Translational Studies in Heart Failure

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Effective Model of Translational Research

Clinical observation

Therapeutic Design

Ex vivo testing

Transgenics

Small animal models

Large animal models

Clinical trials
Non-Effective Translational Research Model
Heart Failure (HF) Statistics

• **Epidemic Proportions**
  >400,000 new cases per year in U.S. (5 million total)

• **Death-rate still Increasing**
  CAD down 49% - CHF up 64% in last 20 years

• **Morbidity and Costs also High**
  #1 cause of all hospitalizations – >$300 Billion per year

• **Therapies not Ideal**
  Improvements but no truly effective therapy
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β-Adrenergic Receptor System in Heart Failure

β₁AR mRNA ↓

β₁AR mRNA ↓

β₂AR mRNA

β₂AR mRNA

βARK mRNA ↑

βARK mRNA ↑

Plasma membrane

Intracellular space

Circulation Vol 87, No 2, Feb 1993
Translational Research

- Clinical observation
- Therapeutic Design
- Ex vivo testing
- Transgenics
- Small animal models
- Large animal models
- Clinical trials
The G Protein-Coupled Receptor Kinases (GRKs)

Serine/Threonine Kinases
3 classes: GRK1 (Rhodopsin Kinase), GRK7
GRK2 (βARK1), GRK3 (βARK2)
GRK4, GRK5, GRK6

Additional Interacting Partners:
Tubulin, Actin, α-actinin

Adapted from Pao CS and Benovic JL, Science's STKE 8 October 2002, pp. pe42
Translational Research

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**βARKct rescues several different murine models of HF**

<table>
<thead>
<tr>
<th>Murine model</th>
<th>Result of βARKct cross</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>MLP</em>&lt;sup&gt;−/−&lt;/sup&gt; Knockout</td>
<td>Complete functional rescue with restored βAR responsiveness</td>
<td>1</td>
</tr>
<tr>
<td>Transgenic Cardiac</td>
<td>Rescue of cardiac function with smaller cardiac dimension and also improved survival</td>
<td>2</td>
</tr>
<tr>
<td>CSQ Overexpression</td>
<td>Rescue of function, prevention of hypertrophy and dimensions and improved exercise tolerance</td>
<td>3</td>
</tr>
<tr>
<td>Transgenic Cardiac</td>
<td>Hypertrophy prevented</td>
<td>4</td>
</tr>
<tr>
<td>Expression of a Mutant Myosin Heavy Chain (HCM)</td>
<td>Only βAR signaling improved with no functional or mortality rescue</td>
<td>5</td>
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<tr>
<td>Transgenic Cardiac</td>
<td></td>
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<tr>
<td>Overexpression of MCP-1</td>
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<tr>
<td>Transgenic Cardiac</td>
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<tr>
<td>Overexpression of dominant-Negative mutant of CREB (CREB&lt;sub&gt;A133&lt;/sub&gt;)</td>
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$\beta$ARKct Rescue of Survival in A Transgenic Mouse Model of HF

- The $\beta$ARKct transgene was cloned into replication-deficient adenoviral vectors
- Attempt intracoronary gene transfer to the hearts of larger animal models
Intracoronary Adenoviral-mediated Myocardial Delivery

Sub-selective coronary artery catheterization

Sub-selective catheter-mediated delivery of $\beta$ARKct: Chronic HF model

Shah et al. *Circulation* 2001;103:1311-1316
Large Animal Gene Therapy

- Cardiopulmonary bypass with RA cannulation
- Cardioplegic arrest (30 min) → gene delivery
- Global transgene expression at 7 days

Ant LV Lat LV Post LV Ant RV Post RV Ant Sept Post Sept Liver NL Liver (+) 36 kDA
Translational Research

1. Clinical observation
2. Therapeutic Design
3. Ex vivo testing
4. Transgenics
5. Small animal models
6. Large animal models
7. Clinical trials
Final Proof of Concept for the $\beta$ARKct

Will $\beta$ARKct be beneficial in failing human myocytes?

Heart arrested with 1L cardioplegia and explanted.

Coronary artery (LAD or graft) cannulated and perfused with collagenase.

Myocytes incubated on Matrigel®-coated plates.

Treated with Adenovirus and single cell contraction measured as well as $\beta$AR signaling.
Restoration of Contractility and βAR Function by βARKct in Failing Human Myocytes

Human myocytes infected with Adeno-GFP/BARKct

dL/dT contraction

* p<0.05

μm/sec

Basal | ISO

Failing (n=5 patients; 10 cells/condition)

Failing+BARKct (n=5 patients; 10 cells/condition)

Basal | ISO

dL/dT relaxation

* p<0.05

μm/sec

Conclusions

Inhibition of GRK2 (βARK1) represents a potential new drug class, targeting βAR and other GPCR systems from “the inside out”.

Molecular Normalization or “Molecular Remodeling” of the βAR System via GRK Inhibition is Beneficial in Heart Failure and a Novel Therapeutic Strategy. Synergistic with current HF therapy with βAR antagonists.

