Bioengineering of Cardiac Pacemaker Cells

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Sinoatrial Node

- Diminutive structure
- $< 10,000$ pacemaker cells
- Slow conduction velocity
- Unique cell morphology
- Beat spontaneously, autonomously
- Membrane clock and $\text{Ca}^{2+}$ clock

Engineering a BioPacer

- Ion channel-based: +/- a gene to make cardiomyocytes beat
- Embryonic stem cells
- Reprogramming: create *de novo* pacemaker cells
Tbx18 dictates embryonic development of the SA node

Tbx18 specifies SAN development

E 9.5–10.5

Tbx18 deficient embryo lacks SAN head

E 12.5

Somatic cell reprogramming:

Ventricular myocyte + Tbx18 \(\rightarrow\) pacemaker?

Modified from Hoogars, Cell. Mol. Life Sci. 2007

Modified from Wiese, Circ Res., 2009
Conversion of adult chamber cardiomyocytes to pacemaker cells in vivo by TBX18

Biological pacemaker created by injection of TBX18

Focal expression of TBX18 in the apex of guinea pig hearts create ectopic pacemaker activity.

TBX18 biological pacemaker activity originates at the focal injection site.


Delineate determinants of trans-differentiation to pacemaker cells

Automaticity
• Voltage clock
• Ca^{2+} clock
• Gap junction

Morphology
• Cytoskeletal rearrangement

Maturation
• Trapped in fetal/neonatal state?
**iSAN**

- Induced SAN-like pacemaker cells
- Beat spontaneously, autonomously
  - Voltage clock and $\text{Ca}^{2+}$ clock
- SAN cell-like morphology
- Responsive to autonomic regulation

Design principles of the iSA node

- **Pace and Drive**
  1. SAN center is relatively uncoupled → protects the spontaneously firing cells from the neighboring cells’ electrotonic inhibition
  2. A transitional region in which the cell type and electrical coupling change from the central SAN region to the peripheral atrial region
  3. Strands of anisotropy facilitate focal activity
Engineering a biological SA node

• Change the fluid we flow through the inlet
• Transparent for optical imaging
• Number of cells can be adapted by the flow rate ratio
Hanging drop Cardiac spheres

- Make Spheres

NRVM

TBX18-induced pacemaker cell
Morphology & Synchronous/Asynchronous beating

Beating Sphere  Non-Beating Sphere  Dead Sphere

Note: independent, asynchronous contractions in multiple cells at 1 week
Spontaneous Ca\textsuperscript{2+} transients in 1 week-old cardiac spheres

90 µm

TBX 18, GFP, TBX 18+Fibroblast, GFP+Fibroblast
MEA data of Cardiac Spheres

TBX 18  GFP  TBX 18+Fibroblast  GFP+Fibroblast

Mean FPD (msec)

Beat Rate

TBX 18  GFP  TBX18 + Fibroblast  GFP + Fibroblast
GFP

Beat Rate (BPM)

Day 8  Day 10  Day 12  Day 14  Day 16  Day 19  Day 21  Day 23

18  21  22  31  41  51  61  71  81
17  27  37  47  57  67  77  87
16  26  36  46  56  66  76  86
15  25  35  45  55  65  75  85
14  24  34  44  54  64  74  84
13  23  33  43  53  63  73  83
12  22  32  42  52  62  72  82
11  21  31  41  51  61  71  81
Conduction velocity from a cluster of spheroids (TBX18 vs. GFP)

A low-conductance gap junction, Cx45, expression increases dramatically in TBX18-spheroids: qPCR
Higher beating rates in TBX18-spheroids correlates with its higher HCN4 expression: qPCR
TBX18-spheroids on 1wk-old NRVM monolayer

Cal590, Ca^{2+} indicator

Green fluorescent TBX18-spheroids
Biological pacing by TBX18-spheroids: in vivo proof-of-concept

TBX18-spheroids

GFP-spheroids

Green fluorescent
Paced mapping EKG at LV apex

Lead II (mV)
-0.003
-0.002
-0.001
0.000
0.001

Lead I (mV)
-2.0
-1.5
-1.0
-0.5
0.0

Calculated Lead III (mV)
-0.0010
-0.0005
0.0000
0.0005
0.0010
0.0015

Normal Sinus Rhythm

Pacing site
Ex-vivo Data in a TBX18-spheroids injected rat model

Escaped Beat after adenosine

Paced mapping EKG at LV apex

Normal Sinus Rhythm

Lead I (mV)

Lead II (mV)

Lead III (mV)

Calculated III (mV)
Spontaneous Beating of TBX18 Spheres

: TBX18 ventricular Beats

**TBX18 Ventricular Beats**

- Lead II (mV)
  - 0.0005
  - 0.0010
  - 0.0015
  - 0.0000
  - 0.0005

- Lead I (mV)
  - 1.5
  - 1.0
  - 0.5
  - 0.0
  - 1.5

- Calculated Lead III (mV)
  - 0.0005
  - 0.0000
  - 0.0005
  - 0.0010

Paced mapping EKG at LV apex

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Tissue Engineering Can Be Incorporated Into Cell Therapy

Biotechnology methods for cell survival and engraftment

- CRISPR/Cas9
- Genetic engineering

Cardiac cell delivery

- Injectable scaffolds
- Cardiac patch/sheet
- Cardiac 3-D tissue construct

Biomaterial scaffolds

Hydrogels

Patricia K. Nguyen, MD; Evgenios Neofytou, MD. Potential Strategies to Address the Major Clinical Barriers Facing Stem Cell Regenerative Therapy for Cardiovascular Disease. JAMA Cardiol. 2016;1(8):953-962
Conclusions

- TBX18-spheroids
  1. Show **automaticity** compared to GFP-spheroids
  2. Demonstrate **long-term pacing** with higher beating rates
  3. Exhibit **features of SA node** including low-conduction and high Hcn4
  4. Can **pace-and-drive** in an in vitro model of tissue engraftment
Acknowledgement

P.I. Cho Hee Cheol, Ph.D

- Jun Li, PhD
- Sandra Gonzalez, BSc
- Wensheng Li, MSc
- David Wolfson, BSc
- Pengcheng Han, PhD
Thank You For Your Attention!