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Human fibro-adipogenic precursor cells as a new tool for investigating antifibrotic drugs in muscular dystrophies.

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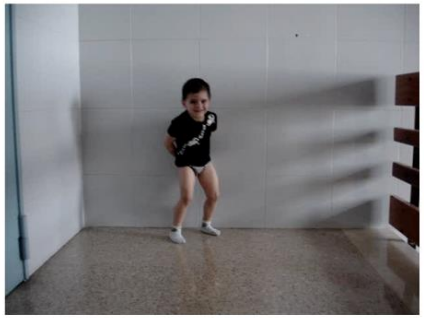
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 777554

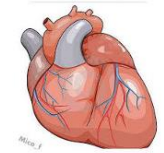
Introduction (I)

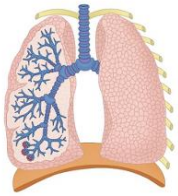
Muscular dystrophies:

-Rare hereditary disorders

-Progressive muscle weakness leading to a variable degree of disability







4-6 years

9-11 years

12-15 years

15-20 years

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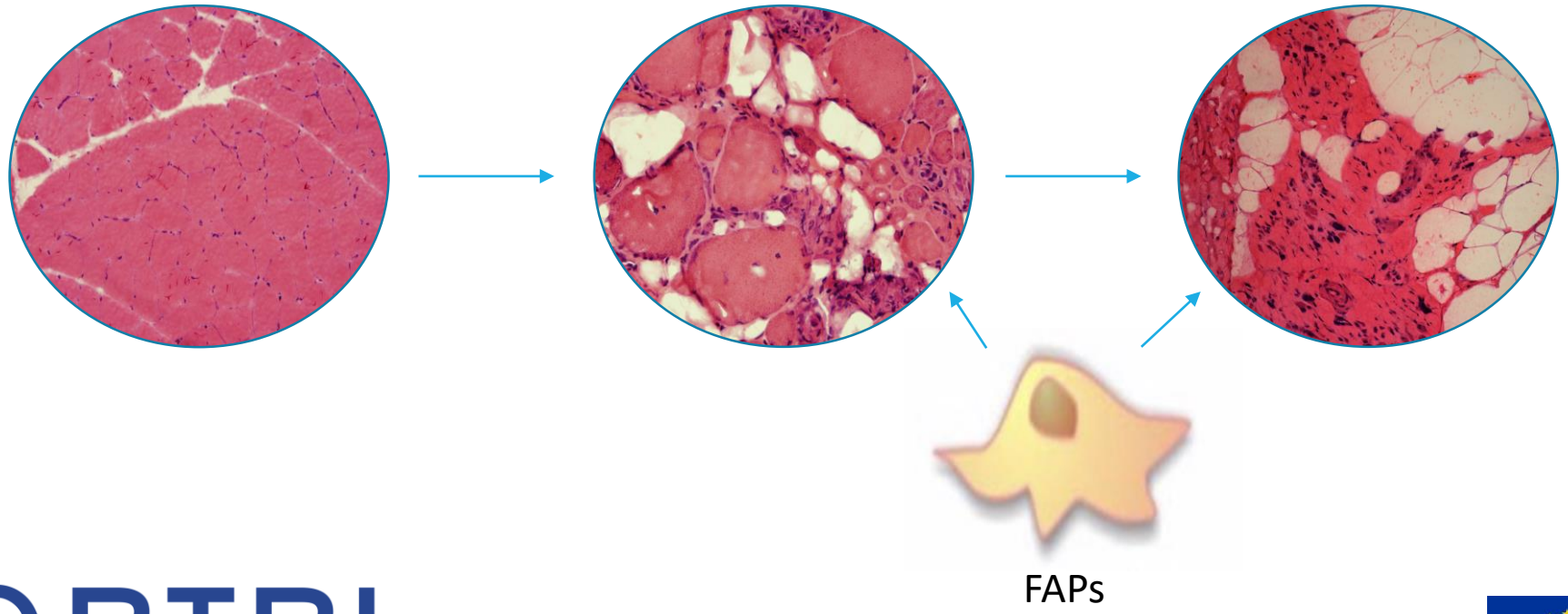
Introduction (II)

Histologic key features:

-Loss of muscular fibers

-Expansion of fibrotic and adipogenic tissue



Introduction (III): FAPs

-FAPs are muscle resident mesenchymal stem cells able to differentiate to profibrotic or proadipogenic cell.

-After an acute muscle damage they activate, proliferate and release components of the extracellular matrix contributing to the regeneration of muscle.

-During chronic muscular damage FAPs are continuously activated leading to fibrosis and fat deposition in skeletal muscles.

Hypothesis and objectives

Human FAPs cells could be a good model to test drugs counteracting its profibrotic and prodadipogenic potential

Objectives:

- 1. To isolate human FAPs from frozen muscle samples
- 2. To compare the differentiation ability of these cells with freshly isolated FAPs.
- 3. To immortalize isolated FAPs for further use in experiments
- 4. To test the ability to decrease FAPs' profibrotic activity using antifibrotic drugs.

