Villains and victims – Deciphering the impact of cell- and mutation-type on cardiac fibrosis using hIPSC derived cardiovascular models.

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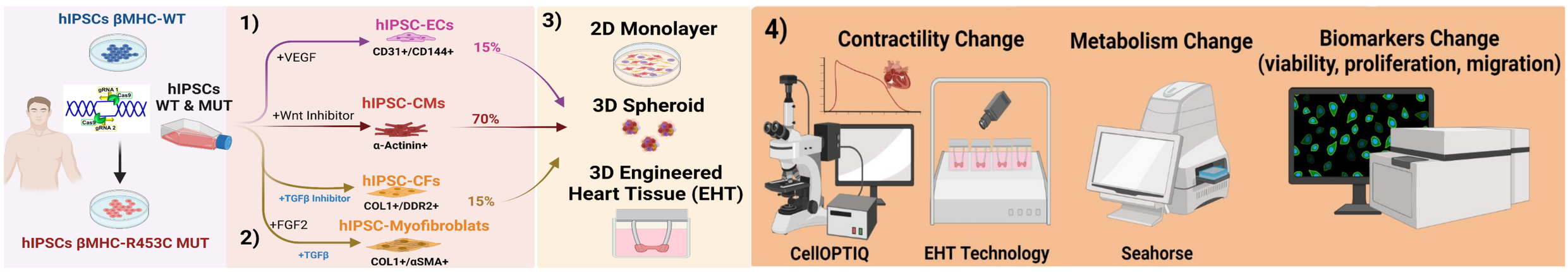
Introduction

Cardiac fibrosis is a significant global health problem, associated with nearly all forms of heart disease, and yet there is no successful therapy. Hypertrophic cardiomyopathy (HCM) is a prevalent genetic cardiovascular disease affecting 1:500 individuals, who exhibit left ventricle thickening and deterioration of cardiac function [1,2]. Although cardiac fibrosis animal model exist, these inadequately model human disease in term of mechanisms, severity and timing, thus limiting translational success.

Modelling cardiac fibrosis *in vitro* using human pluripotent stem cells (hiPSCs) derived models may provide a relevant humanised platform to investigate the disease mechanisms, ultimately enhancing drug discovery efforts. This project aim to develop human physiologically relevant models consisting of the major cell types in the hearts including: cardiomyocytes (CMs), cardiac fibroblasts (CFs) and cardiac endothelial cells (ECs) to study the impact of cell- and HCM mutation-type (R453C in β-myosin heavy chain, βMHC) during cardiac fibrosis progression.

Methods



Workflow overview: 1) Expansion & differentiation of hiPSC wild type (WT), homozygote and/or heterozygote hiPSC mutant (MUT), harbouring βMHC-R453C SNP, into different cardiovascular lineages, including CMs, ECs, CFs; **2)** Control activation of myofibroblast (MFs) by modifying TGFβ signalling; **3)** Generate 2D or 3D cardiovascular tri-linage models **4)** Evaluate phenotypic and functional changes between WT and MUT, including in response to various drug treatment regimes.

