Patient-specific induced pluripotent stem cells to model Ebstein's anomaly (EA) with left ventricular non-compaction (LVNC)

Samudrala, Sai-Suma S (medical student); North, Lauren M; Kim, Min-Su; Strande, Jennifer; Zeeshan, Afzal; Frommelt, Michele A; Earing, Michael; Lough, John W; Mitchell, Michael; and Tomita-Mitchell, Aoy.

Children's Hospital of Wisconsin-Herma Heart Center, Medical College of Wisconsin, Children's Research Institute, Milwaukee, WI.

Background: Ebstein's anomaly (EA) is a rare congenital heart disease of the tricuspid valve and right ventricle. It is typically characterized by the attachment of posterior and septal leaflets to the ventricular septum and apical displacement of the valve.[2] Left ventricular non-compaction (LVNC) is a cardiomyopathy, commonly manifesting with EA and theorized to be due to abnormal compaction of ventricle-specific trabeculations during normal development, leaving behind sponge-like myocardium.[3,4] A multigenerational family with Ebstein's anomaly and LVNC has found variants on chromosome 19 segregate with affected family members. We hypothesize that these variants alter normal cardiac development of atrial and/or ventricular cells. To test this hypothesis, we are interested in studying atrial-like and ventricular-like cardiomyocytes derived from patient-specific induced pluripotent stem cells (iPSCs).
Methods: Cardiomyocytes were differentiated from iPSCs and directed to specific lineages via modulating wnt and retinoic acid pathways.[5,6] Patient-specific iPSCs are being generated from urine samples.[1] Cardiomyocytes and specific lineage formation will be characterized using gene expression profiles, immunohistochemistry, and flow cytometry of atrial or ventricular specific markers (cTNT, MF20, SLN, MLC2a, MLC2v).
Results: We demonstrate successful cardiomyocyte differentiation from patient-specific iPSCs. Early results suggest differences in lineage formation through modulation of retinoic acid pathway. We hope to identify specific lineage formation. D10 and D15 cardiomyocytes show atrial-like cells consistent with prior publications. Furthermore, the disease phenotype of these cells will be characterized to atrial and/or ventricular cardiomyocytes.
Conclusions: EA and LVNC patient-specific iPSCs can be differentiated into cardiomyocytes and directed to specific lineages. iPSCs appear to be a promising disease model for EA and LVNC at the cellular and molecular level. Our approach validates the importance of translational research in medicine when approaching complex diseases.

References

1. Afzal et al. Generation of human iPSCs from urine derived cells of a patient with a novel homozygous PAI-1 mutation. *Stem Cell Research.* 17: 657–660, 2016.
2. Attenhofer et al. Ebstein's Anomaly. *Circulation.* 115(2): 277-85, 2007.
3. Benson et al. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. *J Clin Invest*. 104(11): 1567–1573, 1999.
4. Kelle et al. Ebstein anomaly, left ventricular non-compaction, and early onset heart failure associated with a de novo α-tropomyosin gene mutation. *Am J Med Genet A.* 170(8):2186-90, 2016.
5. Kim et al. Activin-A and Bmp4 levels modulate cell type specification during CHIR-induced cardiomyogenesis. *PLoS One.* 10(2):e0118670, 2015.
6. Zhang et al. Direct differentiation of atrial and ventricular myocytes from human embryonic stem cells by alternating retinoid signals. *Cell Research.* 21:579-587, 2011.