GENETICS GUIDED DRUG DISCOVERY USING HUMAN INDUCIBLE PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES AND HUMAN ENGINEERED CARDIAC TISSUES

SCOTT MACDONNELL
ASSOCIATE DIRECTOR – CARDIOVASCULAR AND RENAL THERAPEUTIC AREA
AGENDA:

• GENETIC GUIDED DRUG DISCOVERY

• IPSC DERIVED HUMAN CARDIOMYOCYTES

• USING IPSC CARDIOMYOCYTES TO EVALUATE FUNCTION OF R403Q CELLS AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY
  ➢ 2D vs. 3D Models

Contracting iPS-Derived Human Cardiomyocytes
GENETICS GUIDED DRUG DISCOVERY

Drug Development

- Target Selection
  - Literature, human genetics, animal studies, clinical data
- Lead Discovery
  - Binding affinities, selectivity, biophysical characterization
- Lead Prioritization
  - Ensure that models are organ/cell + species specific. Also are relevant to disease.
- In-Vitro POC
- In-vivo POC
- Preclinical Safety & Clinical Studies

Real World Application:

Genetic and therapeutic antagonism of ANGPTL3 in humans and of ANGPTL3 in mice was associated with decreased levels of all three major lipid fractions and decreased odds of atherothrombotic cardiovascular disease.

Actionable Scientific Discoveries
- Peer reviewed publications (e.g., NEJM)
- Translational medicine (e.g., new targets, functional models, biomarkers, potential new therapies)
- Return of results to collaborators
MICE ARE NOT HUMANS....
OF MICE NOT HUMANS…
FUNDAMENTAL PHYSIOLOGIC DIFFERENCES BETWEEN HUMAN AND MOUSE CARDIOMYOCYTES

General Characterization

Mouse Cardiomyocytes

Properties
Mass 0.15 g
600 beats per minute
Energy Production 1350 kcal/kg/day
Sarcomeres : mitochondria 2:1

Human Cardiomyocytes

Properties
Mass 300 g
70 beats per minute
Energy Production 700 kcal/kg/day
Sarcomeres : mitochondria 2:1

Electrophysiologic Differences

MYH6 > MYH7

Genes with single identifiable orthologue ~80%6
May restrict regenerative potential6
90% bi-nucleated7
α myosin heavy chain (fast)
Impact tension contraction and power10,11
which can impact cardiac biology and disease12
β myosin heavy (slow)

MYH7 > MYH6

This impacts how tension and power are generated during contraction, affecting the mechanisms underpinning cardiac disease and function

Mice are less reliant on hERG channel. Challenges modeling arrhythmia in mice.
START WITH HUMANS - IPSC TECHNOLOGY

Human Donor(s) → Blood Sample → Reprogramming → iPS Cell Lines → Differentiation → Terminally-differentiated Cell Types

Genome Editing
iPSC-Derived Cardiomyocyte Monolayer

**Opportunity**
- Human Cells (patient derived or engineered)
- Near limitless source of material – well powered studies
- Exhibit functional properties (contractile and electrical) of human cardiomyocytes
- Evaluate the impact of variants across multiple genetic backgrounds in parallel
- Unable to acquire and culture human primary cardiomyocytes
- In-vitro – mechanistic evaluation in the absence of sympathetic tone

**Limitations**
- Disorganized sarcomere structure
- Immature phenotype
- Variation - Require multiple lines to define phenotype
- Isolated system
Impedance measurements to assess contractile function and extracellular field potential (EFP) to evaluate action potential duration are collected in parallel.
Analysis of Impedance Traces

10

Contractility – Trough-to-Peak

Arrhythmia – Inter-spike interval variation

Beat width – duration of contraction (Calcium handling)

Time and Velocity of Upstroke – Calcium influx/SR release and contractile protein activation – Systolic function

Time and Velocity of Relaxation – SR calcium uptake - Diastolic function
Hypertrophic cardiomyopathy (HCM) affects 1 in 500 people worldwide, with no discrimination for race or gender and is caused by the enlargement of cardiomyocytes, resulting in thickening of ventricular walls in the absence of increased external load. This thickening reduces left ventricle chamber size and impairs relaxation, eventually resulting in reduced cardiac output, fibrosis, arrhythmia, and failure.

The most prevalent mutations implicated in familial hypertrophic cardiomyopathy are in the MYH7 gene.

Decrease in myosin motor function with the MYH7-R403Q mutation leads to a compensatory asymmetric hypertrophic response.

Patients carrying the MYH7-R403Q mutation are at increased risk for arrhythmia, heart failure, and sudden cardiac death. 50% mortality by age 40


Table 1. Sarcomere mutations in hypertrophic cardiomyopathy

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac β-myosin heavy chain</td>
<td>MYH7</td>
<td>14q12</td>
<td>~40%</td>
</tr>
<tr>
<td>Cardiac myosin binding protein C</td>
<td>MYBPC3</td>
<td>11p11.2</td>
<td>~40%</td>
</tr>
<tr>
<td>Cardiac troponin T</td>
<td>TNNT2</td>
<td>1q32</td>
<td>~5%</td>
</tr>
<tr>
<td>Cardiac troponin I</td>
<td>TNNI3</td>
<td>19q13.4</td>
<td>~5%</td>
</tr>
<tr>
<td>α-Tropomyosin</td>
<td>TPM1</td>
<td>15q22.1</td>
<td>~2%</td>
</tr>
<tr>
<td>Myosin regulatory light chain 2</td>
<td>MYL2</td>
<td>12q21.11</td>
<td>~1%</td>
</tr>
<tr>
<td>Myosin, light chain 3</td>
<td>MYL3</td>
<td>3p21.3-p21.2</td>
<td>~1%</td>
</tr>
<tr>
<td>Actin</td>
<td>ACTC1</td>
<td>15q14</td>
<td>~1%</td>
</tr>
<tr>
<td>Titin</td>
<td>TTN</td>
<td>2q31</td>
<td>Rare</td>
</tr>
<tr>
<td>Myozin</td>
<td>MYOZ2</td>
<td>4q26-q27</td>
<td>Rare</td>
</tr>
<tr>
<td>α-Myosin heavy chain</td>
<td>MYH6</td>
<td>14q28.01CM</td>
<td>Rare</td>
</tr>
</tbody>
</table>
Decrease in myosin motor function with the MYH7-R403Q mutation leads to a compensatory asymmetric hypertrophic response.

