, day 7 and day 10
8. Analysed to see if the cells adapted to surviving and replicating in an environment only conducive to oxidative phosphorylation

Patient Cell Model

1. Repeated steps 1-8 but using 6 patient cell lines (Extra Information) instead of wild-type cells.

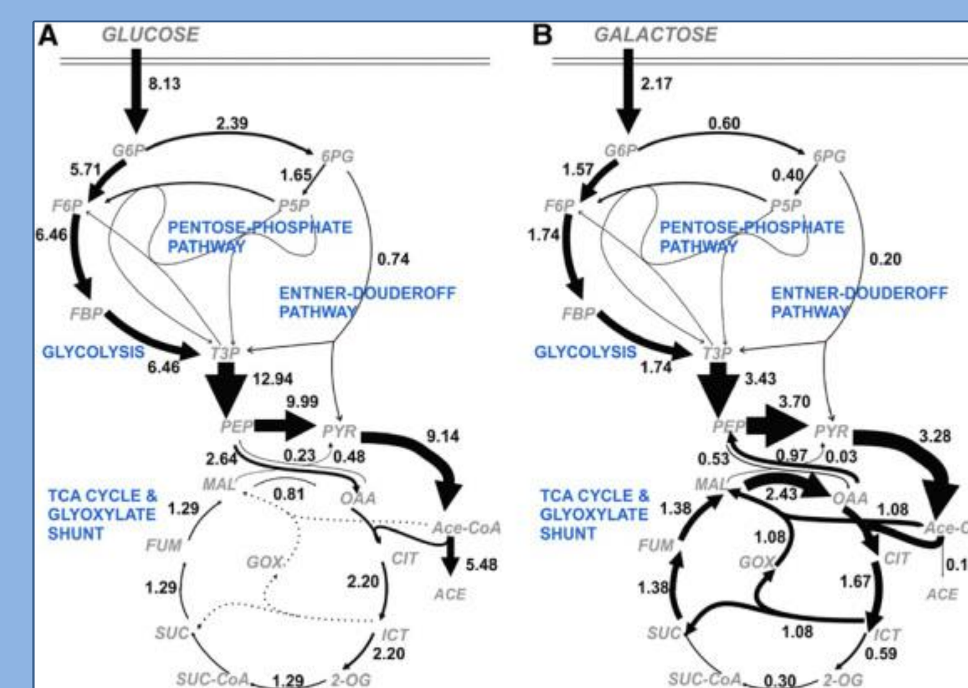
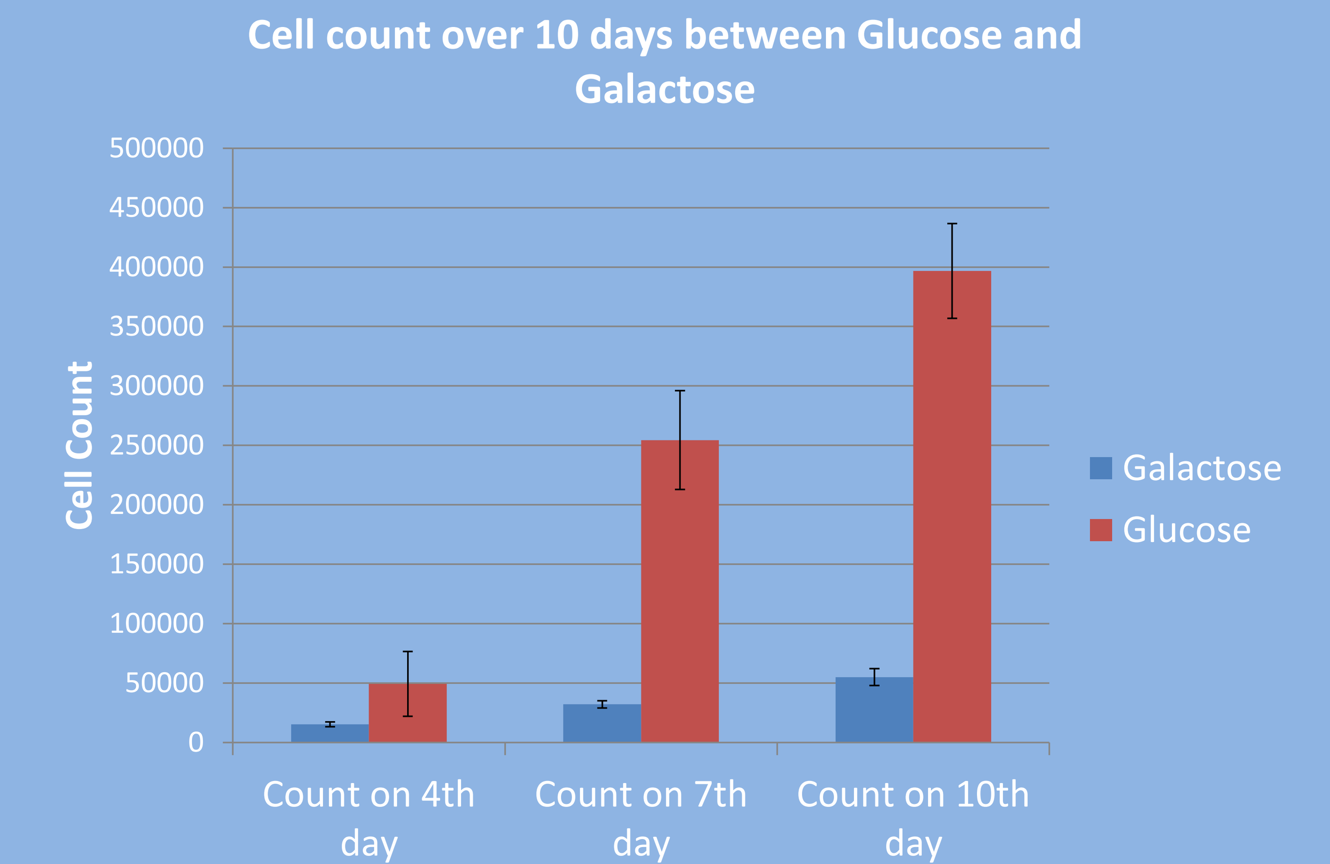


