iPSC derived cardiomyocytes: A powerful tool for promoting translational cardiac research

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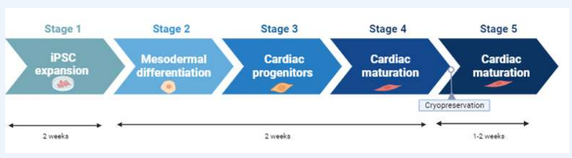
INTRODUCTION

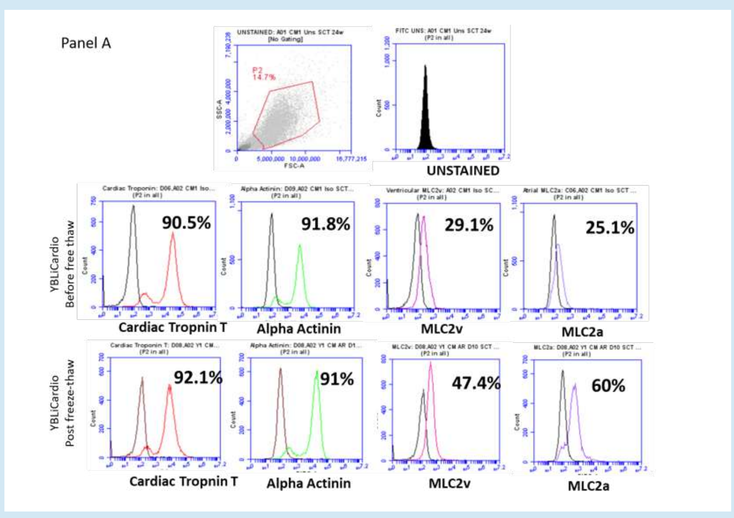
- iPSC derived cardiomyocytes (iPSC CM) have proven to be an exciting platform for translation cardiac research.
- However, a common limitation lies in the development of mature myocyte like cells.
- To address this concern, we curated a range of media and components to differentiate iPSCs from an in house line YBLi001 to ventricular cardiomyocytes. Samples were obtained on Day0, Day 6, Day 15 and Day 30 for in depth characterization using RNA sequencing, RT qPCR, flowcytometry, immunofluorescence and electrophysiological assessment.
- Results demonstrated that the developed iPSC CMs showed a post thaw viability of ≈ 90% and a plating efficiency of 70%.
- Expression of mature markers such as CTNT2, MYH7, HCN4, IRX4 were found to be in comparison with the primary myocyte cells.
- An electrophysiological characterization conducted utilizing specific acute and chronic compound dosage revealed the cells to be robust while showing an excellent reaction to gold standard test compounds.

METHODS

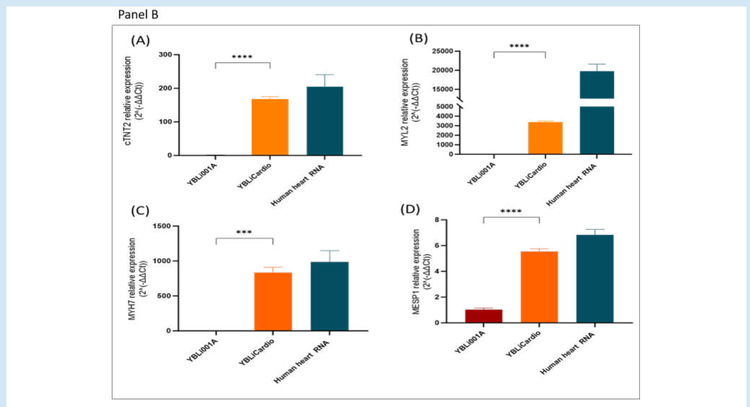
We cultured and expanded YBLi001 hiPSCs in STEMCELL technologies' mTeSR Plus medium and seeded 3.1*10^5 cells per well to generate ventricular cardiomyocytes using carefully curated in-house media. Cryopreserved cardiomyocytes were revived in a curated media that aids the maturation of cardiomyocytes showing a spike in the cardiac troponin T2 expression.