Results

1. Serum-free differentiation of hIPSC-ECs and hIPSC-CFs

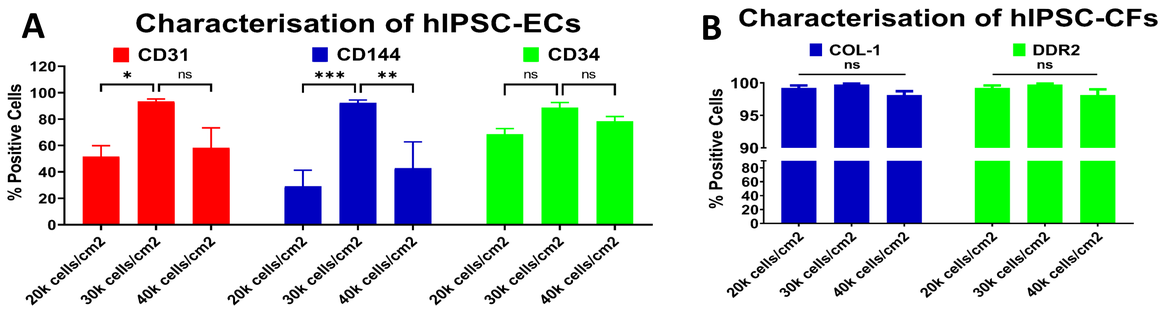


Fig 1. Optimisation of high efficiency hIPSC-ECs and hIPSC-CFs differentiation using a in serum-free protocol. (A) Summary of average hIPSC-ECs differentiation efficiency, EC-specific markers CD31, CD34 & CD144 **(B)** hIPSC-CFs differentiation efficiency, CF-specific markers COL1 and DDR2. N=3 biological replicates.

2. Generation of the tri-linage cardiovascular culture within hIPSC-βMHC-R453C isogenic set using serum-free culture system.

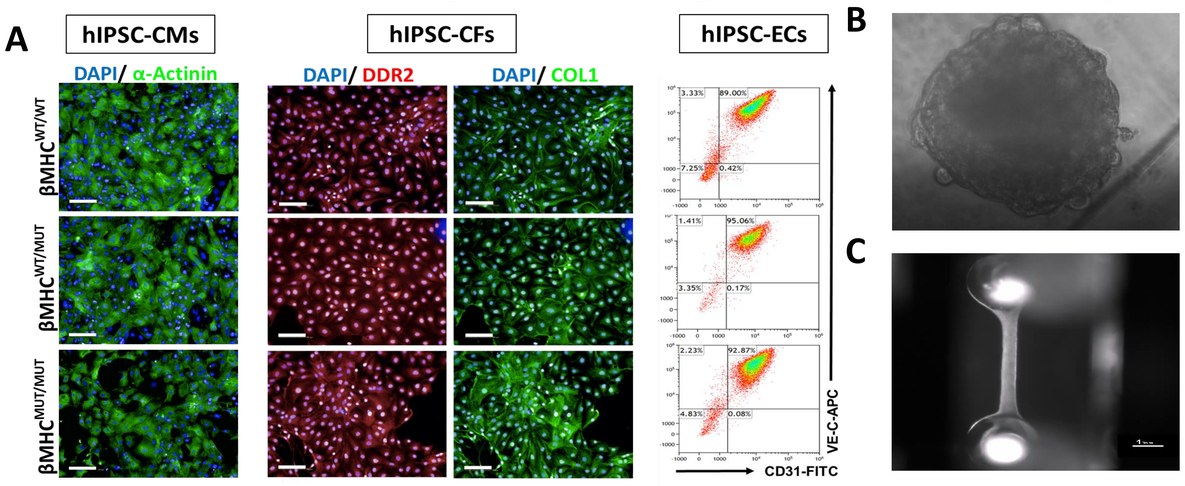


Fig 2. Characterisation of the tri-linage cardiovascular culture within the hIPSC-βMHC-R453C isogenic set. (A) All hIPSCs cell lines exhibited > 90% cells positive to cell type specific markers. Scale bar = 100 µm. Representative flow cytometry plots for EC-specific markers CD31/CD144 (VE-Cadherin). (**B,C**) Representative images 3D formation of hPSC-CMs spheroid and mini EHTs

