HKU Discovers the Application of Human Induced Pluripotent Stem Cells in Precision Medicine for Hereditary Diseases

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What is Precision Medicine?

“An emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person.”*

– Enables physicians to tailor medical treatment for each patient
– Supports the development of molecularly targeted drugs based on biologic pathways
– Identifies at-risk populations for targeted prevention prior to disease onset

In precision medicine, the patients' disease risks, prognoses, and treatment responses can be predicted based on the behaviors of their hiPSC derivatives in cell culture.

Patient-specific hiPSC derivatives recapitulate the phenotypes of their in vivo counterparts.

Differences in patients' clinical phenotypes are the result of their unique DNA sequences.

Differential gene expression patterns cause different cellular and tissue phenotypes.
Human Induced Pluripotent Stem Cell (hiPSC)-Based Platforms for Drug Development

Patient recruitment
- Healthy
- Diseased

Somatic cell isolation
- Phlebotomy, skin biopsy, urine collection

Reprogramming factors

iPSC reprogramming
- Co-culture, embryoid bodies, monolayer, +GF/SM

iPSC differentiation

Comprehensive drug testing
- Contractility
- Morphology
- Arrhythmia
- Synthetic function
- Metabolism

Common cell source
- PBMCs
- Fibroblasts
- Renal epithelial cells

Method
- Retrovirus
- Lentivirus
- Sendai virus
- Epstein–Barr virus
- Adenovirus
- Plasmids
- Minicircles
- Co-MIP
- Synthetic mRNAs
- Proteins
- Small molecules

Cell type
- Cardiomyocytes
- Endothelial cells
- Smooth muscle cells
- Fibroblasts
- Adipocytes

Nature Reviews | Cardiology
Skin biopsy and dermal fibroblast expansion

Lentiviral Transduction

Switch to Matrigel™ coated plate

bFGF supplement

ROCK kinase inhibitor

Emergence of ES cell-like colonies

Manual picking and subculturing of iPS cell colonies

Day

-10

0

1

2

7

14

21

28

Skin cells

Reprogramming genes

ROCK kinase inhibitor

Micro-dissection & clonal expansion

hiPSCs

Branching into various cell types of the body

Embryoid bodies
Smooth muscle cells
Skin and nerve cells
Liver cells
Heart cells
Blood vessel cells

Beating Heart Muscle Generated from hiPSC
• Alternative splicing of *LMNA* gene generates lamin A and C, the intermediate filament protein of nuclear lamina.
• Serve as a matrix to maintain chromosome and genome integrity
• **Mutations in LMNA** referred to as “laminopathies, which cause Hutchinson Gilford Progeria (premature aging syndrome) and muscular dystrophy, to familial dilated cardiomyopathy (DCM).
Laminopathies are rare human degenerative disorders with a wide spectrum of clinical phenotypes.

Hutchinson-Gilford Progeria Syndrome
In HGPS patients the cell nucleus has dramatically aberrant morphology (Scaffidi et al., 2005).
LAMIN A/C Related Cardiomyopathy

Asymptomatic Conduction block
1st degree AV block
• Sick Sinus Rhythm

2nd to 3rd AV nodal block

0-20 years

Atrial fibrillation

20-40 years

Cardiomyopathy

30-60 years

VT/VF

40-60 years

SIU CW Aging (2011)
Pedigree of $\text{LMNA}^{\text{R225X/WT}}$ probands with two females (II-5 & II-9) and one male (II-7)
Schematic diagram of LMNA/C structure presenting mutation sites of our enrolled patients