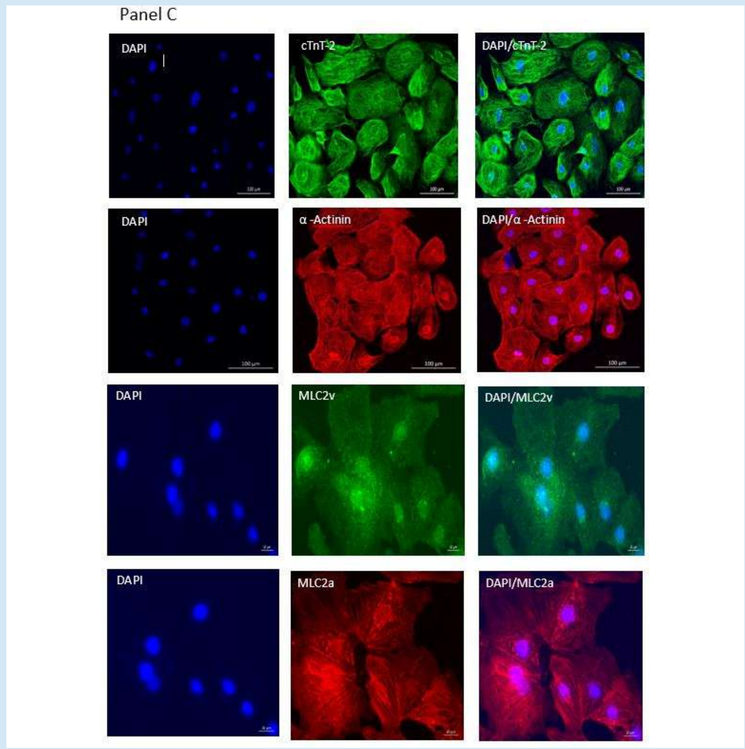
RESULTS



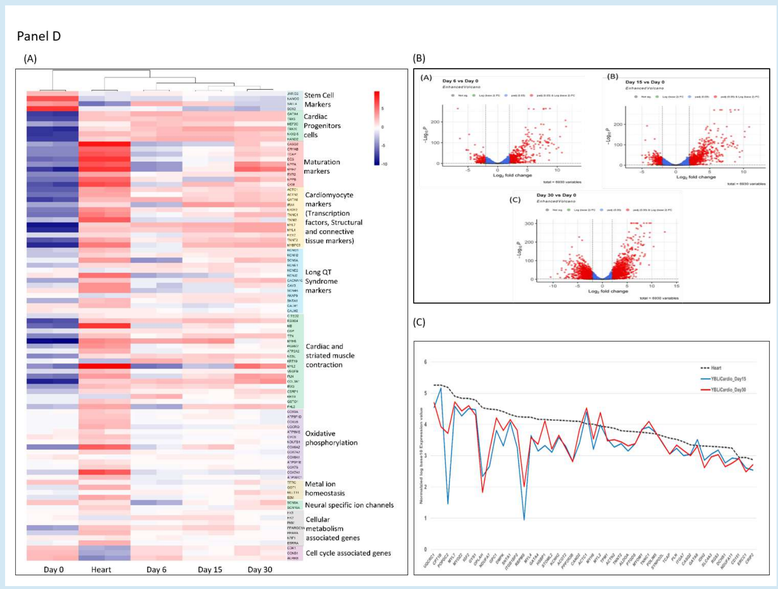
Panel A: Characterization by flow-cytometry. Representative flow cytometry panel showing A) Gating strategy used for cardiomyocytes analysis, B) Histogram of unstained samples. Representative overlays showing expression of C) Cardiac Troponin, D) Alpha-Actinin, E) MLC2v, and F) MLC2a of YBLiCardio Pre and Post cryopreservation cultures for day 11.



