The effect of SMN deficiency in lysosomal and mitochondrial activity

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INTRODUCTION

Spinal Muscular Atrophy (SMA) is a neurodegenerative disease that affects lower motor neurons, and in most cases leads to death. The frequency of this pathology is 1 in 6000 people with 2% of the global population being a carrier. This disorder is caused by mutations in the SMN1 gene leading to the decrease of the amount of Survival of Motor Neurons (SMN) protein, which is directly associated with motor neuron survival.

Studies show that deficiency in SMN protein impairs the functionality of several cellular organelles, which leads to cell death.

The main goal of our project is to investigate if SMN plays a major role in controlling lysosomal and mitochondrial homeostasis contributing to the pathology of SMA.

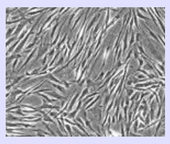
OBJECTIVE

To see how lysosomal and mitochondrial activities are affected in two SMA cellular models.

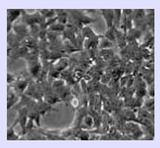
METHODS

MODEL SYSTEMS

Fibroblasts
(Human skin cells)



HEK293T cells7
(Human embryonic kidney)



FLOW CYTOMETRY & IMMUNOFLUORESCENCE

To measure lysosomal and mitochondrial activity, we used these dyes:
- Lysotracker: stains lysosomes
- Lysosensor: stains lysosomes, the brighter the more acidic they are (healthy)
- Mitotracker: stains all mitochondria
- JC-1: stains mitochondria. Dual marker: the healthier, the redder because JC-1 aggregates inside.

RNA INTERFERENCE & WESTERN BLOT

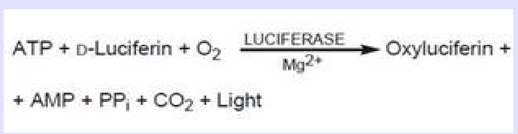
- To reduce SMN mRNA translation in HEK293T cells to generate an induced, accute SMA model.
- To quantify protein concentrations and separate them based on molecular weight in different cell compartments and SMA models.

CELLULAR FRACTIONATION

Separate proteins from the nucleus and cytoplasm to identify the localization of transcription factor involved in lysosomal activity (TFEB).

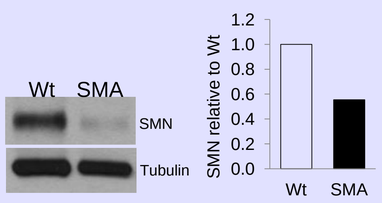
ATP QUANTIFICATION

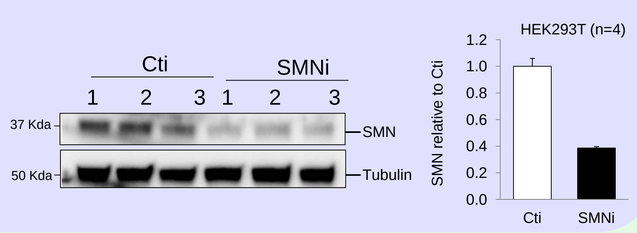
To measure ATP production by mitochondria



OUR MAIN MODEL SYSTEMS

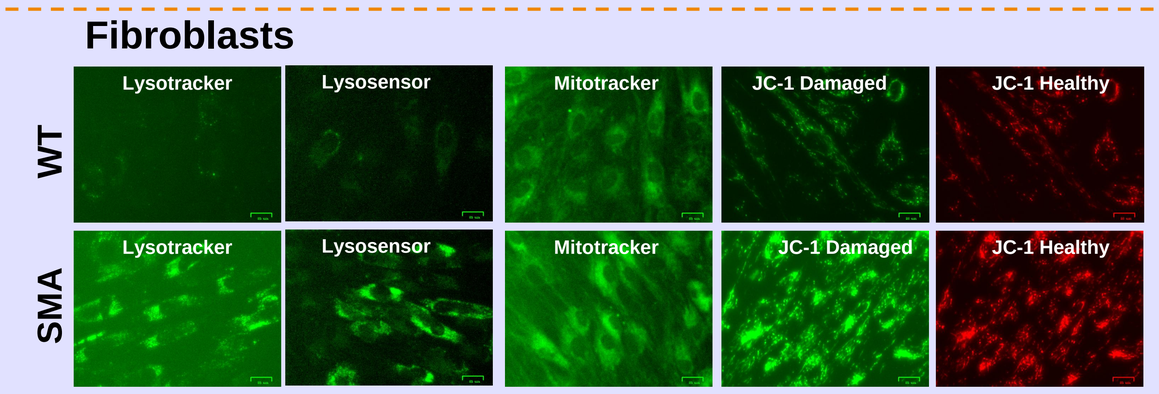
1 Healthy and diseased fibroblasts **2** Control and SMN knock-down (KD) HEK293T cells





