Role of SMN in organelle homeostasis and response to stress

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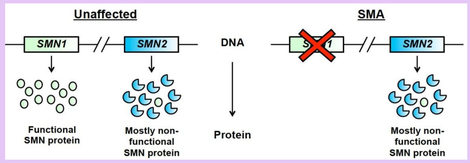
BACKGROUND

Studying of Spinal Muscular Atrophy (SMA)
Изучение Спинальной Мышечной Атрофии (СМА)

- SMA is a neurodegenerative disease caused by a gene mutation. It is the most common genetic cause of infant mortality.
- SMA patients have decreased levels of SMN protein, which functions in RNA splicing and axon growth.
- СМА – это генетическое нейродегенеративное заболевание, вызванное отсутствием белка SMN, отвечающего за сплайсинг и рост аксонов.



- The most sensitive to the lack of SMN cells are motor neurons
- The mechanism of neuron death is unknown.
- В первую очередь от понижения уровня SMN страдают моторне нейроны. Механизм данного явления не изучен.

Main Aim: to examine the pattern of survival and death in SMN-deficient cells under different stress conditions.
Цель проекта: изучить влияние SMN на выживаемость и смертность клеток при разных условиях.

MATERIALS AND METHODS

Techniques used

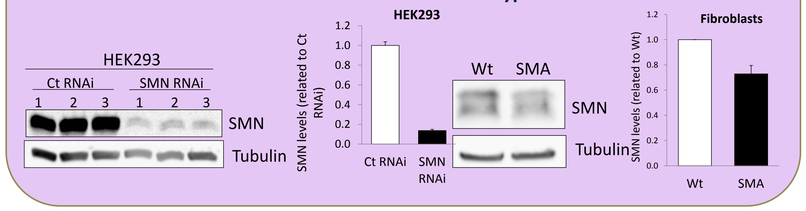
- **RNA interference:** to generate a HEK SMA model.
- **Western blot:** to confirm lower SMN expression in SMA fibroblasts and validate the knock-down of SMN after RNAi.
- **ATPlite:** to measure cell viability as a function of intracellular ATP level.

$$ATP + D\text{-Luciferin} + O_2 \xrightarrow[Mg^{2+}]{\text{LUCIFERASE}} \text{Oxyluciferin} + \text{+ AMP + } PP_i + CO_2 + \text{Light}$$

- **Fluorescence Microscopy:** to observe the morphology and distribution of mitochondria and lysosomes.

Cell models: HEK293 cell line (WT and RNAi) and Fibroblasts from patients (WT and SMA).

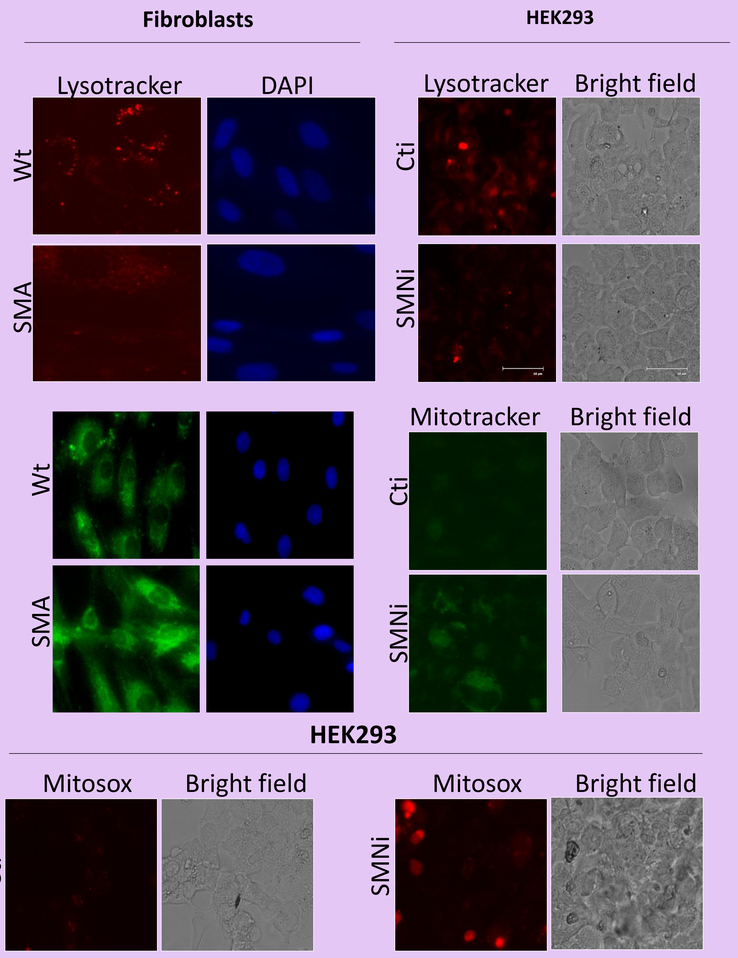
Decreased levels of SMN after RNAi
РНК интерференция уменьшает уровень SMN