Objective I: Isolation of FAPs

Frozen explants

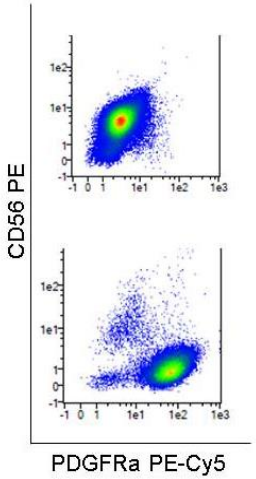
Heatlhy controls

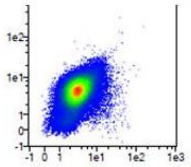
DMD patients

Digestion

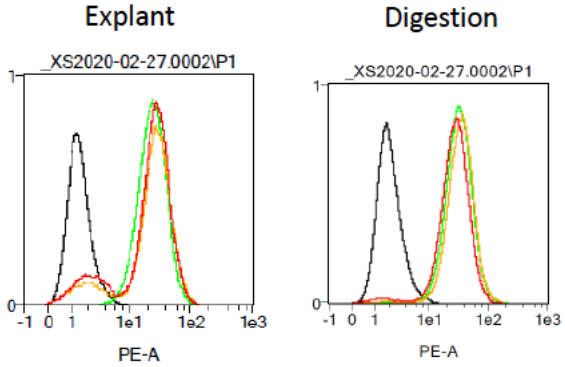
Heatlhy controls

Cytometry





PDGFRc-aplha cells



-Isotype control
-Low passage
-Medium passage
-High passage

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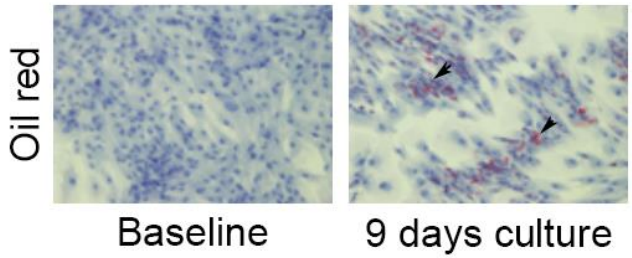
Objective II: Differentiation of FAPs

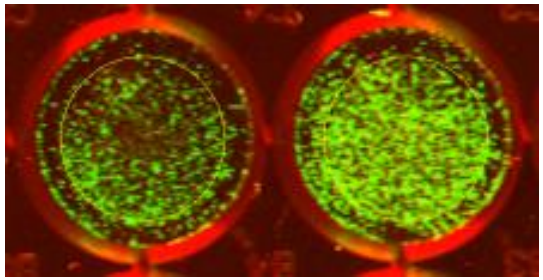
FAPs obtained from frozen explants

Adipogenic differentiation

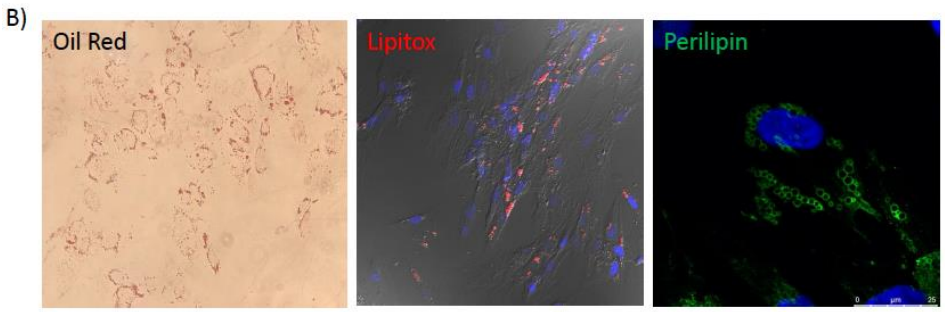


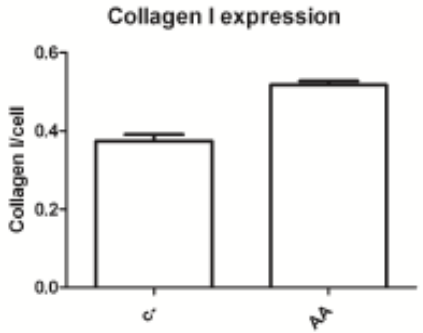
Fibrogenic differentiation

Collagen I



Untreated PDGF-AA





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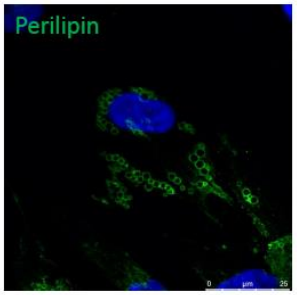
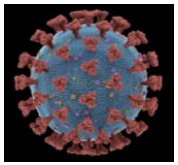
Objective III: Inmortalization of FAPs

