Cardiac modeling of desmin related myofibrillar myopathy in vitro and assessment of effects on mitophagy

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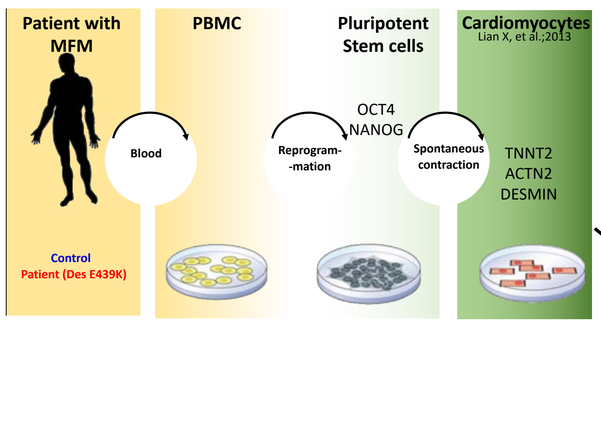
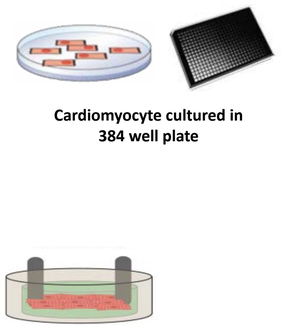
Summary

Mutations of DES, the gene encoding desmin, the major intermediate filament of muscle cells, can lead to Myofibrillar Myopathy (MFM). MFM is a rare disease that is mainly characterized by a loss of muscle mass (muscular dystrophy) but also, in many cases, by a progressive cardiac disease[1,2]. It is well established that desmin is linked to mitochondrial trafficking and, during heart failure, its loss is accompanied by mitochondrial dysfunction[3]. Moreover, it is also known that autophagy is perturbed in desmin-related cardiomyopathy. However, a link between desmin and mitophagy, which could contribute mechanistically to the pathophysiological mechanisms involved in MFM, has not been established. Here we have developed a method to study mitochondrial health and mitophagy. It is based on a 2D model of desmin-related cardiomyopathy : (i) cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs)[4] obtained from patients carrying a DES mutation (E439K) or control, and (ii) an automated image analysis method (in-house software). In a second line of experiments, we employed two 3D models generated from hiPSC-CMs associated with fibroblast - either forming muscle rings[6] or cardiac spheroids[5] - to better mimic the high degree of complexity of the human myocardium.

Workflow

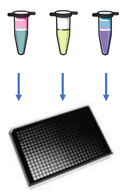
In vitro Disease Modeling

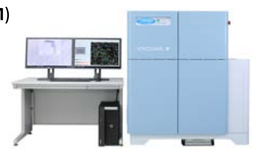




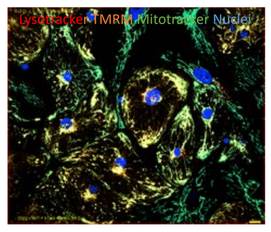
Staining and Automated image acquisition

Live Imaging :
Tetramethylrhodamine, methyl ester (**TMRM**)
MitoTracker^{FM}
LysoTracker™

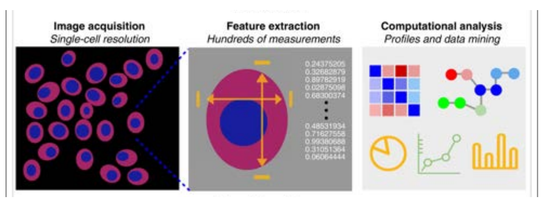




Automated Confocal Microscope

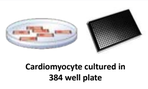


Automated image analysis (in-house software)