**TGA premature stop**

R225X  
C to T  
heterozygous nonsense mutation

**TAG premature stop**

Q354X  
C to T  
heterozygous nonsense mutation

**Frameshift**

T518fs  
heterozygous frameshift mutation

WT: CGTCATGAGACCCGACGGTGG  
MT: CGTCATGAGACCTGACTGGTGG  
stop

WT: ATGCAGCAGCAG  
MT: ATGCAGTACGGCAG  
stop

WT: GCACAGAACAACCTGGGGC  
MT: GCACAGAACACTGGGGC  
Deletion of ‘c’
Schematic Diagram Illustrating the LMNA Mutations Involved in Current Study: Nonsense and Frame-Shift Mutations in LMNA
<table>
<thead>
<tr>
<th>Affected subjects</th>
<th>Sex</th>
<th>heart block</th>
<th>AF</th>
<th>VT/VF</th>
<th>Cardiomyopathy</th>
<th>AICD/Pacemaker</th>
<th>Age of death</th>
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<tbody>
<tr>
<td>SF5* (II-7)</td>
<td>M</td>
<td>CHB (49)</td>
<td>+ (49)</td>
<td>+ (50)</td>
<td>-</td>
<td>PPM (49); AICD (52)</td>
<td>57</td>
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<tr>
<td>LMNA&lt;sup&gt;R225X/WT&lt;/sup&gt;</td>
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<tr>
<td>SF29*(II-9)</td>
<td>F</td>
<td>CHB (48)</td>
<td>+ (48)</td>
<td>-</td>
<td>-</td>
<td>PPM (49); AICD (53)</td>
<td>-</td>
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<tr>
<td>LMNA&lt;sup&gt;R225X/WT&lt;/sup&gt;</td>
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<tr>
<td>SF36*(II-5)</td>
<td>F</td>
<td>CHB (51)</td>
<td>+ (52)</td>
<td>+ (60)</td>
<td>+ DCM (60)</td>
<td>ICD (60)</td>
<td>-</td>
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<tr>
<td>LMNA&lt;sup&gt;R225X/WT&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>SF11</td>
<td>M</td>
<td>3° HB (46)</td>
<td>+ (49)</td>
<td>-</td>
<td>-</td>
<td>Pacemaker (50)</td>
<td>-</td>
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<tr>
<td>LMNA&lt;sup&gt;frameshift/WT&lt;/sup&gt;</td>
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<tr>
<td>SF26</td>
<td>M</td>
<td>CHB (50)</td>
<td>+ (54)</td>
<td>+ (54)</td>
<td>+ DCM (57)</td>
<td>ICD (?)</td>
<td>64</td>
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<tr>
<td>LMNA&lt;sup&gt;E422X/WT&lt;/sup&gt;</td>
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<tr>
<td>SF30</td>
<td>M</td>
<td>CHB (50)</td>
<td>+ (50)</td>
<td>+ (56)</td>
<td>+ DCM (50)</td>
<td>PPM: AICD (50)</td>
<td>56</td>
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<td>LMNA&lt;sup&gt;Q534X/WT&lt;/sup&gt;</td>
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<td>SF39</td>
<td>M</td>
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<td>+ (47)</td>
<td>+ DCM (47)</td>
<td>AICD (47)</td>
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Table 1. Cardiac manifestations in affected subjects bearing LMNA mutation in present study

Abbreviations: 1° HB: first degree heart block; 2° HB: second degree heart block; 3° HB: third degree heart block; AF: Atrial fibrillation; AICD: automatic implantable cardioverter defibrillator; PPB: permanent pacemaker; AV block: atrio-ventricular block; CHB: complete heart block; PR: P-R interval; VT: ventricular tachyarrhythmia, DCM: dilated cardiomyopathy.

* Three probands were come from the same family.
hiPSC Heart muscle cells from patients with Cardiac Laminopathy exhibit nuclear blebbing upon electrical stimulation.

SIU CW Aging (2011)
PTC 124 (Ataluren) is a novel small molecular CFTR-G542X nonsense allele inhibitor.

In safety pharmacology studies in rats and dogs, oral administration of PTC124 (Ataluren) induces no adverse neurological, pulmonary, or cardiovascular effects at doses through 1500 mg/kg.

In toxicology studies in rats and dogs at oral doses through 1500 mg/kg for 28 days, PTC124 (Ataluren) has shown good tolerability.

FDA approved a new indication for orphan drug PTC 124 (made by PTC Therapeutics, Inc.), allowing its use in the treatment of Duchenne muscular dystrophy (MD) caused by a nonsense mutation in the dystrophin gene.

