Agenda

Journal Club overview

Elements of an outstanding journal club presentation
Objectives: 1) develop and foster communication skills, 2) critically assess the literature, and 3) encourage Bioengineering graduate students to stay abreast of current knowledge in the field.

Format: 2 presenters each week

Journal citation submission via email 1 week prior to the presentation.

Policies: Laptops and cell phones turned off; Academic honor code applies.
<table>
<thead>
<tr>
<th>Date</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul 1</td>
<td>Introduction</td>
</tr>
<tr>
<td></td>
<td>Dr. Renee Cottle</td>
</tr>
<tr>
<td>Jul 8</td>
<td>Tanner Rathbone</td>
</tr>
<tr>
<td></td>
<td>Ilayda Ates</td>
</tr>
<tr>
<td>Jul 15</td>
<td>Volunteer 3</td>
</tr>
<tr>
<td></td>
<td>Volunteer 4</td>
</tr>
<tr>
<td>Jul 22</td>
<td>Volunteer 5</td>
</tr>
<tr>
<td></td>
<td>Volunteer 6</td>
</tr>
<tr>
<td>Jul 29</td>
<td>Volunteer 7</td>
</tr>
<tr>
<td></td>
<td>Volunteer 8</td>
</tr>
<tr>
<td>Aug 5</td>
<td>Volunteer 9</td>
</tr>
<tr>
<td></td>
<td>Volunteer 10</td>
</tr>
<tr>
<td>Presentation Skills</td>
<td>Subscore =</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Was the presenter organized?</td>
<td></td>
</tr>
<tr>
<td>Was the presenter clear and understandable?</td>
<td></td>
</tr>
<tr>
<td>Was the presenter confident?</td>
<td></td>
</tr>
<tr>
<td>Was the presenter succinct?</td>
<td></td>
</tr>
<tr>
<td>Was the organization of the slides logical?</td>
<td></td>
</tr>
<tr>
<td>Presentation Elements</td>
<td>Subscore =</td>
</tr>
<tr>
<td>Did the presenter identify the authors’ central hypothesis/question?</td>
<td></td>
</tr>
<tr>
<td>Did the presenter summarize the background to the central hypothesis/question?</td>
<td></td>
</tr>
<tr>
<td>Did the presenter explain uncommon methods used by the authors?</td>
<td></td>
</tr>
<tr>
<td>Did the presenter identify the important findings in the article?</td>
<td></td>
</tr>
<tr>
<td>Did the presenter accurately summarize the authors’ conclusions?</td>
<td></td>
</tr>
<tr>
<td>Did the presenter identify the significant limitation(s) of the study?</td>
<td></td>
</tr>
<tr>
<td>Did the presenter identify future directions, unanswered questions, or controversial issues?</td>
<td></td>
</tr>
<tr>
<td>Questions</td>
<td>Subscore =</td>
</tr>
<tr>
<td>Did the presenter demonstrate a strong understanding of the topic by his or her responses to questions from the audience?</td>
<td></td>
</tr>
<tr>
<td>Was the presenter courteous to the audience?</td>
<td></td>
</tr>
</tbody>
</table>
How to choose article

- **Research**: Find a research article related to your thesis work or research in your laboratory
- **Audience**: Find an article the audience can learn from
- **Quality**: Find an article from a respected peer reviewed journal
- **Current**: Find an article from recent literature
- **Interest**: Find an article that is engaging
Presentation outline

- Background
- Significance
- Results
- Conclusion
- Article critique
- Application to your research
Background

Information on the authors

What relevant work has been done previously

What is the current consensus in the field

Similar work published by author

What makes this study different

Use pictures wherever possible

Cite everything
Significance

Identify the gap in the knowledge

How the aims of this paper will contribute to field
Results

- Title should be the conclusion from data
- Don’t clutter slides
- Go through every figure
  - Explain dependent and independent variables
- Include supplementary data necessary
- Mind your audience
  - Spend extra time on difficult or unfamiliar data
  - Spend less time on simple/obvious figures
Conclusion

Summarize key points from article

Future work
Article critique

• Discuss whether the experimental design addressed the scientific question
• Identify sources of bias (funding sources, authors affiliations, previous work) and how has it influenced the study validity
• Identify weaknesses or inconsistencies
• Points that were unclear in the article
• Evaluate the likely impact of the study on the field
• Reference editorial commentary
Application to research

