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CRISPR engineered human iPSC cardiac reporter line

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Topic(s):
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Background: Human induced pluripotent stem cell (hiPSC) derived cardiomyocytes are currently emerging as a valuable experimental platform in cardiovascular research. Despite being widely used as an alternative for animal models to study disease specific drug responses, fully functional and mature cardiomyocytes suitable for drug discovery and toxicity screenings remain to be developed.

Purpose: Using the CRISPR-Cas9 genome editing strategy, we generated a cardiac specific hiPSC fluorescent reporter line for real-time monitoring of cardiomyocyte differentiation from hiPSCs. This reporter line offer a promising tool to isolate a purified population of cardiomyocytes and will provide better insights in the biology and function of hiPSC-derived cardiomyocytes.

Method: The myosin heavy chain locus (MYH6), enocoding a cardiac muscle specific protein, is targeted before the stop codon by knocking-in a bicistronic 2A-mScarlet reporter cassette carrying an excisable selection marker (PGK-Puro) using homologous recombination. For validating the reporter activity, a homozygous reporter iPSC clone free of predicted off-target mutations and a normal karyotype was differentiated to cardiomyocytes by both mouse visceral endodermal-like (END-2) co-culture method and small molecule differentiation method. Immunostaining of the mature cardiac markers, Ca2+ imaging and action potential measurements by conventional patch clamping functionally characterized the reporter hiPSC-derived cardiomyocytes.

Result: The expression profile of the mScarlet fluorescent reporter monitored during the course of cardiomyocyte differentiation by a time-lapse incubator imaging system demonstrate a faithful cardiac reporter activity of the CRISPR engineered hiPSC reporter line. Beating areas expressing mScarlet fluorescent protein was observed at day 14 for END-2 differentiation and day 10 for small molecule differentiation method. Video-based beating analysis, Ca2+ handling properties and electrophysiological measurements of the disassociated cardiomyocytes confirmed the functional use of this reporter line to define cardiac cell lineage.

Conclusion: Our cardiac specific hiPSC mScarlet reporter line is a versatile tool that could allow the optimization of efficient cardiac differentiation protocols, tracking cells in cardiac cell-based therapies and explore the molecular mechanisms during cardiomyocyte differentiation. It holds great potential to further advance the use of hiPSC-derived cardiomyocytes as in vitro systems for disease modeling and drug discovery.
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