PTC124 reverses or alleviates nonsense mutation
Effects of PTC124 on Expression of Lamin A/C Proteins in Dermal Fibroblasts and hiPSC-Derived Cardiomyocytes

<table>
<thead>
<tr>
<th>Skin Fibroblast</th>
<th>iPSC-CMC</th>
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<tbody>
<tr>
<td><strong>LMNA&lt;sup&gt;wt/wt&lt;/sup&gt;</strong></td>
<td><strong>LMNA&lt;sup&gt;Q354X/wt&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td>PTC (µM)</td>
<td>0</td>
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<tr>
<td>Lamin A/C</td>
<td><img src="Lamin_A/C_wt_wt.png" alt="Image" /></td>
</tr>
<tr>
<td>ACTB</td>
<td><img src="ACTB_wt_wt.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>LMNA&lt;sup&gt;R225X/wt&lt;/sup&gt;</strong></td>
<td><strong>LMNA&lt;sup&gt;T518fs/wt&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td>PTC (µM)</td>
<td>0</td>
</tr>
<tr>
<td>Lamin A/C</td>
<td><img src="Lamin_A/C_R225X_wt.png" alt="Image" /></td>
</tr>
<tr>
<td>ACTB</td>
<td><img src="ACTB_R225X_wt.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Nuclear blebbing in the hiPSC-derived cardiomyocytes

LMNA<sup>WT/WT</sup>  
CTR

LMNA<sup>R225X/WT</sup>  
Electrically stressed

LMNA<sup>Q354X/WT</sup>  
Electrically stressed

LMNA<sup>T518fs/WT</sup>  
Electrically stressed + PTC124
## Evaluation of TUNEL-Positive Apoptotic Cell in Electrically Stressed and PTC124-Treated Cardiomyocytes

### DNA content (PI)

<table>
<thead>
<tr>
<th>Condition</th>
<th>LMNA$^{wt}$/wt</th>
<th>LMNA$^{Q35}$/wt</th>
<th>LMNA$^{T518F}$/wt</th>
<th>LMNA$^{Q35}$/wt</th>
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<tbody>
<tr>
<td>Control</td>
<td>6.32 ± 1.72</td>
<td>33.78 ± 1.72</td>
<td>28.20 ± 13.70</td>
<td>59.85 ± 13.79</td>
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<tr>
<td>E-Stim</td>
<td>7.48 ± 3.94</td>
<td>69.96 ± 7.49</td>
<td>17.38 ± 7.23</td>
<td>43.78 ± 15.30</td>
</tr>
<tr>
<td>E-Stim + PTC</td>
<td>8.78 ± 3.38</td>
<td>20.6 ± 3.91</td>
<td>26.13 ± 5.75</td>
<td>54.58 ± 16.28</td>
</tr>
</tbody>
</table>

### Apoptotic cell (TUNEL positive: BrdU-FITC) → FITC Log → DNA content (PI) →

- **Control**: 6.32 ± 1.72
- **E-Stim**: 7.48 ± 3.94
- **E-Stim + PTC**: 8.78 ± 3.38

### Graphical Analysis

- **Control**: 6.32 ± 1.72
- **E-Stim**: 7.48 ± 3.94
- **E-Stim + PTC**: 8.78 ± 3.38

*Significance levels: *

- *p < 0.05
- **p < 0.01
- #p > 0.05
Simultaneous Recording of Cardiac Cell Contractile Force and Calcium Transients of Single Cardiomyocytes

**Cell shortening:** Video based edge detection used to record cell shortening

**Ca²⁺ Transient:** the calcium indicator, Fura-2 AM was loaded into cells

Field electrical pacing at 40 V at the frequency of 0.5 Hz, 1 Hz, 1.5 Hz and 2 Hz

**Diastolic Ca²⁺ concentration**

<table>
<thead>
<tr>
<th>Amplitude</th>
<th>LMNA^{WT/WT}</th>
<th>PTC124</th>
<th>LMNA^{R225X/WT}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 Hz</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 Hz</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1.5 Hz</td>
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<tr>
<td>2 Hz</td>
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Conclusions

• Precision medicine is a new trend in modern medicine. It aims to allow tailing disease treatment and prevention according to individual variability in genes, environment, and lifestyle for each person.

• hiPSC technology provides an unique platform for clinicians and scientists to study the underlying mechanisms of various diseases.

• More importantly, while the clinical manifestations are very similar in patients with the same disease, hiPSC technology allow better prediction to clinical responses prior to real clinical trials. This is particularly important for rare diseases, whose number of patients often too small for meaningful clinical trials.
Conclusions

- In fact, since 2008, we have generated more than 20 disease-specific hiPSC line. This hiPSC bank is a unique platform for innovative biomedical research and drug development in Hong Kong and Mainland China.
- The present work demonstrate the feasibility of hiPSC technology in precision medicine for rare disease, representing a step forwards to its clinical applications.