Patients carrying the MYH7-R403Q mutation are at increased risk for arrhythmia, heart failure, and sudden cardiac death.

50% mortality by age 40

Determine if iPSC-derived cardiomyocytes from patients carrying the R403Q variant demonstrate a phenotype consistent with the clinical manifestations.

Can cells be used to model/explore the impact of genetic variants on cardiac function?
Two myosin heavy-chain genes, MYH6 and MYH7, are expressed in mammalian hearts in opposing patterns during cardiac development and remodeling.

Cardiac injury shifts this ratio toward MYH7.

**Link to HCM:** Compensatory hypertrophic signaling
MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Conclusion: MYH7-R403Q cardiomyocytes demonstrate a slower intrinsic beat rate, slower relaxation times and a larger beat amplitude compared to isogenic control CMs. This phenotype persists when paced at 1Hz.

Link to HCM: Increased beat width, fall time, and reduced velocity suggests impaired diastolic performance in these R403Q derived cardiomyocytes.
MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Question: Can the MYH7 (R403Q) cells respond to a pacing challenge?

Conclusion: A lower intrinsic beat rate was observed in the MYH7-R402Q vs. control.

Interestingly, while both MYH7-R403Q and control cells displayed rhythmic beating at 1Hz, the MYH7-R403Q cells were unable to increase rate up to 2Hz.

Link to HCM: Increased arrhythmia risk, limited reserve, reduced ability to adapt to stress.
Conclusion: MYH7-R403Q cardiomyocytes demonstrate slower electrical beat rates, larger beat amplitude, and longer field potential durations compared to isogenic controls.

Link to HCM: Increased action potential duration can be a trigger for arrhythmia.
MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Conclusion: MYH7-R403Q demonstrate increased Ca$^{2+}$ transient amplitude with reduced fall time suggesting impaired diastolic performance.

Link to HCM: Potential trigger for arrythmia, Ca$^{2+}$ induced hypertrophic signaling
IPSC-DERIVED CARDIOMYOCYTES – OPPORTUNITY AND LIMITATIONS

Opportunity

• Human Cells (patient derived or engineered)
• Near limitless source of material – well powered studies
• Exhibit functional properties (contractile and electrical) of human cardiomyocytes
• Evaluate the impact of variants across multiple genetic backgrounds in parallel
• Unable to acquire and culture human primary cardiomyocytes
• In-vitro – mechanistic evaluation in the absence of sympathetic tone

Limitations

• Disorganized sarcomere structure
• Immature phenotype
• Variation - Require multiple lines to define phenotype
• Isolated system (no fibroblasts)
Using human induced pluripotent stem cell derived cardiomyocytes, the patented Biowire™ II platform generates engineered adult human ventricular cardiac tissues, called Cardiotype™ tissues.

iPSC Cardiomyocytes and 10% Fibroblasts

TARA Bioscience
3D Cardiac Tissue
The Biowire™ II platform biomimetically matures the tissues using electromechanical stimulation resulting in Cardiotype™ tissues that exhibit characteristic hallmarks of *adult human ventricular myocardium*.

**Properties of Adult Myocardium**

- The positive force-frequency relationship is a property intrinsic to adult human myocardium.
- Post-rest potentiation and no spontaneous beating, properties intrinsic to adult human myocardium.

**Functional Data Collected from CardioType Tissues**

- **Arrhythmia** – Inter-spike interval variation
- Time and Velocity of Relaxation – SR calcium uptake - **Diastolic function**
- Beat width – duration of contraction (**Calcium handling**)
- Time and Velocity of Upstroke – Calcium influx/SR release and contractile protein activation – **Systolic function**
MYH7-R403Q 3D tissues have decreased structural alignment compared to control 3D tissues (cTroponin in green, F-actin in red, nuclei in blue).
Conclusion: MYH7-R403Q 3D tissues display a negative force frequency relationship in response to external increasing electrical stimulation (1 to 4Hz).

MYH7-R403Q 3D tissues display a higher active force at 1Hz external stimulation.

Consistent with 2D impedance data

Platform provides an opportunity to further profile targets of interest based on 2D findings
CONCLUSION: MYH7(R403Q) VS. ISOGENIC CONTROL

- Cardiomyocytes generated using iPS cells derived from a patient carrying the MYH7-R403Q variant maintain a hypertrophic phenotype in-vitro.
- Increased impedance amplitude suggest enhanced contractility frequently observed in hypertrophied cardiomyocytes as a compensatory mechanism.
- Increased contraction beat width and fall time suggest impaired diastolic performance and sarcoplasmic reticulum Ca\textsuperscript{2+} uptake.
- The diseased phenotype observed is maintained at both intrinsic and paired beat rates.
- Reduced FFR was observed in 3D Biowire tissues.

These data suggest that distinct differences in iPSC-CM from patients carrying diseased associated genetic variants can be observed in-vitro.

Functional studies using iPSC-CM may thus be used to define mechanisms involved in cardiovascular diseases and screen for novel therapeutics.