ATM KO HUMAN NEURAL PROGENITOR CELLS AS A VALUABLE CELLULAR PLATFORM FOR IDENTIFYING NOVEL DRUG TARGETS IN ATAXIA-TELANGIECTASIA

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BACKGROUND

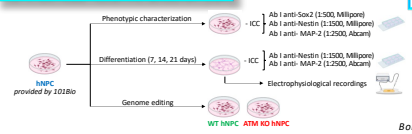
Ataxia-telangiectasia (A-T) is an autosomal recessive multi-organ rare disease characterized by early childhood onset. Clinically, A-T is marked by progressive cerebellar atrophy, cognitive impairment, telangiectasia, immunodeficiency and predisposition to cancer development (1). A-T is caused by mutations in the Ataxia-Telangiectasia Mutated (ATM) gene, located on chromosome 11q22-23, which encodes for ATM, a serine/threonine protein kinase that phosphorylates a plethora of substrates implicated in DNA damage response (DDR), regulation of response to oxidative stress and autophagy (2). The main neuropathological signs include loss of cerebellar Purkinje and granule neurons (3).

AIM

Although the role of ATM has been widely described in A-T patients and murine models, molecular mechanisms of neural/neuronal dysfunction associated with this rare disease still deserve further investigation.
Since **a specific treatment is not available yet**, the goal of this project is to identify novel cellular and molecular pathways which may be disrupted in absence of ATM in human iPSC-derived NPC and its neuronal progeny and to propose novel potential targets for pharmacological interventions.

METHODS

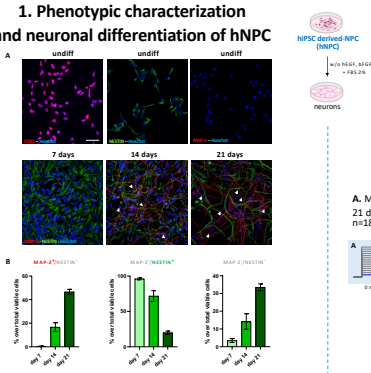
Our *in vitro* model: human iPSC derived-NPC (hNPC)



Bortolotto V. et al., 2017 (4)

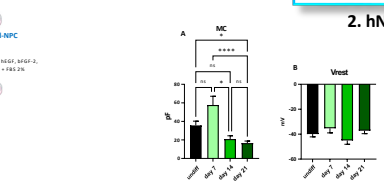
RESULTS

1. Phenotypic characterization and neuronal differentiation of hNPC

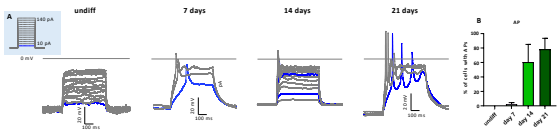


A. Representative confocal images for Sox2 (red), nestin (green) and MAP-2 (red) of hNPC; nuclei were stained with Hoechst (blue); obj 40x; scale bar 20 µm. **B.** Data are expressed as mean ± SD of n=2 experiments run in triplicates.

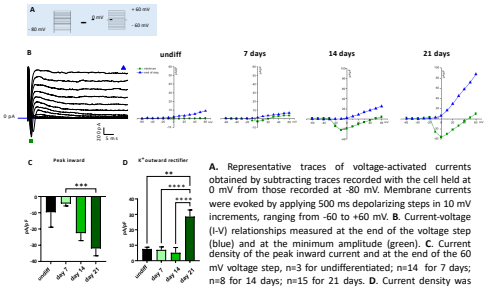
2. hNPC are able to generate functionally active neurons



A. Membrane capacity (MC). Error bars represent SEM, n=40 for undifferentiated; n=26 for 7 days; n=12 for 14 days; n=24 for 21 days, Kruskal-Wallis test, * p < 0.05; **** p < 0.0001. **B.** Resting potential (Vrest), n=42 for undifferentiated; n=28 for 7 days; n=18 for 14 days; n=26 for 21 days, Kruskal-Wallis test.

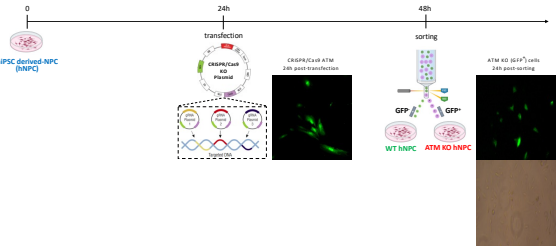


A. Current-clamp recordings. Cells were held around −65 mV. **B.** Bar plot showing the percentage of cells generating at least one evoked action potential (AP). Data are represented as mean ± SEM, n represents distinct cultures: n=2 for undifferentiated; n=4 for 7 days; n=3 for 14 days; n=3 for 21 days.