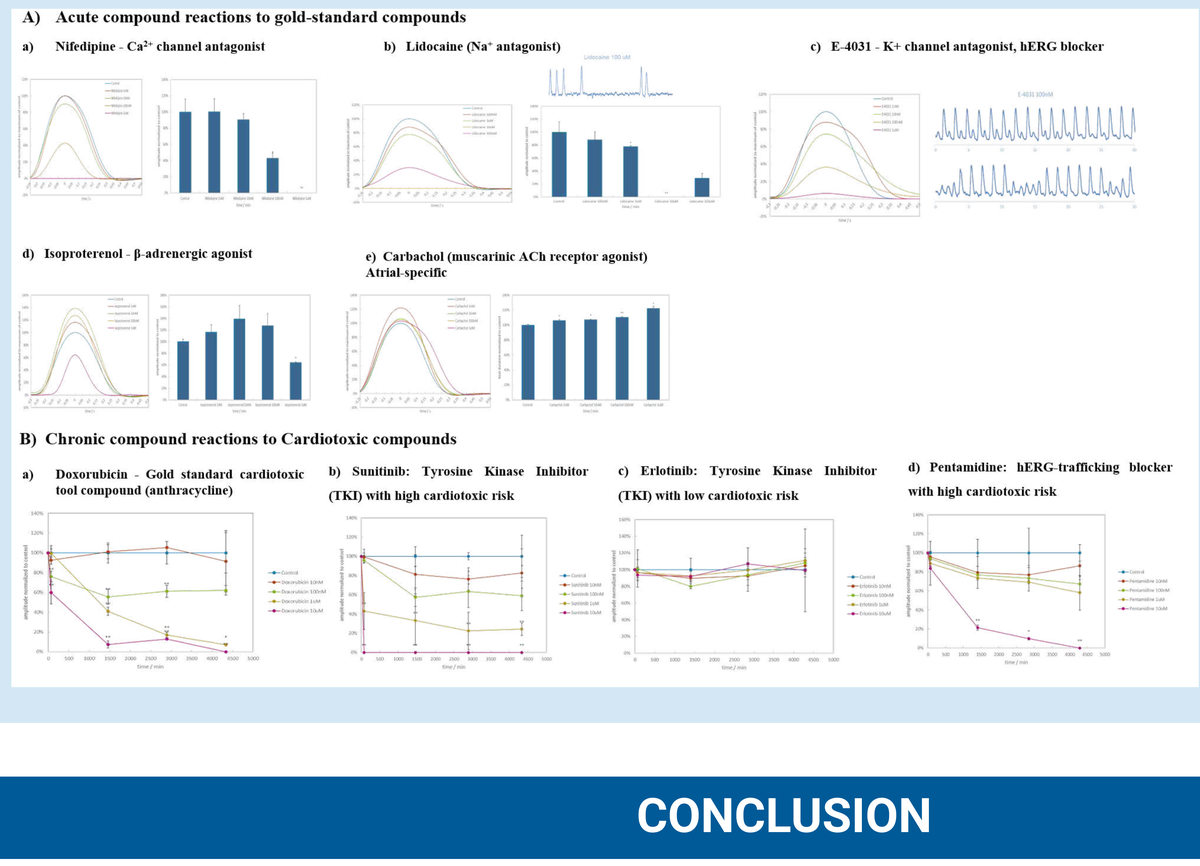
Panel B: Gene expression of Cardiomyocytes genes. Graph showing the expression of cardiac related genes A) CTNT2, B) MYL2, C) MYH7, D) MESP1. Data points are presented as the mean ± standard error of the mean (SEM) from three distinct experimental replicates; Unpaired two-tailed t-tests were conducted to assess statistical significance. Significant differences are denoted as follows: ***p≤0.0005; ****p≤0.0001.



Panel C: Characterization using Immunofluoresence. Representative confocal images of YBLiCardio showing expression of cardiac specific markers A) Cardiac Troponin T (green), B) Alpha Actinin (red), C) MLC2v (green), and D) MLC2a (red). Nucleus was counterstained with DAPI (blue). Images were taken at 63X.



Panel D: Data from RNA Sequencing
A) Differentially expressed genes were identified between pairs of clusters corresponding to the undifferentiated hiPSC population (Day 0) and Heart, Day 6, Day 15, and Day 30. Heat map shows up- and down-regulated genes from each pairwise cluster comparison. B) Volcano plot of expressed RNA at different time points of Cardiomyocytes samples illustrates the differential gene expression in YBLiCardio samples at A) Day 6, B) Day 15, and C) Day 30. Each point represents a gene. Upregulated genes are indicated in red, while downregulated genes are indicated in blue. The size of each point reflects the magnitude of the fold change. C) Data for more than 40 cardiac genes demonstrate a stable genomic expression profile for YBLi Cardio that trends well with that of adult human cardiac tissue.



Panel E: Electrophysiological assessment of YBLiCardio:
A) Acute compound reactions to gold-standard compounds and B) Chronic reactions to cardiotoxic compounds.The y-axis denotes the normalized beating amplitude, while the x-axis represents the logarithmic scale of drug concentrations applied. Data points are presented as the mean ± standard error of the mean (SEM) from four distinct experimental replicates.

CONCLUSION

YBLiCardio ventricular cardiomyocytes show a high expression of cardiac troponin T2, alpha actinin and MLC2v. The cells show outstanding responses to test compounds.

We believe that YBLiCardio hold the potential to aid in:
1) Drug discovery
2) Toxicity assays
3) Safety assays

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