Establishment of a self-assembling human 3D cardiac organoid model to investigate LEMD2 associated cardiomyopathy

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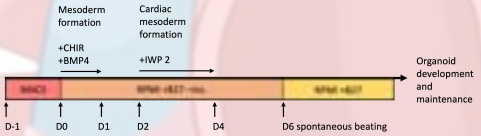
Abstract

The heart is the first fully functional organ to develop in the embryo and the organ most commonly affected by disease and congenital defects. Human induced pluripotent stem cell derived cardiomyocytes are a promising strategy to study human cardiac development and disease in vitro. Conventional 2D cell cultures are not suitable to understand complex interactions of different cell types within the cardiac tissue context; therefore, realistic 3D tissue models, such as organoids, that recapitulate cardiac development and morphogenesis are required.

Here, we present a protocol to generate self-assembled, chambered human iPSC-derived 3D cardiac organoids with functioning myocardium displaying a robust spontaneous heartbeat. Structural differences of cardiomyocytes in early and mature organoids indicate a maturation process. The myocardium is embedded into a mesenchymal tissue interspersed with a branching vascular network. In mature organoids, macrophages are observed in the vicinity of the muscle. We utilize this model to study dilated left ventricular cardiomyopathy caused by a mutation in the LemD2 gene. In the patient, this cardiomyopathy is characterized by fibrosis, dysfunctional systolic function, and arrhythmias eventually leading to cardiac death.

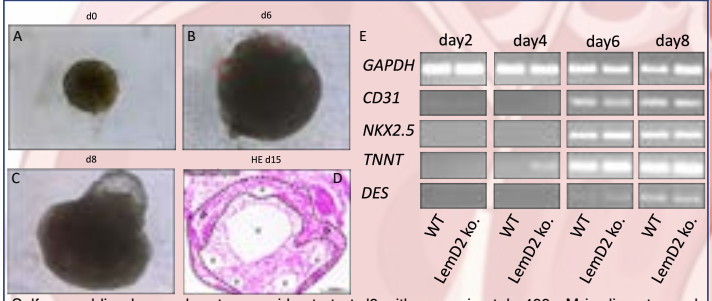
Methods

To generate cardiac organoids the precise modulation of WNT and BMP4 signaling pathways is necessary. Both are important for the formation of the mesodermal germ layer in which the cardiac progenitors are specified. The formation of the mesoderm is dependent on the activation of the canonical Wnt signaling pathway. With the inhibition of the Wnt/β-Cat Signaling pathway at later differentiation time points the formation of cardiomyocytes is favored. Optimal conditions of activation and inactivation timings as well as concentrations of small molecules and their interaction are to be determined to modulate cardiac differentiation in a human-derived iPSC organoid model.



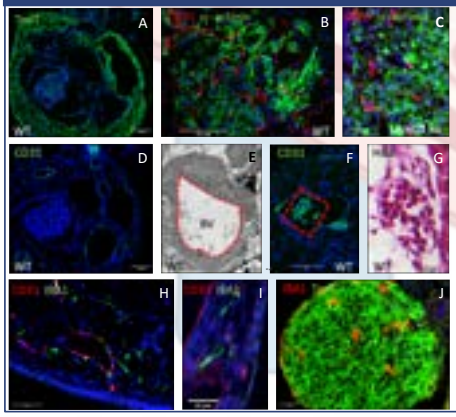
A schematic illustration of the three step WNT modulation protocol. Starting with the activation, processing with the active inactivation of the WNT signaling pathway by adding IWP2 and finishing with an activation again to generate a more complex tissue organoid model

Cardiac differentiation process



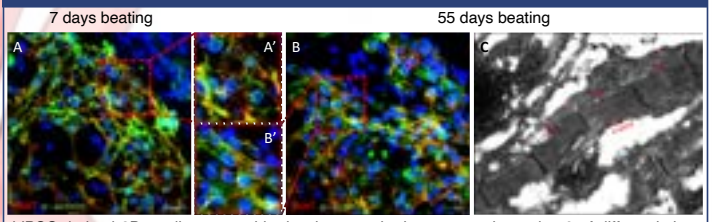
Self-assembling human heart organoids start at d0 with approximately 400 μM in diameter and increase their size to up to 3 mm by day 30 (A-C). Spontaneous beating starts 6 days after induction (QR1) and cavities appear (B-red dotted line). H&E staining shows the tissue architecture. Mesenchymal tissue and cardiac tissue (black dotted line) can be distinguished. Moreover, vessel like structures (V) are observed (D). PCR results confirm the differentiation process. Cardiac markers *NKX2.5*, *TNNT* and *DES* are expressed as soon as the cardiac mesoderm is formed. Vascularization starts as early as day 6 as confirmed by *CD31* expression in the PCR (E).