SMA fibroblasts show lower SMN levels than Control as expected
У фибробластов с SMA подтвержден низкий уровень белка SMN

RESULTS

Figure 1 . Lack of SMN affects lysosomes and mitochondria
Отсутствие SMN влияет на лизосомы и митохондрии

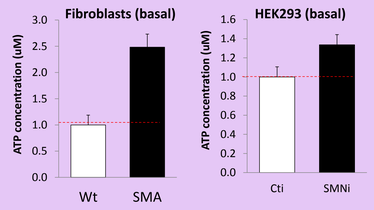


- Fewer and smaller lysosomes upon SMN deficiency.
- Higher number of mitochondria in SMA cells.
- Increased levels of mitosox in SMA cells, suggesting mitochondrial damage.
- Лизосомы редкие и маленькие. Митохондрий больше, но они подвергнуты оксидации

CONCLUSIONS

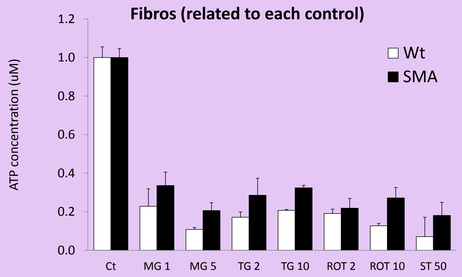
SMN deficiency results in a higher mitochondrial activity combined with mitochondrial damage. SMN deficient cells are more vulnerable to certain stressors. A reduced number of lysosomes in SMA cells may indicate a defect in autophagy (especially mitophagy), decreasing their viability.
Отсутствие SMN вызывает аккумуляцию поврежденных митохондрий и уменьшение количества лизосом.

Figure 2. SMN-deficient fibroblasts and HEK cells have more ATP
Отсутствие SMN в фибробластах и HEK293 увеличивает АТФ



Stressors: to mimic pathological conditions observed *in vivo.*

Compound	Mechanism of cell death
MG132 (MG)	inhibit proteasome
Thapsigargin (TG)	ER stress (Ca++)
Rotenone (Rot)	mitochondria
Stausporine (St)	Calpain

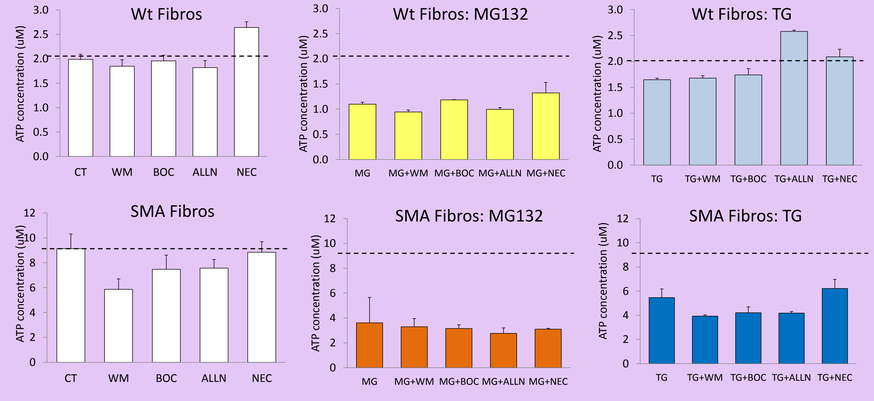


- SMN deficient cells show higher ATP levels compared to control under basal conditions and upon exposure to different stressors.
- АТФ увеличивается в нормальных и стрессовых условиях когда нет SMN.

Figure 3. Cell death inhibitors (ATPlite) to dissect the cell death pathway that cells undergo upon stress in absence of SMN.

Specific inhibitors of cell death pathways: to elucidate the type of cell death in the absence of SMN.

Inhibitors	Cell death pathway targeted
Necrostatin (Nec)	Inhibits necroptosis
ALLN	Inhibits apoptosis and necrosis
BOC	Inhibits apoptosis
Wortmanin (WM)	Inhibits autophagy



- MG132 induces a similar decrease in ATP levels in SMA and in control cells, and cannot be rescued by any tested inhibitor. Whereas TG induces a decrease in ATP that can be rescued by ALLN and NEC and is not rescued in SMA.
- MG132 уменьшает АТФ и эффект не убирается ингибиторами. TG уменьшает АТФ но эффект убирается ALLN и Nec только в здоровых клетках (но не в SMA).