Novel Signaling Pathways Related to Nuclear Translocation of GRK5

Wally Koch, PhD.
W.W. Smith Professor of Medicine

Disclosures: None
**G Protein-Coupled Receptor Kinases (GRKs)**

- **RK subfamily**
  - **GRK1 (RK)**
  - **GRK7**

- **βARK subfamily**
  - **GRK2 (βARK1)**
  - **GRK3 (βARK2)**

- **GRK4 subfamily**
  - **GRK4**
  - **GRK5**
  - **GRK6**

**Diagram Details**:
- **RGS domain**: Recoverin binding
- **Catalytic domain**: Clathrin binding, PKC binding
- **C-terminal domain**: MAPK binding
- **PH domain**: PKA binding
- **CaM binding**: PKC binding
- **PIP2 binding**: PKC binding
Highly expressed GRK in muscle – Second to GRK2 in myocardium.

Found up-regulated in animal models of heart failure and also in human HF patients.

Human polymorphism (Q41L) in amino-terminus associated with altered outcomes to β-AR blocker treatment in HF

Overexpression in the heart causes βAR desensitization and KO mice are viable with no obvious cardiac phenotype.
GRK5 Has Functional NLS

Johnson et al (Julie Pitcher) Mol Cell Biol, 2004
Pressure Overload Causes GRK5 Nuclear Accumulation In Vivo

Martini et al., PNAS, 2008
Overexpression of GRK5 Accelerates Hypertrophy

N=8,9,8,9
*p<0.05 vs NLC sham, **p<0.05 vs GRK5 Sham, # p<0.05 vs NLC post-TAC
(one-way ANOVA, Bonferroni MCT)

ANF, BNP and Myocyte Size Similar Results

Martini et al., PNAS, 2008
Nuclear GRK5 is Responsible for Pathology Post-TAC

Martini et al., PNAS, 2008
Nuclear GRK5 is Responsible for Pathology Post-TAC

N=19, 7, 14, 5
*p<0.05 vs NLC sham, **p<0.05 vs GRK5 Sham, # p<0.05 vs NLC post-TAC (one-way ANOVA, Bonferroni MCT).

Martini et al., PNAS, 2008
GRK5 is an HDAC Kinase
Nuclear GRK5 Activates MEF2 In Vitro

Martini et al., PNAS, 2008
GRK5 is an HDAC5 Kinase


GRK5 Interacts with HDACs In Vivo

Martini et al., PNAS, 2008
Myocardial overexpression of GRK5 leads to exaggerated hypertrophy and early onset of HF after TAC

This pathology is not observed when a nuclear deficient mutant of GRK5 (ΔNLS) is overexpressed in the heart

Chronic Gq signaling leads to nuclear GRK5 accumulation both \textit{in vitro} and \textit{in vivo}

GRK5 can act as a novel class II HDAC kinase leading to increased MEF2 activity
Our results demonstrate the ability of GRK5 to act in a previously unappreciated manner downstream of Gq mediated hypertrophic signaling.

This finding is significant since it assigns physiological relevance to a non-receptor substrate for GRK5.

Because GRK5 has been shown to be upregulated in several animal models of HF as well as in human HF studies, insights into GRK5’s role as a nuclear kinase and role in maladaptive hypertrophy should be continued to be explored.
What is Mechanism for GRK5 Nuclear Translocation?

Epinephrine, Endothelin, Angiotensin

Epinephrine → Gq → CaMK → MEF2

Endothelin → Gq → PKC → MEF2

Angiotensin → Gq → PKD → MEF2

GRK5

HDAC5

Cardiac Hypertrophy Genes
# Requirements for GRK5 Nuclear Localization

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target</th>
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<tbody>
<tr>
<td>Bis1</td>
<td>PKC</td>
</tr>
<tr>
<td>Go6976</td>
<td>PKD</td>
</tr>
<tr>
<td>CDZ</td>
<td>Calmodulin</td>
</tr>
<tr>
<td>Bapta</td>
<td>Ca(^{2+})</td>
</tr>
<tr>
<td>CamK-DN</td>
<td>Dominant neg.</td>
</tr>
<tr>
<td>CamK-CAM</td>
<td>Constitutively Active</td>
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<table>
<thead>
<tr>
<th>Mutant</th>
<th>Description</th>
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<tbody>
<tr>
<td>GRK5</td>
<td>WT</td>
</tr>
<tr>
<td>PKC (-)</td>
<td>PKC unable to phos</td>
</tr>
<tr>
<td>ST/AA</td>
<td>S484A, T485A</td>
</tr>
<tr>
<td>ST/DD</td>
<td>S484D, T484D</td>
</tr>
<tr>
<td>W30A, K31Q</td>
<td>Calmodulin binding mut</td>
</tr>
<tr>
<td>NTPB</td>
<td>N-term polybasic motif</td>
</tr>
<tr>
<td>CTPB</td>
<td>C-term polybasic motif</td>
</tr>
<tr>
<td>W30A, K31Q, ST/DD</td>
<td>Calmodulin + phosphomimetic</td>
</tr>
</tbody>
</table>
Inhibiting CaM Decreases Nuclear GRK5 Accumulation

* p<0.05 vs. veh, # p<0.05 vs. CDZ, n=3

Jessica Gold, Unpublished Results
CaM Increases GRK5 Nuclear Accumulation

Jessica Gold, Unpublished Results
Requirements for GRK5 nuclear localization

Jessica Gold, Unpublished Results
GRK5 activity, especially when enhanced, appears pathological in the heart and can cause maladaptive hypertrophy through nuclear activity.

Normal levels of GRK5 in the heart appears to play a role in cardiac hypertrophy. Perhaps a role for Q41L mutation?

GRK5 nuclear translocation and activity appears to be dependent on Ca/CaM downstream of Gq activation.

This nuclear activity may be a novel therapeutic target in maladaptive hypertrophy and HF.
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