A Novel Small Molecule Modulating Mitochondrial Activity Resolves Inflammation and Fibrosis in a Diet-Induced NASH Model

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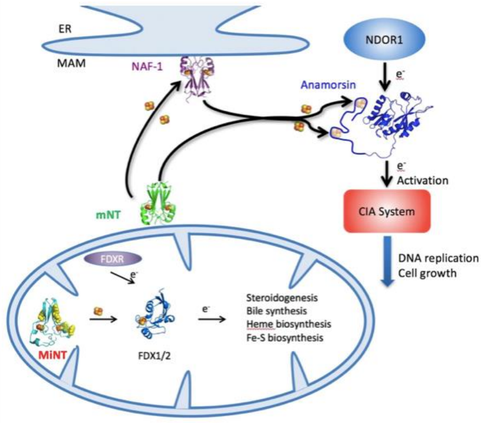
INTRODUCTION

ENYO has developed a novel, potent chemistry that regulates the function of 3 mitochondrial proteins called the NEET proteins. They are iron-sulfur transfer proteins containing a redox-active 2Fe-2S cluster that they can reversibly bind. This enables an important biological role for NEET proteins in regulating:

- Fe transfer to proteins that require it as a co-factor
- Fe and ROS homeostasis in mitochondria
- Stress and innate immune responses from mitochondria
- The carbon utilization and energetic output of mitochondria



AIM

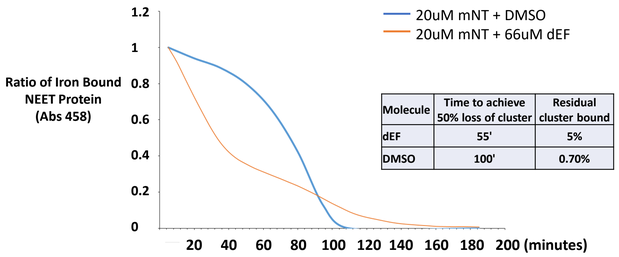
Literature reports therapeutic importance of NEET proteins in metabolic diseases, ageing, Wolfram Syndrome type 2 and oncology. Hence, we want to assess the effects of ENYO's molecule on :

- NEET proteins function
- Mitochondrial activity (Respiration, MMP)
- Pathology associated with a diet-induced obesity model of NASH in mice

RESULTS

Cluster assay stabilization by ENYO's molecule

Assay = Time-dependent Stability of Iron binding to NEET proteins

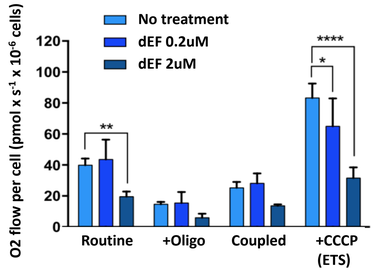


Molecule	Time to achieve 50% loss of cluster	Residual cluster bound
dEF	55'	5%
DMSO	100'	0.70%

- UV-vis spectroscopy was used to observe the 2Fe-2S cluster stability of NAF-1 or mNT or MiNT in the absence and presence of ENYO's molecule (dEF).

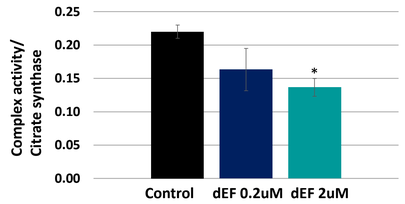
Our molecule destabilizes the 2Fe-2S cluster of the 3 NEET proteins. As an example we show here the result on mNT.

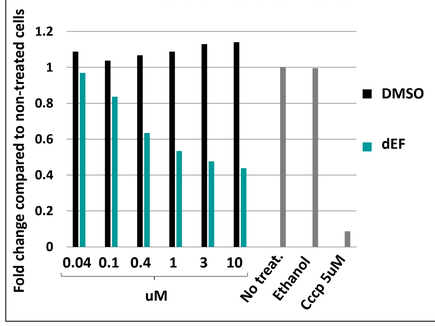
Modulation of Mitochondrial Respiration



- We performed High-resolution respirometry studies on A549 cells (cellular oxygen consumption in a closed-chamber system).
- We observe an inhibition of routine respiration and spare respiratory capacity (+CCCP).
- ENYO's molecule inhibits the ability of a cell to enhance oxidative phosphorylation upon stimulation (as induced by metabolic, oxidative or other cellular stress).