3. Control transactivation of hIPSC-MFs via TGFβ signalling

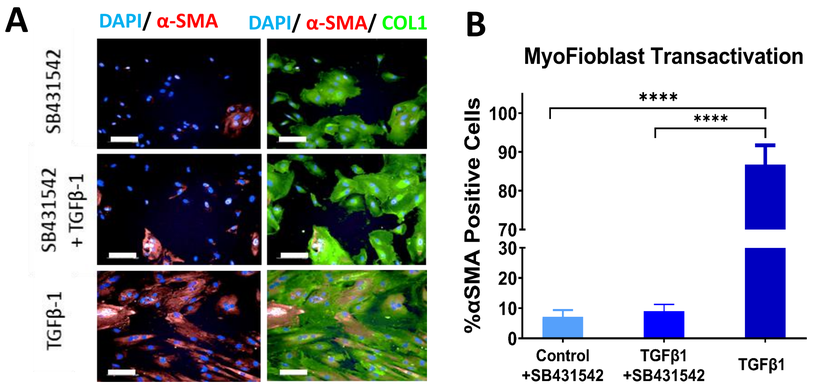


Fig 3. Management of myofibroblast transactivation from CFs using TGF-β stimulation or inhibition (A) Cells were grown in the presence and absence of 10 ng/ml TGFβ1 (activator) and/or 10 µM TGFβ inhibitor over 72 hours. Scale bar = 100 µm **(B)** Summary of α-SMA positive CFs indicate for activated hIPSC-MFs, N=2 biological replicates.

4. Impact of βMHC-R543C mutation in contractility response in 2D of hIPSC-CMs during drug evaluation.

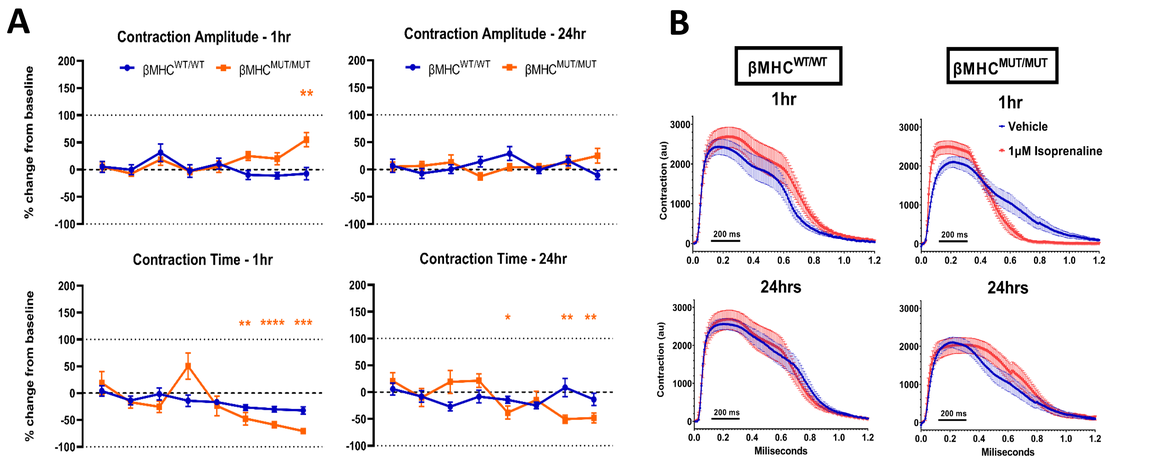


Fig 4. Differential contraction during drug challenge between WT and MUT hiPSC-CMs. At 1 h or 24 h after drug treatment, data were collected and analysed. **(A)** Analysis of contraction amplitude and contraction time **(B)** Contraction traces of hIPSC-βMHC-R453C CMs at vehicle control (blue), and the highest tested concentration (red). N=3 biological replicates.

Conclusions & Future works

- Reproducible and consistent high differentiation efficiency of hIPSC-ECs and hIPSC-CFs confirms for the possible replacement of animal serum products in the standard medium system by serum replacement product.
- Managed activation of MFs from CFs by TGF-β1 and TGF-β inhibitor validates the potential use of the hIPSC-derived model during fibrosis development.
- Evaluation of contractility phenotype of hIPSC-βMHC-R453C CMs in 2D showed proof of principle for the value of the isogenic model in identifying differences and understanding the cell responses due to the genetic mutation(s).
- Next steps are development of functional assays to establish the specific phenotypic tool kit for both 2D and 3D (spheroid & EHT) tri-linage models during cardiac fibrosis development.
- This work will benefit development of improved platforms for drug validation in the cardiovascular field and result in translational applications in future clinical practice. The validation of the humanised platform in serum-free conditions will help to remove the need for animal use in the field.

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Reference: 1. Semsarian C . *et al.* J Am Coll Cardiol 2015; 65:1249–1254. 2.Diogo M. *et al*, European Heart Journal (2018); 39(43): 3879–3892.

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