NPC1 gene reintroduction rescues cell death in *Npc1*$^{-/-}$ MEFs

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Introduction

Autophagy is an intracellular mechanism involved in degradation of damaged or dysfunctional proteins. A failure in autophagy results in cellular toxicity linked to several neurogenerative disorders. Niemann-Pick disease (NPC) is an autosomal recessive lipid storage disorder, caused by the mutation of *NPC1* gene, which presents with mitochondrial dysfunction and stalled autophagy [1]. The protein encoded by the *NPC1* gene is an important cellular transporter and mutations lead to accumulation of glycolipids. In my research project the correlation between the NPC1 protein and cell death is investigated by assessing the levels of autophagy and cell death related proteins upon culture in galactose medium. Metabolism of galactose to pyruvate yields no net ATP, forcing MEF cells to rely on oxidative phosphorylation (OXPHOS), whereas the production of pyruvate from glucose yields 2 net ATP molecules[2]. Indeed, switching *NPC1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) from glucose to galactose medium shows increased oxygen consumption, followed by the induction of autophagy[3]. It is important to remember, that NPC1 disease results from the gene mutation, whereas the mouse model cell line used in this experiment has the whole gene knock-out (KO).

Aims

- Compare the behaviour of cells under two different conditions (glucose/ galactose environment).
- Provide evidence that introduction of the gene into the KO mouse cells restores autophagy and prevents cell death.

Methods

Tissue culture

Cell splitting	Cell seeding	Cell treatment

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Laboratory

Cell lysis	Sample preparation	Western blotting

↓

Office

Data analysis

Results

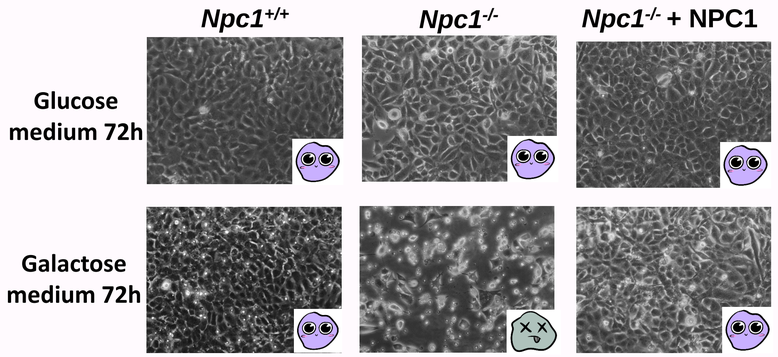

Figure 1. Capturing cell death after 72 hours of treatment. In galactose media elevated cell death is seen in *Npc1*$^{-/-}$ compared to *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ + NPC1. In glucose media all three cell lines show the same level of survival.

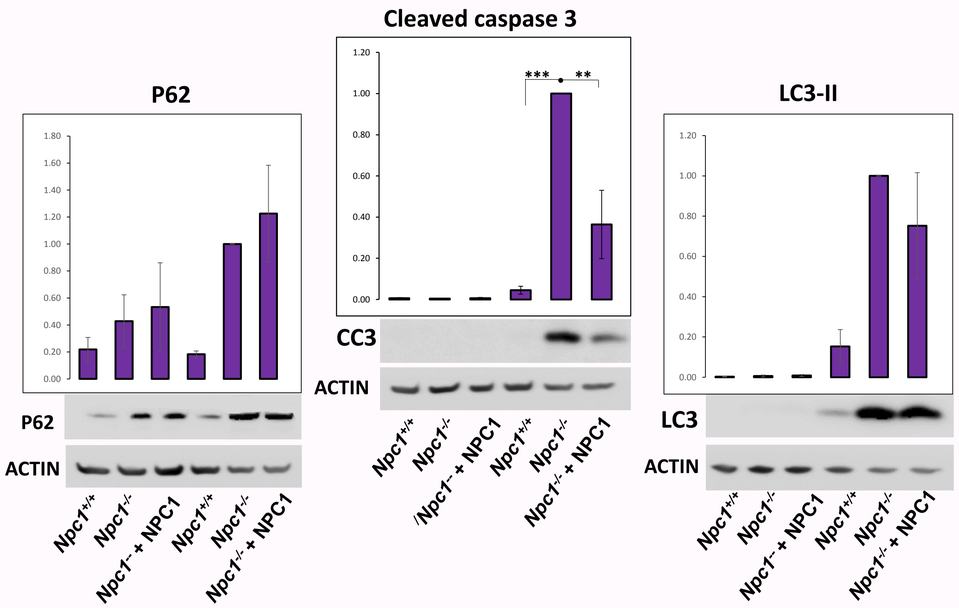

Figure 2. Immunoblot analysis of protein levels with anti-p62 (autophagy), anti-cleaved caspase 3 (cell death) and anti LC3 (autophagy) antibodies in Npc1$^{+/+}$, Npc1$^{-/-}$ and Npc1$^{-/-}$ + NPC1 MEFs cultured in glucose and galactose medium.
Graphical mean +/- SEM, p>0,001 (***) , p>0,01 (**) , p>0,05 (*).

Conclusions

- *NPC1*$^{-/-}$ cells do not survive in galactose medium when forced to respire through mitochondria
- p62 accumulates in *NPC1*$^{-/-}$ due to blockage of autophagy
- LC3 levels appear to increase in *NPC1*$^{-/-}$ when the autophagy is induced by culture in galactose medium
- Reintroduction of NPC1 rescues cell death shown by the immunoblot analysis
- Cell death rescue can be observed by significantly lower levels of cleaved caspase 3 in *NPC1*$^{-/-}$ +NPC1 MEFs.
- Reintroduction of NPC1 does not affect the levels of p62
- Levels of p62 show similar trends in both glucose and galactose media

Discussion

- Galactose media is known to be an excellent model of detecting mitochondrial dysfunction as it enhances mitochondrial metabolism. In this experiment it was used to show how the stalling of autophagy affects a change in cell metabolism and cellular fitness.
- Additionally, reintroduction of NPC1 resulted in increased fitness of autophagy induction in *NPC1*$^{-/-}$ + NPC1 MEFs. It was shown that the reintroduction of NPC1 shows a trend of autophagy restoration and rescues cell death. From Figure 1 it appeared that the reintroduction of the gene had an effect on autophagy through lower, though not significantly, LC3 levels.
- Unfortunately, the length of the project might have affected the levels of autophagy restoration. Further work should be done to investigate if a longer culture and antibiotic selection would lead to full restoration of autophagy in the *NPC1*$^{-/-}$ +NPC1 MEFs.

References

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3. Robinson BH, Petrova-Benedict R, Buncic JR, Wallace DC. Nonviability of cells with oxidative defects in galactose medium: a screening test for affected patient fibroblasts. Biochem Med Metab Biol. 1992;48:122–126.

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