Gene Therapy with βARKct will be first but also a definite need for small molecule.
Potential Targets for Heart Failure Gene Therapy

1. β agonist stimulation of βARs
2. Dissociation of Gs protein
3. Activation of AC
4. βARK1 inhibition

βARK1 overexpression

Translocation of βARK1 and phosphorylation of β1ARs and β2ARs

SERCA2a PLB inhibition S100A1

Ca2+ cycling/induction of contraction

G495 L689

βARKct

β-AR-binding catalytic domain

↑cAMP

PKA
Hurdles to Human Application

• Target validation present for βARKct as well as other targets (S100A1, SERCA, Adenylyl Cyclase)

• Choice of vector
  – Advanced Adenovirus vs. AAV (or Lentivirus?)

• Route of vector administration
  – Invasive vs. non-invasive (CPB, coronary cath. or intra-ventricular)

• Choice of patient population
  – End-stage, +/- LVAD? Or Class III/V, post CPB dysfunction
GRK2 as a Novel Biomarker for Heart Failure

Another Translational Approach – Clinical Research
Can GRK2 be a Biomarker for Human HF?

- A biomarker for heart failure is much needed
- More therapeutic tools are needed for the treatment of this condition
- Evidence available in animals indicating this molecule as a key player in experimental HF where its levels are regulated by the activation of the sympathetic nervous system
- To be exported in human settings we need confirmation that in HHF
  1) GRK2 is pathophysiologically relevant
  2) is dysregulated during the disease
  3) is GRK2 important for prognosis in HHF
  4) its reduction can be beneficial
- To answer these and more questions we need a way to monitor cardiac GRK2 repeatedly over the time
Cardiac GRK2 Tracks with Levels Found in White Blood Cells

\[ y = 0.5741x - 0.4527 \]
\[ R^2 = 0.5686, \ p < 0.02 \]

Iaccarino et al., Eur Heart J, 2005
Lymphocyte GRK22 Negatively Associated with Cardiac Function

\[ y = -0.21784x + 19.265 \]

\[ R^2 = 0.193, \ p < 0.02 \]

Ejection Fraction (%)

NYHA Class

\[ F = 4.272, \ p < 0.02 \ \text{ANOVA} \]
If High GRK2 is Associated with Worsened Function - is GRK2 Lowered With Treatment?

Is GRK2 Involved in Reverse Remodeling Associated with LVAD Treatment?
• ~3000 cardiac transplants performed per year, LVADs are commonly used as a “bridge to cardiac transplant”.
• LV unloading by LVAD support leads toward normalization of myocardial structure and function (“reverse remodeling”) including restoration of βAR responsiveness.
• Long-term LVAD support leads to enhanced survival in patients not eligible for transplant compared to optimized medical treatment (REMATCH Trial, Rose, et al, NEJM, 2001) …however, 1-year mortality ~50%.

• Will LVAD support in HF induce significant changes in myocardial GRK2 expression and GRK activity to support improved βAR responsiveness as a positive component to reverse remodeling?
GRK2 Levels After Mechanical Unloading in the Failing Human Heart

**Myocardial βARK1 protein**

- Core LV
- Core LV
- Core LV
- Core LV
- LV (+)

**Myocardial βARK1 mRNA**

- Pre-LVAD N=12
- Post-LVAD N=12
- Pre-LVAD N=12
- Post-LVAD N=12

- Z.C. Pre
- Z.C. Post
- I.B. Pre
- I.B. Post

- 80 kDa

Graphical representation showing densitometric units/ug protein and picograms RNA for Pre-LVAD and Post-LVAD conditions, with statistical significance indicated by *p<0.005 and *p<0.05.
Cardiac (LV) GRK2 Tracks with Levels Found in White Blood Cells

Hata et al., JCF, 2006
Summarizing GRK2 (βARK1) and GRK Activity as a Novel Biomarker in HF

• Alterations in GRK2 expression and GRK activity seen in failing myocardium mirrored in lymphocytes and appears to be associated with severity of disease and decreased GRK2 associated with cardiac functional improvement.

• Potential for GRK2 levels and GRK activity in lymphocytes to be used as a biomarker in HF (surrogate marker for response to therapy currently being tested).
Active Clinical Studies at Jefferson
Does Lymphocyte GRK2 Represent a Novel Biomarker for HF

Measurement of lymphocyte GRK2 in acutely decompensated (hospitalized) HF patients and comparison to BNP for acute volume reduction.

ACKNOWLEDGMENTS

– Collaborators
Andrea Eckhart – Jefferson
David Whellan – Jefferson
Paul Mather – Jefferson
Terry Hyslop - Jefferson

Hugo Katus – Heidelberg
Jorg Heierhorst – Melbourne

Carmelo Milano – Duke
Howard Rockman – Duke
Bob Lefkowitz - Duke

– Koch Lab
Kurt Chuprun
Sven Pleger
Patrick Most
Jeff Martini
Erhe Gao
Brent DeGeorge
Natalie Patch
Maggie Shapiro
Liz Mandel
Matt Kuhn
Wiebke Pleger
Tasos Lymperopoulos
Matt Williams
Jonathan Hata
Amit Mittal
Noah Bloomgarden
Matthieu Boucher

- Support
NIH/NHLBI
Genzyme