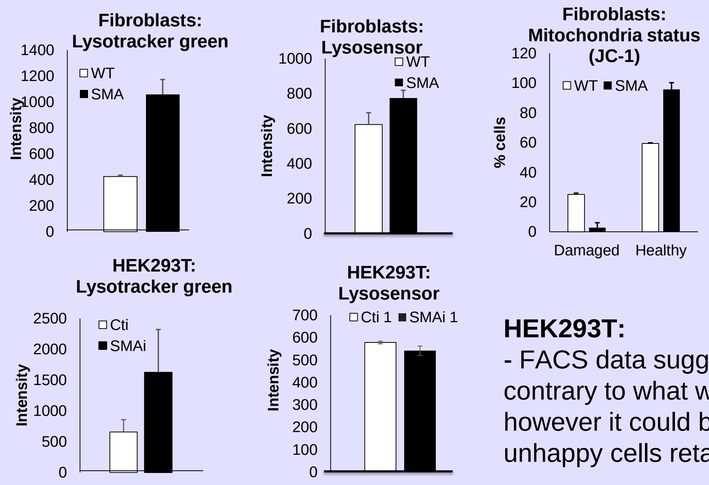
RESULTS

1. Changes in mitochondrial and lysosomal homeostasis determined by immunofluorescence and FACS.



Fibroblasts:
- Higher amounts of lysosomes and more acidic in SMA than Wt.
- No clear differences in the mitochondrial mass, but maybe mitochondria are more active in SMA (JC-1 signal).

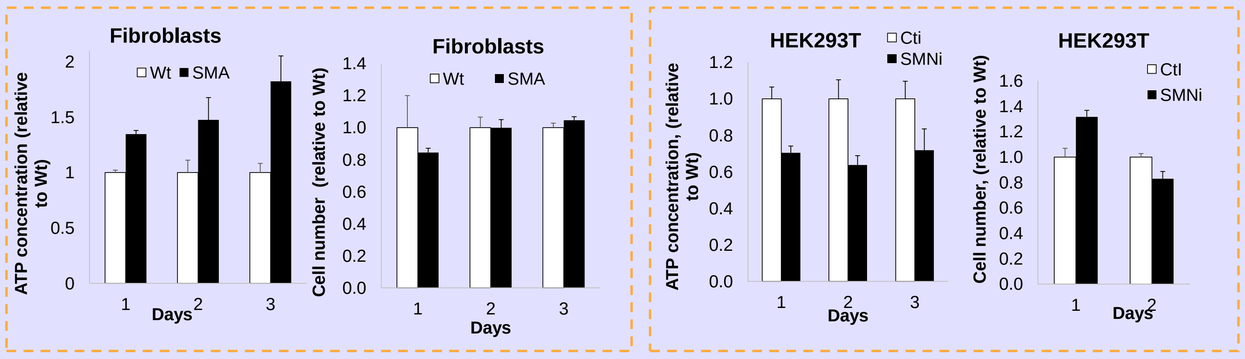
HEK293T:
-Less number of lysosomes in SMNi compared to Cti but probably more acidic.
- No clear changes in mitochondrial mass.



Fibroblasts:
- Increased intensity of Lysotracker signal (either more lysosomes or bigger) in SMA vs control. Also higher acidification, confirming results obtained by immunofluorescence.
- Higher percentage of healthier mitochondria (higher mitochondrial membrane potential) in SMA compared to control.

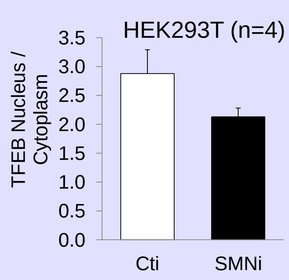
HEK293T:
- FACS data suggest higher lysosomal mass/size in SMNi than Cti, contrary to what we have seen by immunostaining (see above), however it could be due to the brightest cells seen in Ct (possibly unhappy cells retained in the Ct condition but lost in SMNi).

2. SMN-deficient fibroblasts show increased ATP levels compared to control.



Fibroblasts: Higher amounts of ATP in SMA compared to Control despite the similar number of total cells at all time points studied.
HEK293T: Inconclusive results because even though it seems to be a decrease in ATP in SMNi compared to Cti it may be due to a very different number of cells between conditions and technical complications at counting cells numbers.

3. SMN-deficient cells show decreased nuclear localization of the transcription factor TFEB –master regulator of lysosomal gene expression– compared to control.



Successful cellular fractionation (nuclear membrane protein LaminB1 only present in nuclear fraction and cytoplasmic protein GAPDH highly enriched in cytoplasmic fraction).

CONCLUSIONS

- We have obtained different results for fibroblasts and HEK293T cells, maybe due to the fact that fibroblasts are a natural SMA model, whereas the SMA model in HEK293T cells must be induced, and is therefore subjected to a higher variability.
- We have been able to show that lysosomal mass, mitochondrial mass and activity are enhanced in SMA cells increased as compared to the control by multiple techniques (Immunostaining, FACS and ATP measurements).
- The already known defects in autophagy activity might be the cause of higher amount and acidic lysosomes, and an enlarged mitochondrial activity possibly to compensate this metabolic defect.
- The defect in the nuclear TFEB translocation upon SMN deficiency may be the responsible factor for all of these irregularities.