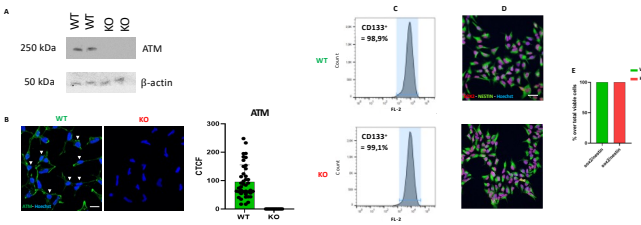
A. Representative traces of voltage-activated currents obtained by subtracting traces recorded with the cell held at 0 mV from those recorded at -80 mV. Membrane currents were evoked by applying 500 ms depolarizing steps in 10 mV increments, ranging from -60 to +60 mV. **B.** Current-voltage (I-V) relationships measured at the end of the voltage step (blue) and at the minimum amplitude (green). **C.** Current density of the peak inward current and at the end of the 60 mV voltage step, n=3 for undifferentiated; n=14 for 7 days; n=8 for 14 days; n=15 for 21 days. **D.** Current density was calculated by normalizing the currents to cell capacitance, n=23 for undifferentiated; n=21 for 7 days; n=10 for 14 days; n=17 for 21 days, Kruskal-Wallis test, ** p = 0.0032; *** p = 0.0005; **** p < 0.0001.

3. A CRISPR/Cas9 KO strategy to generate ATM KO cellular model



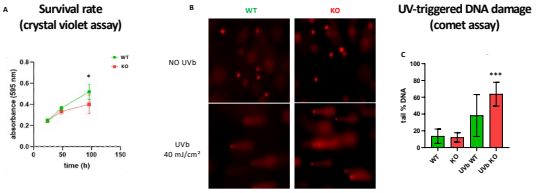
Schematic representation of the experimental steps to generate, by genomic editing, human iPSC derived-NPC (hNPC) with ATM gene knock out and their wild type counterpart.

4. Validation and phenotypic characterization of ATM KO hNPC



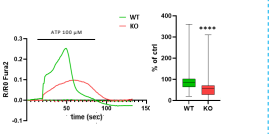
A. Representative images of western blots using WT and ATM KO hNPC. **B.** WT and ATM KO hNPC representative confocal images for ATM immunoreacticity (green); nuclei were counterstained with Hoechst (blue); obj 63x; scale bar 20 µm **C.** Cytofluorimetric analysis for CD133 expression in WT and ATM KO hNPC. **D.** Representative confocal images for Sox2 (red) and nestin (green) of WT and ATM KO hNPC; nuclei were counterstained with Hoechst (blue); obj 40x; scale bar 20 µm. **E.** Data are expressed as mean ± SD of n=2 experiments run in triplicates.

5. ATM KO hNPC recapitulate canonical defects associated with ATM dysfunction

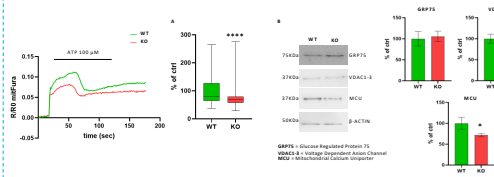


A. Data are expressed as mean ± SD of n=2 experiments run in triplicates and analyzed by Student's *t* test, * p < 0.05 vs WT. **B.** Representative images of a comet assay where UVb-exposed WT and ATM KO hNPC were stained with propidium iodide (red), obj 20x. **C.** Quantitative analysis of comet assay: data are expressed as mean ± SD of n=40 cells from n=1 experiment run in triplicates and analyzed by One way Anova, Tukey *post-hoc*, *** p < 0.001 vs WT.

6. ATM KO hNPC display reduced cytosolic Ca^{2+} signalling and mitochondrial Ca^{2+} up-take



Data are expressed as mean ± SD of 203 WT and 203 ATM KO hNPC from n=3 experiments run in triplicates and analyzed by Student's *t* test, **** p < 0.0001 vs WT.



A. Data are expressed as mean ± SD of 187 WT and 213 ATM KO hNPC from n=3 experiments run in triplicates and analyzed by Student's *t* test, **** p < 0.0001 vs WT. **B.** Representative images and densitometry analysis of western blots in WT and ATM KO hNPC. Data are expressed as mean ± SD of n=3 experiments and analyzed by Student's *t* test, * p < 0.05 vs WT.

SUMMARY

- ✓ hNPC are able to generate functionally active neurons, whose electrophysiological properties can be measured;
- ✓ By CRISPR/Cas9 technology we generated ATM KO hNPC;
- ✓ ATM KO gene does not affect expression of stem cell markers (Sox2, nestin, CD133);
- ✓ As expected, ATM KO hNPC show reduced survival rate and higher sensitivity to UVb-induced DNA damage compared to WT hNPC;
- ✓ ATM KO hNPC display reduced cytosolic and mitochondrial Ca^{2+} signalling;
- ✓ Preliminary data suggest reduced MCU protein levels in ATM KO hNPC.

CONCLUSIONS & FUTURE PERPECTIVES

ATM KO hNPC and their neuronal progeny (under evaluation) may represent **valuable cellular platforms** for better understanding molecular mechanisms associated with ATM dysfunction and for **drug screening activities**.

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4. Bortolotto V. et al. Proneurogenic Effects of Trazodone in Murine and Human Neural Progenitor Cells. *ACS Chem Neuroscience*. 2017.

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