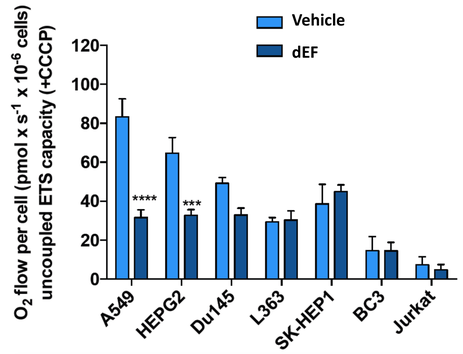
Inhibition of Electron Transport Chain Complex 1





- Rotenone dependent NADH-ubiquinone oxido-reductase activity rate is measured spectrophotometrically on cell homogenate and complex I specific function is verified using rotenone, a specific Complex 1 blocker. A significant inhibition of respiratory chain complex 1 was seen.
- TMRM localizes in mitochondria and detects mitochondrial membrane depolarization. We measured TMRM incorporation by flow cytometry and observed that our molecule induces a dose-dependent decrease in mitochondrial membrane potential. This effect is associated with a decrease in intracellular ATP (not shown) as expected in condition of complex 1 inhibition.

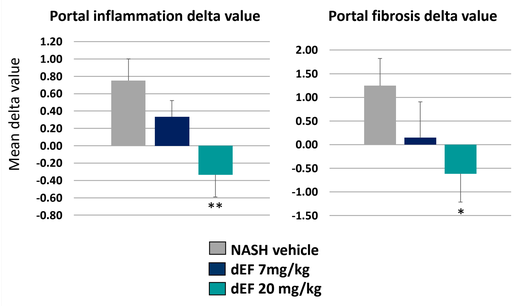
High OxPhos Cell Lines Are More Sensitive To ENYO's Molecule



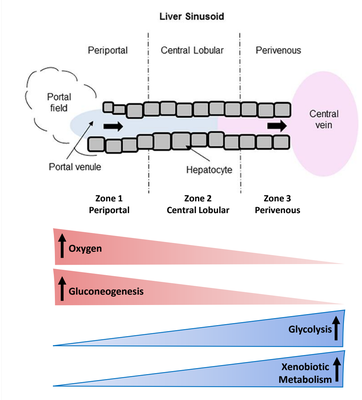
- Evaluation of the bioenergetic modulation effect of dEF was performed on seven human cancer cell lines. This analysis revealed that dEF alters stimulated mitochondrial respiration in cells with the highest respiratory capacity (as revealed in the presence of an uncoupler).
- This result suggests cells most reliant upon inducible reserve respiratory capacity (OxPhos^{+}) may be more sensitive to the effects of ENYO's molecule

Reduction of Inflammation and Fibrosis *in vivo*

Portal inflammation and fibrosis in diet-induced NASH mice



- Mice were fed with a high-fat diet for 30 weeks and then treated by oral administration, twice daily with compound for 8 weeks. Effects were determined by comparing liver biopsy pathology immediately before treatment and upon study completion.
  - Inflammation is graded by number of H&E positive inflammatory foci per field.
  - Fibrosis is graded by image analysis and quantification of PSR positive staining in the region 100 µm from the portal area.
- Therapeutic use of ENYO's molecule resolves portal inflammation and fibrosis following 8 weeks of treatment in a NASH mouse model.



From Tomlinson et al, Front. Bioeng. Biotechnol., 18 February 2019

CONCLUSION

ENYO's lead molecule is well tolerated *in vivo* and resolves inflammation and fibrosis in a NASH mouse model. Improved pathology is biased to the hepatic periportal zone which is perfused with blood with highest oxygen content. We propose hepatocytes, pro-inflammatory & pro-fibrotic cells in the well-perfused periportal zone are most sensitive to ENYO's molecule by virtue of their OxPhos^{+} status and metabolic inflexibility.

ENYO's lead series offers a novel approach to regulate the activity of a family of proteins of critical importance in mitochondrial biology. It may offer new perspectives in the treatment of NASH and other diseases with an important inflammatory component.