Enter senescence after 8-9 passages

Continuous proliferation
Effective differentiation

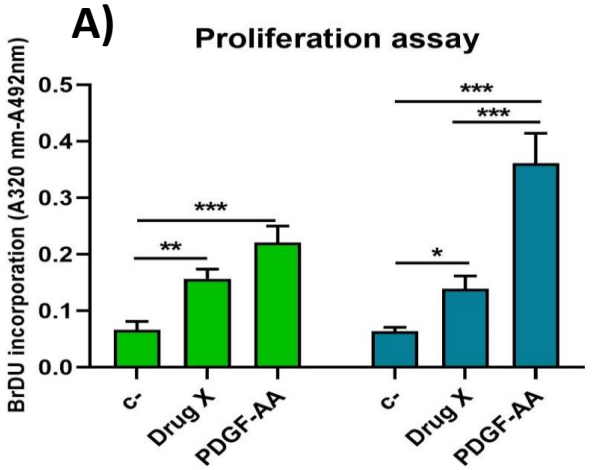




Cyclin-dependent kinase-4 (cdk4)
Telomerase reverse transcriptase (hTERT)

Objective IV: Testing drugs

A)



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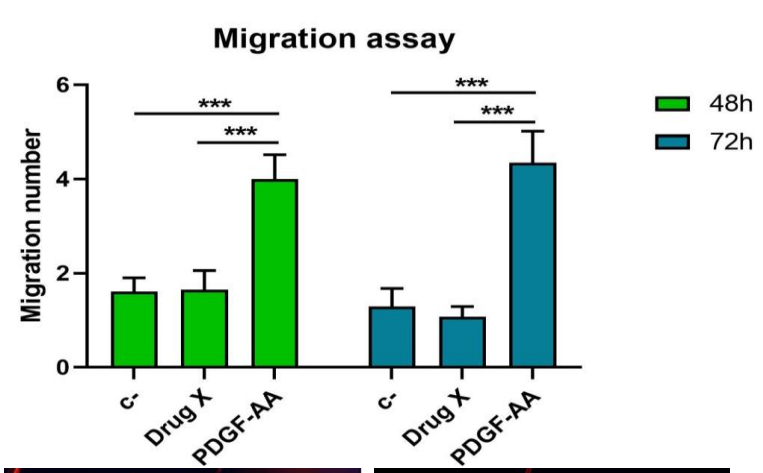
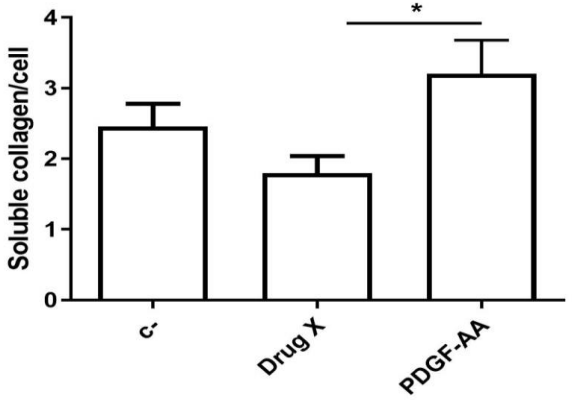
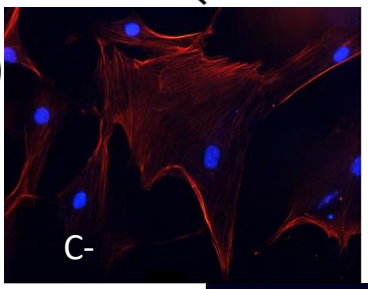
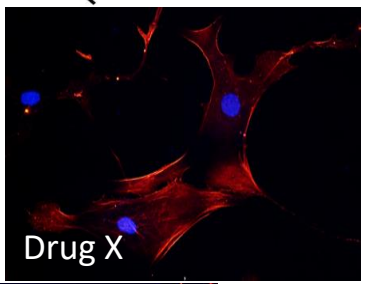


C)

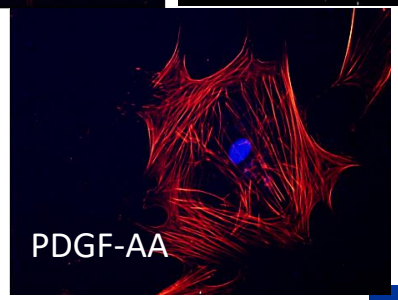


D)





actin



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Conclusions

1. We have been able to develop a protocol to isolate FAPs from already frozen muscle biopsies of patients with MD and controls

2. These FAPs can be immortalized easily and effectively, and therefore can be used in further experiments.

3. h-iFAPs can be used to test the effectivity of antifibrotic and antiadipogenic drugs in vitro analysing their collagen-I expression and their production of fat.

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The views and opinions expressed in the following PowerPoint slides are those of the individual presenter and should not be attributed to EPTRI or the EC

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