The important part of the presentation

How is this article important to your research
Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies

Gang Wang, Megan L McCain, Luhan Yang, Aibin He, Francesco Silvio Pasqualini, Ashutosh Agarwal, Hongyan Yuan, Dawei Jiang, Donghui Zhang, Lior Zangi, Judith Geva, Amy E Roberts, Qing Ma, Jian Ding, Jinghai Chen, Da-Zhi Wang, Kai Li, Jiwu Wang, Ronald J A Wanders, Wim Kulik, Frédéric M Vaz, Michael A Laflamme, Charles E Murry, Kenneth R Chien, Richard I Kelley, George M Church, Kevin Kit Parker & William T Pu
Primary Principal Investigators

- William T. Pu, M.D.
  - Professor of Pediatrics at Harvard Medical School
  - Research interests: epigenetics heart failure, heart regeneration, iPSC heart models

- Kevin Kit Parker, Ph.D.
  - Tarr Family Professor of Bioengineering and Applied Physics at Harvard School of Engineering
  - Research interests: cardiac cell biology, traumatic brain injury, micro- and nanotechnologies

- George M. Church, Ph.D.
  - Robert Winthrop Professor of Genetics at Harvard Medical School
  - Research interests: genome engineering, multiplex and nanopore genome sequencing, bio-omics, genes vs environmental traits, DNA chip libraries
Pathobiology of Barth Syndrome

Barth syndrome (BTHS):  
• X-linked recessive disorder  
• Occurs in 1:300,000 births  
• Enlarged weakened heart, poor muscle tone, recurrent infections, small stature  
• Mutations tafazzin gene (TAZ) encoding acyltransferase  
• Aberrant cardiolipin (CL) remodeling and impaired mitochondrial function

Knowledge Gaps:  
• Role of human tafazzin  
• Relationship between genotype and phenotype  
• CL role in cardiomyopathy and potential therapeutic targets

Houtkooper, Biochimica et Biophysica Acta 2009
Cardiolipin has critical role in mitochondrial function

Wang, Nature 2014
Goal

To provide insight into pathophysiology of BTHS and suggest new therapeutic strategies using novel *in vitro* models of BTHS
Paper Outline

Part 1. Characterize an iPSC-derived cardiomyocyte (CM) model of BTHS

Part 2. Recapitulate BTHS phenotype in isogenic iPSC-CMs using genome editing tools and extend understanding of BTHS abnormalities

Part 3. Replicate contractile pathophysiology of BTHS and evaluate TAZ modRNA using in vitro BTHS ‘heart-on-chip’ model

Part 4. Assess potential treatments using BTHS iPSC-CMs
iPSCs Disease Models

Yamanaka, Nature Medicine 2009
Takahashi, Cell 2007
Warren, Cell Stem Cell 2010
Validation of BTHS iPSCs

Wang, Nature 2014
iPSC-CM Differentiation Protocol

Wang, Nature 2014
Shiba, Nature 2012
Mitochondrial Functional Assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ETC Target</th>
<th>Effect on OCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligomycin (oligo)</td>
<td>ATP synthase complex V</td>
<td>decrease</td>
</tr>
<tr>
<td>FCCP</td>
<td>inner mitochondrial membrane</td>
<td>increase</td>
</tr>
<tr>
<td>Rotenone/Antimycin (R/A)</td>
<td>complex I and II</td>
<td>decrease</td>
</tr>
</tbody>
</table>
Genome Editing

• Engineering model organisms

• Crops and plants

• Therapeutic gene correction

• iPSC disease model

Meng, Biotechnology 2008
Wood, Science 2011
Nekrasov, Nature Biotechnology 2013
Genovese, Nature 2014
Hoban, Blood 2015
Nucleases-Mediated Gene Modifications

- Double Strand Break
  - Non-homologous end joining (NHEJ)
  - Gene Disruption
  - Homology directed repair (HDR)
  - Gene Modification
RNA-Guided Nucleases

- Clustered regularly interspaced short palindromic repeats (CRISPR) systems
  - Complex with Cas9
  - Target desired sequence by altering guide RNA
  - Substantial off-target activity