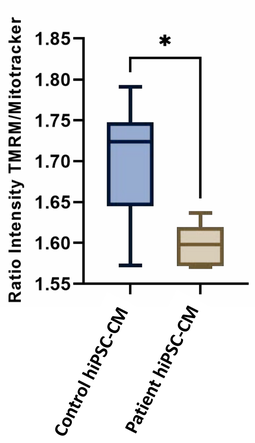


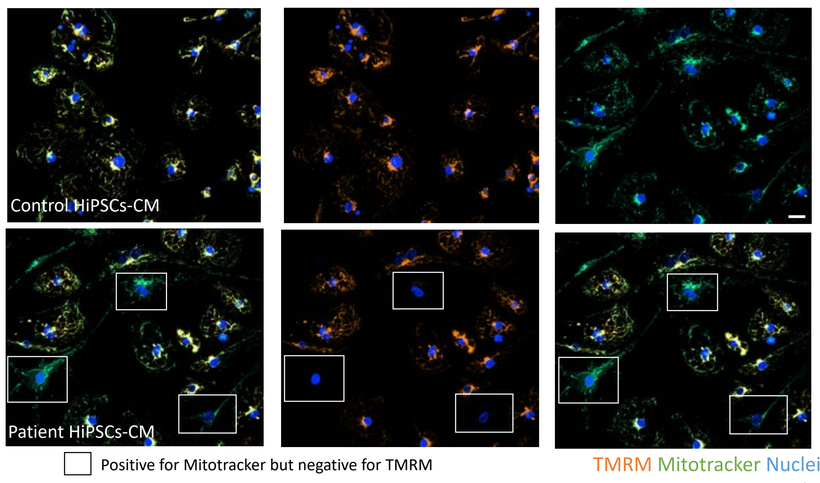
1. Nuclei Identification
2. Cropping
3. Feature extraction (Surface, Intensity, Puncta counting)
4. Downstream analysis (Ratio intensity)
5. Data visualisation (Graph)

Analysis of mitochondrial health by immunofluorescence



Mitochondrial potential membrane analyzed by immunofluorescence





n=6, *, p< 0,1, $paired\ t-test$

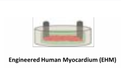
Positive for Mitotracker but negative for TMRM

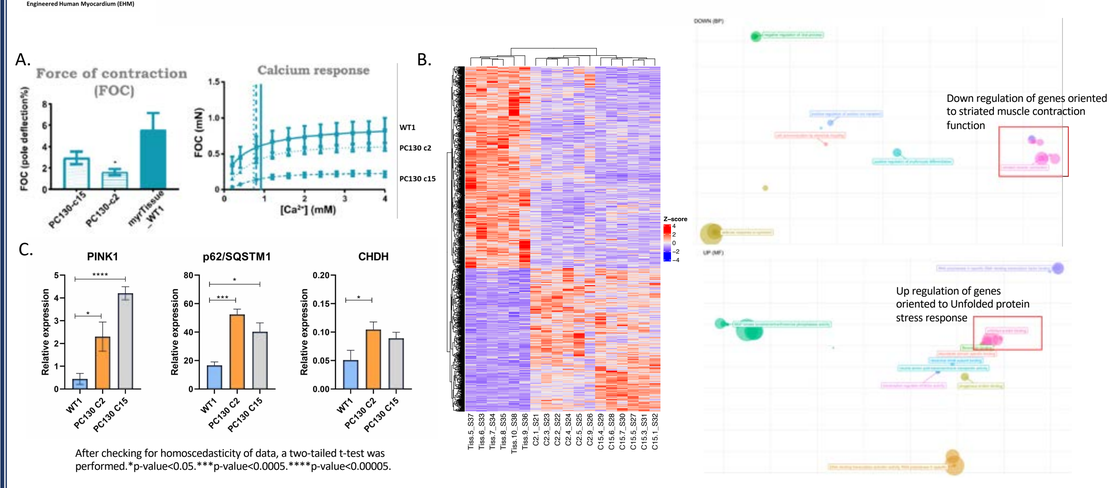
TMRM Mitotracker Nuclei

Scale bar 10μm

Live imaging of cardiomyocytes derived from human pluripotent stem cells (hiPSCs-CM, Patient carrying the mutation DES E439K or control patient) with TMRM (active mitochondria staining) and Mitotracker (All mitochondria staining). The framed cells indicate cells with mostly inactive mitochondria. The intensity ratio of TMRM signal over the Mitotracker signal highlight a defect in mitochondrial health of Patient hiPSCs-CM in comparison with the control.

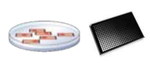
Functional defect and transcriptomic response