Figure 1
This rather in depth diagram was taken from Haverkorn van Rijsewijk et al. 2011 (Ref 2). The sole purpose is to demonstrate the idea that while galactose metabolism does in part involve glycolysis, this is comparatively small compared to glucose. Also relative to glucose the oxidative phosphorylation pathway (represented by the lower circle) is upregulated. This suggests that cells metabolising galactose rather than glucose will be much more reliant on oxidative phosphorylation for survival, making galactose a good medium to help select for increased mitochondrial oxidative phosphorylation.

Results

When working on a model for testing efficacy and toxicity it was shown that wild-type cells survived in galactose media and could also proliferate, albeit at a much slower rate. These results are displayed in the graph below. Further tests were carried out with patient cell lines, however the cells were not re-suspended after performing a cell count leading to unpredictable concentrations of cells. This was particularly detrimental for the galactose cell lines where cell death and low proliferation rates were expected based on the wild-type results. As a result this second set of data was not interpretable.



Conclusion/Discussion

The use of a galactose media model can provide an effective method of selecting for cells able to metabolise sufficiently primarily through oxidative phosphorylation. This is an essential pre-requisite to check for the ability of any compound to provide therapeutic value. The results confirm that these wildtype fibroblast cells, chosen for their ability to be easily imaged, can grow in galactose media conditions. As a result if the right mutation was later isolated, (equivalent to a reasonably severe phenotype but allowing the cells to grow to sufficient concentrations) then tests could be carried out to see if the increased mitochondrial biogenesis was allowing the cell to survive primarily using oxidative phosphorylation. This combined with imaging of the cells could verify both that the mitochondrial density had increased alongside ATP production essential for survival and reproduction.

Extra Information

The 6 cell patient lines included; M0412-11 (deletion affecting SURF1 gene involved in assembly of COX), M0050-10 (Large mtDNA deletion), M0607-09 (A>G point mutation in MTND5 gene causing complex 1 defect), M0737-10 (mutation in LRPPRC gene causing COX defect), M304-06 (Arg>Gln and Met>Thr mutationsin NDUFS2 gene cause complex 1 assembly defect), M541-06 (C>A point mutation in MTND4 gene causing complex 1 defect)

References

1) Jones K et. al (2013) The effects of high intensity interval training on clinical symptoms and functional capacity in adults with neuromuscular disease. J Neurol Neurosurg Psychiatry.
2) Haverkorn van Rijsewijk et. al (2011) **Large-scale 13C-flux analysis reveals distinct transcriptional control of respiratory and fermentative metabolism in Escherichia coli. Molecular Systems biology**