Mali, Science 2013
Cradick, Nucleic Acids Research 2013
Heart-on-Chip Model

Batch fabricate substrates

Coated with PIPAAm and PDMS

Myocytes seeded on micropatterned surface form anisotropic monolayers

Films cut PIPAAm dissolved

Paced or spontaneous contraction

Myocytes contract Films bend up

Grosberg, Lab Chip 2012
Paper Outline

Part 1. Characterize an iPSC-CM model of BTHS
Mitochondrial Abnormalities in BTHS iPSC-CMs

Wang, Nature 2014

Inefficient F1F0 ATP synthase activity
TAZ modRNA Rescues Mitochondrial Function in IPSC-CMs

High transfection efficiency

Wang, Nature 2014
Paper Outline

Part 2. Recapitulate BTHS phenotype in isogenic iPSC-CMs using genome editing tools and extend understanding of BTHS abnormalities
Engineered Isogenic iPSCs with BTHS Disease Mutations

Wang, Nature 2014
TAZ Mutant iPSC-CMs Have Disease Phenotype

Wang, Nature 2014
Impaired Sarcomere Organization in BTH-H and TAZ Mutant iPSC-CMs

Wang, Nature 2014
Part 3. Replicate contractile pathophysiology of BTHS and evaluate TAZ modRNA using in vitro BTHS ‘heart-on-chip’ model
Depressed Contractile Stress Generation Engineered BTHS Myocardial Tissues

Wang, Nature 2014
Part 4. Assess potential of treatments using BTHS iPSC-CMs
Treatments Tested

• Bromoenol lactone (Bel): inhibits mitochondrial phospholipase A$_2$ catabolism of cardiolipin
• Linoleic acid (LA): precursor for cardiolipin and ROS scavenging
• Arginine/cysteine (A/C): deficient in BTHS patients
• TAZ modRNA: wild type TAZ gene delivered
• MitoTEMPO: ROS scavenging
Linoleic Acid Corrects BTHS Phenotype in iPSC-CMs

Wang, Nature 2014
Conclusion

Applied iPSC-CM models to show that TAZ mutations causes abnormal mitochondrial function, sarcomere assembly and contractile stress generation. Demonstrate ‘heart on a chip’ to study contractile dysfunction in BTHS and identify therapeutic molecules, including ROS scavenging agents.
Significance

Demonstrates *in vitro* models that recapitulates cardiovascular disease by combining heart on a chip, iPSC, and gene editing technologies and shows that it can be used to study pathophysiology and test new therapeutic strategies. Opens new possibilities for personalized medicine.
Novelty

First to engineer heart tissue with patient specific disease causing mutation that recapitulates many aspects of disease phenotype.
Unsolved Questions & Future Directions

• Can other disease relevant \( \text{T} \text{A} \text{Z} \) mutations result in sarcomere disorganization?
• How do other \( \text{T} \text{A} \text{Z} \) isoforms contribute to the sarcomere organization?
• Is the sarcomere disorganization relevant to \textit{in vivo} conditions?
• How will results from myocardium tissue models change if iPSC-CMs having a mature phenotype were used?
• What is the mechanism by which \( \text{T} \text{A} \text{Z} \) mutations lead from mitochondrial dysfunction to sarcomere disassembly and reduced contractile stress generation?
• Use of ROS scavenging agents or \( \text{T} \text{A} \text{Z} \) modRNA for cardiomyopathy \textit{in vivo}?
• Differences between iPSC lines made by modRNA vs traditional (retroviral) approaches?
• How to tease out abnormal ATP synthase quantity vs activity?
Critiques

**Pros**
- Abundant data
- Interesting mitochondrial respiration studies
- Interesting sarcomere assembly studies
- Combined basic science with engineering approaches
- Studied BTHS at single cell and bulk cell level

**Cons**
- Immature phenotype of iPSCs-CMs in tissue engineered models (physiologically relevant?)
- Superficial discussion
- Missing pertinent methods information (genome editing)
- Low n values (sarcomere assembly studies)
- Inconclusive data
- Contradictory data
- Unessential gene modification
Inconclusive Data

Why is the peak systolic stress substantially higher for BTH-C and PGP iogenic mutants vs BTH-H?
Contradictory Data

Conclusion: sarcomere assembly was sensitive to mitochondrial function independent of whole-cell ATP levels.

What other variables between cell lines?

Why different experimental controls and conditions?
Unessential Gene Modification

Why use HDR-mediated gene modification to generate $\text{TAZ}^{517\text{delG}}$?