Vasculogenesis and Hematopoiesis in cardioid



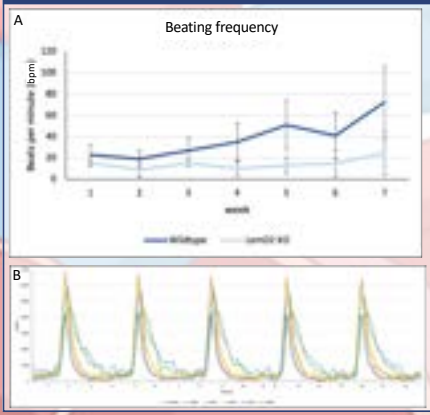
Immunofluorescence analyses show the developing myocardium in WT tissue and LemD2 KO tissue (α-actinin/TNNT) (A-C), which is surrounded by mesenchymal tissue interspersed with endothelial lined chambers ($CD31^+$) and a branching vascular network ($CD31^+$) (B-G). The vascular network gives rise to hematopoietic progenitor cells ($CD31^+$) (F) and different blood cells (G) (QR-3). In mature organoids, macrophages ($IBA1^+$) are observed in the vicinity of the vessels and the muscle (H-J).

Cardiac muscle maturation



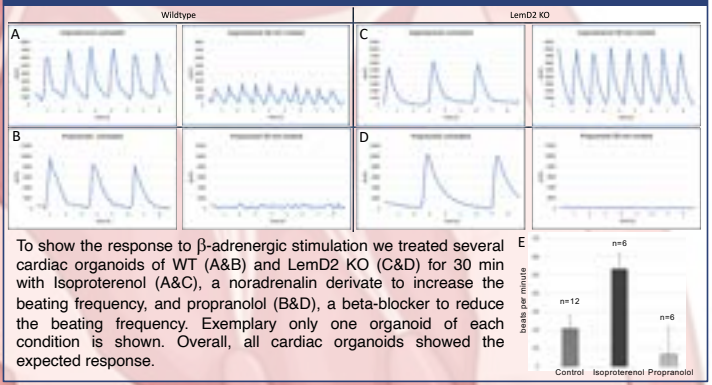
hiPSC-derived 3D cardiac organoids develop muscle tissue as early as day 6 of differentiation. Cardiac muscle tissue shows structural differences of cardiomyocytes in early and mature organoids (α-actinin/TNNT) which indicates a maturation process. After 7 days of beating the first sarcomere structures emerge (A&A'), which get organized after 55 days of beating (B&B'). Electron microscopic analyses revealed an approximately sarcomere length of 1.6 μm, with regular Z-lines, consistent A and I-bands and the start of M-band formation (C).

Beating frequency



Beating frequency of cardiac organoids was determined by counting beats of 8 different cardiac organoids for WT and LemD2 KO cell line. First measurement was taken as soon as the cardiac organoids started beating at day 6 of differentiation. Counting was repeated every 7 days. WT organoids started with ca. 20 bpm and increased up to 70 bpm, whereas LemD2 KO Organoids started with ca. 15 bpm and increased a frequency to ca. 25 bpm (A). The organoid's myocardium displays a robust spontaneous heartbeat. Dynamic fluorescent changes of the calcium indicator Fluo-4 were captured in 30 days old organoids, showing a frequency of 23 bpm and repetitive beating (B).

β-adrenergic receptor stimulation



To show the response to β-adrenergic stimulation we treated several cardiac organoids of WT (A&B) and LemD2 KO (C&D) for 30 min with Isoproterenol (A&C), a noradrenalin derivate to increase the beating frequency, and propranolol (B&D), a beta-blocker to reduce the beating frequency. Exemplary only one organoid of each condition is shown. Overall, all cardiac organoids showed the expected response.

QR Codes for videos

QR-code 1 of a beating 30 days old cardiac organoid, in brightfield

QR-code 2 of calcium changes within a cardiac organoid in IF

QR-code 3 of beating organoid with blood cells in brightfield

Discussion and outlook

Here we present a protocol to generate self-assembling, chambered human iPSC derived 3D cardiac organoids, with robust beating and functioning myocardium. A branched vascular network forms within the organoid model, which gives rise to different hematopoietic cell lines. We use this model to study the LemD2 induced dilated left ventricular cardiomyopathy. Next, these organoids will be analyzed by single-cell RNA-sequencing. We expect a more severe effect of the cardiomyopathy in a later/older cardiac organoid culture.

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