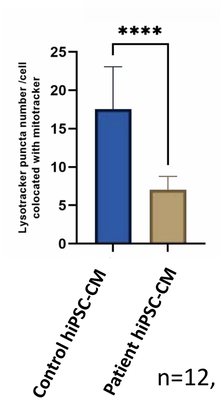


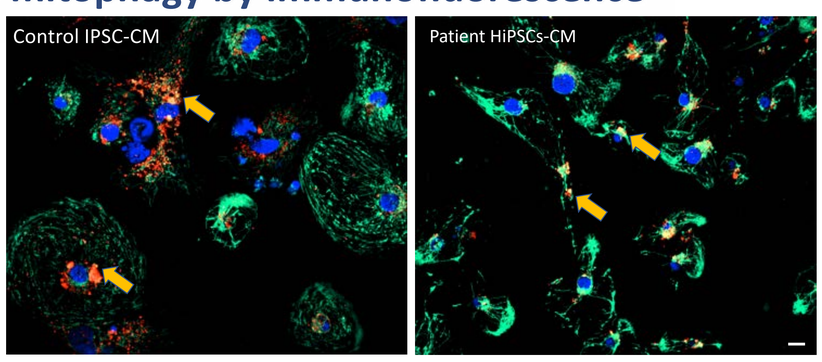


Functional analysis of the force of contraction and transcriptomic response investigation in the 3D model Engineered Human Myocardium for 2 patients lines (PC130C2 and PC130C15) carrying the mutation DES and 1 control (WT1).
A. The functional analysis show a defect in the force of contraction from the patient lines in comparison with the control even with an increase of the calcium concentration. B. RNAseq that highlight a difference in the transcriptomic response between patients and control especially in the expression of gene related to the striated muscle contraction function and the unfolded protein stress response. C. qRT-PCR showing an upregulation in gene related to mitophagy.

Analysis of mitophagy by immunofluorescence







n=12, ****, p< 0,0001, $paired\ t-test$

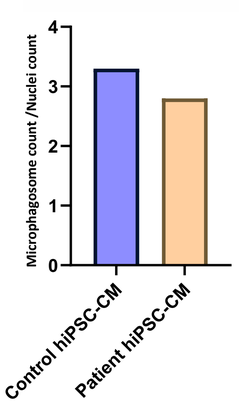
: Mitophagy

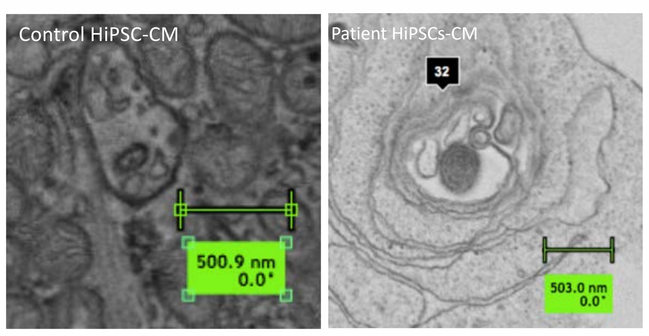
Lysotracker Mitotracker Nuclei

Scale bar 10μm

Live imaging of hiPSC-CM (Patient and control) with Mitotracker (Mitochondria staining) and Lysotracker (Lysosome staining). The counting of the lysotracker puncta into the mitotracker mask allow a measure of the mitophagy system. The yellow arrows indicate mitophagic structures. These results show a defect in the mitophagy from patient hiPSC-CM in comparison with control.

Analysis of mitophagy by electronic microscopy



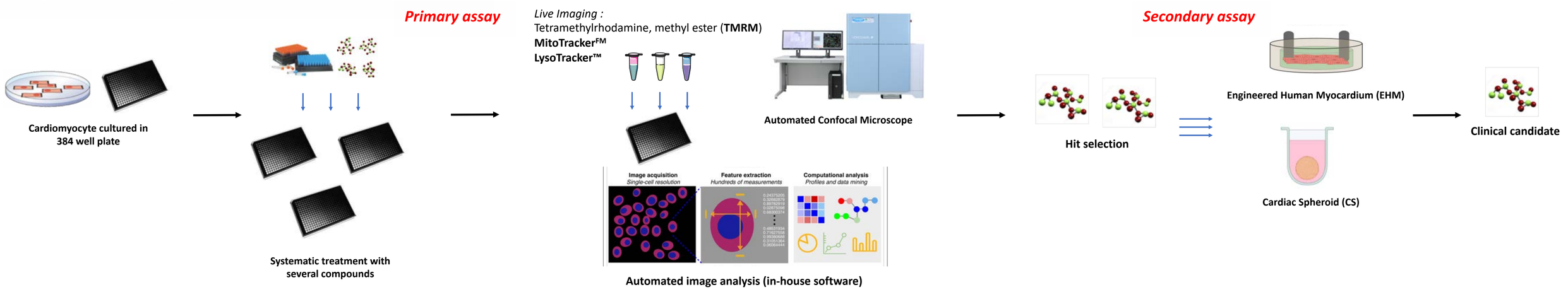


Electronic microscopy of the 3D model cardiac spheroid allow the counting of the mitophagic structure. This result highlight a lower number of mitophagic structure in the patient spheroid.

Conclusion

Our results confirm that *DES* mutations induced a mitochondrial and mitophagy defects that can be measured by an *in vitro* 2D model assay based on a Live imaging with TMRM, Mitotracker and Lysotracker, an automated confocal microscope for the image acquisition and an automated image analysis by in-house software. Indeed, It would be interesting to use this workflow for primary assay in a High content screening context for drugs discovery. Moreover, we showed functional defect on 3D models (you can also See the Poster 067 for complementary data on cardiac spheroid) that can be use for secondary assay in a High content screening for the validation of the selected compounds.

Perspective : High content screening assay



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