Wang, Nature 2014
Yang, Nature Communications 2014
Questions
Synthetic Modified RNA

Linear DNA template → in vitro transcription → Modified mRNA

5′-methyl-cytidine
pseudouridine

Stability
Immunogenicity
Negligible Off-Target Activity Depending on Method

Off-Target Sites for TAZ-targeting gRNA

<table>
<thead>
<tr>
<th>Off-target site</th>
<th>Total reads #</th>
<th>Indel reads #</th>
<th>Indel frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>362679</td>
<td>257</td>
<td>0.09%</td>
</tr>
<tr>
<td>2</td>
<td>23636</td>
<td>4</td>
<td>0.15%</td>
</tr>
<tr>
<td>3</td>
<td>270107</td>
<td>107</td>
<td>0.05%</td>
</tr>
<tr>
<td>4</td>
<td>3851</td>
<td>16</td>
<td>0.44%</td>
</tr>
<tr>
<td>5</td>
<td>434</td>
<td>4</td>
<td>0.92%</td>
</tr>
<tr>
<td>6</td>
<td>110549</td>
<td>663</td>
<td>0.60%</td>
</tr>
<tr>
<td>7</td>
<td>48643</td>
<td>204</td>
<td>0.42%</td>
</tr>
<tr>
<td>8</td>
<td>4243</td>
<td>20</td>
<td>0.47%</td>
</tr>
<tr>
<td>9</td>
<td>2190</td>
<td>11</td>
<td>0.50%</td>
</tr>
<tr>
<td>10</td>
<td>182504</td>
<td>744</td>
<td>0.41%</td>
</tr>
<tr>
<td>11</td>
<td>3404</td>
<td>3</td>
<td>0.09%</td>
</tr>
<tr>
<td>12</td>
<td>150138</td>
<td>579</td>
<td>0.39%</td>
</tr>
<tr>
<td>13</td>
<td>4201</td>
<td>5</td>
<td>0.12%</td>
</tr>
<tr>
<td>14</td>
<td>10020</td>
<td>52</td>
<td>0.52%</td>
</tr>
<tr>
<td>15</td>
<td>1454</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>16</td>
<td>268206</td>
<td>238</td>
<td>0.11%</td>
</tr>
<tr>
<td>17</td>
<td>2012</td>
<td>5</td>
<td>0.25%</td>
</tr>
<tr>
<td>18</td>
<td>1022</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>19</td>
<td>787</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>20</td>
<td>193618</td>
<td>272</td>
<td>0.14%</td>
</tr>
<tr>
<td>21</td>
<td>26706</td>
<td>28</td>
<td>0.10%</td>
</tr>
<tr>
<td>22</td>
<td>353484</td>
<td>680</td>
<td>0.25%</td>
</tr>
<tr>
<td>23</td>
<td>4438</td>
<td>4</td>
<td>0.01%</td>
</tr>
<tr>
<td>24</td>
<td>366306</td>
<td>1145</td>
<td>0.30%</td>
</tr>
<tr>
<td>25</td>
<td>454280</td>
<td>352</td>
<td>0.08%</td>
</tr>
<tr>
<td>26</td>
<td>2012</td>
<td>13</td>
<td>0.65%</td>
</tr>
<tr>
<td>27</td>
<td>41240</td>
<td>29</td>
<td>0.70%</td>
</tr>
<tr>
<td>28 (Chr. 5)</td>
<td>16682</td>
<td>333</td>
<td>18.91%</td>
</tr>
<tr>
<td>29</td>
<td>412222</td>
<td>463</td>
<td>0.11%</td>
</tr>
<tr>
<td>30</td>
<td>2313</td>
<td>12</td>
<td>0.52%</td>
</tr>
<tr>
<td>31</td>
<td>5185</td>
<td>16</td>
<td>0.31%</td>
</tr>
</tbody>
</table>

Yang, Nature Communications 2014
Glucose Doesn’t Correct Respiratory Capacity

Wang, Nature 2014
Contractile Activity Independent of Mitochondrial ATP Production

How is TAZ mutations influencing the contractile stress generation?
Analysis of Sarcomeric α-actinin

Wang, Nature 2014
Contraction Stress Measurement

Stoney’s Equation

\[ \sigma_{cell} = \frac{E t_s^2}{6(1 - \nu^2) R t_c (1 + \frac{t_c}{t_s})} \]

Grosberg, Lab Chip 2012
Wang, Nature 2014