Single-Cell Transcriptomics of Engineered Cardiac Tissues from Patient-Specific iPSC-CMs Reveals Abnormal Developmental Trajectory and Intrinsic Contractile Defects in Hypoplastic Right Heart Syndrome

Yin-Yu Lam¹, Wendy Keung², Chun-Ho Chan¹, Lin Geng², Nicodemus Wong¹, Ronald A Li¹,²,³, Yiu-Fai Cheung¹

¹Department of Paediatrics and Adolescent Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong
²Dr Li Dak-Sum Research Centre, The University of Hong Kong-Carolinska Institute Collaboration in Regenerative Medicine, The University of Hong Kong, Hong Kong
³Ming-Wai Lau Centre for Reparative Medicine, Karolinska Institute, Sweden

Introduction

Pulmonary atresia with intact ventricular septum (PAIVS):
- A subset of hypoplastic right heart syndrome (HRHS) affecting 1 in 100,000 live births¹.
- The exact cause is unknown, but is believed to be of multifactorial origin.
- Genetic mutations is only identified in individual PAIVS patients².
- No animal models are able to recapitulate the disease phenotype.
- Patient-specific iPSC-CMs and bioengineered cardiac tissues can evaluate PAIVS cardiomyocyte’s intrinsic functional and transcriptomic properties in the absence of in vivo secondary remodelling to the primary anatomical defect.

Induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs)³:
- Morphologically and functionally resemble first-trimester human foetal cardiomyocytes.
- In vitro cardiac differentiation is analogous to that of in vivo human cardiac development.
- Bioengineered cardiac tissues provide biomimetic stimulus for iPSC-CM maturation.

Single cell RNA-sequencing (scRNA-seq):
- Permits the examination of individual iPSC-CM transcriptomes.
- Permits the identification of Differentially-expressed genes (DEGs) and its biological relevance can be identified via gene ontology (GO) analysis.

Objectives
- To characterize the intrinsic functional properties of PAIVS iPSC-CMs in the absence of secondary in vivo remodeling to the primary defect.
- To dissect the molecular mechanisms responsible for the functional defects in PAIVS iPSC-CMs.

Materials & Methods
- iPSCs were generated with peripheral CD34+ haematopoietic progenitors of two healthy controls (One male, one female) and three PAIVS patients.
- iPSC-CMs were generated from iPSCs with an established protocol and harvested at day 14 post-differentiation to bioengineer tissue constructs³.
- Cardiac tissue strips (CTS) were constructed by embedding iPSC-CMs in a collagen-based matrix, which were then used to evaluate contractility, contraction kinetics and calcium sensitivity⁴.

Results

PAIVS CTS shows impaired contractility:

PAIVS CTS shows impaired contractile kinetics:

PAIVS CTS shows increased calcium sensitivity:

EC₅₀ Concentration (mM ± 95% CI):
Control – 1.777 ± 0.067
PAIVS – 1.624 ± 0.088

Conclusion

- PAIVS CTS demonstrates reduced contractility, prolonged contraction kinetics and increased calcium sensitivity, indicating primary defect in the cardiac contractile apparatus.
- Ventricular development and cardiac contractile apparatus were downregulated with upregulation of primitive isoforms in PAIVS CTS and iPSC-CMs, which accounts for the functional defects observed in PAIVS CTS.

References

Acknowledgements

We would like to thank the Innovation and Technology Fund for their support towards this project (Project Code: ITS/195/05FP).