A β1-adrenergic receptor/β-arrestin1-regulatable microRNA, miR-150 protects the mouse heart from ischemic injury by repressing pro-apoptotic egr2 and p2x7r

Il-man Kim, Assistant Professor, Ph.D.
Vascular Biology Center
Department of Biochemistry & Molecular Biology
Medical College of Georgia
Georgia Regents University

Financial Disclosure : None
Unlabeled/Unapproved Uses Disclosure : None
Research Interest

Transcriptional regulation and microRNA regulatory network by GPCR signaling in heart failure
Signal transduction by GPCRs (7TMRs): New paradigm

**Signalling** by GRK5/6
- Kinases (MAPK, PI3K, AKT)
- Transcriptional control
- Transactivation (EGFR)

** Trafficking** by GRK2/3
- Internalization
- Translocation

Concept of GPCR biased signaling & β-arrestin-biased ligands in therapy for HF

Ventricular dilation
Myocyte apoptosis
Cardioprotective
Antiapoptotic

HF: heart failure
β1AR: β1-adrenergic receptor
Alp: alprenolol
Car: carvedilol

Gap in the knowledge base to be addressed:

The precise molecular mechanisms in downstream pathways by which β-arrestin-biased ligands promote cardioprotective signaling pathways are not well understood.
Statement of need & objective evidence for its existence:

A proteomics study suggests that β-arrestins may regulate miR biogenesis (Xiao K. et al., 2007 *PNAS* 104:12011-12016).

MiR, which is increasingly recognized as a major regulator during cardiac remodeling, may be an underlying downstream mechanism by which 7TMR-mediated β-arrestin signaling pathways confer cardioprotection.
Statement of need & objective evidence for its existence:

Our published data using carvedilol suggest regulation of miR biogenesis by β1AR-mediated β-arrestin cardioprotective signaling pathways.

β-arrestin1-biased β₁-adrenergic receptor signaling regulates microRNA processing

β-arrestin1 is required for carvedilol-induced miR activation in vivo, which occurs at a post-transcriptional step.
Carv-dependent in vivo interaction of β-arrestin1 with hnRNPA1, a component of Drosha microprocessor complex.

β-arrestin1-biased β1AR signaling stimulates the processing of a subset of miRs in the heart

β1AR/β-арrestin1-regulatable miRs function as protective miRs in response to simulated IR of CMs

manuscript in preparation
MicroRNA-214 protects the mouse heart from ischemic injury by controlling Ca^{2+} overload and cell death.
Aurora AB^{1}, Mahmoud AI, Luo X, Johnson BA, van Rooij E, Matsuzaki S, Humphries KM, Hill JA, Bassel-Duby R, Sadek HA, Olson EN.


miR-199a-3p
MiR-150 KO mice have normal cardiac function and structure at baseline.
MiR-150 protects the heart against MI

manuscript in preparation
MiR-150 regulates pro-apoptotic *egr2 & p2x7r*

3' gugaccAUGUU-CCCAACCUCu 5' mmu-miR-150

339:5' uuuuuucUACAAUAGGUUGGGAGu 3' Egr2

792:5' uuaagcuauacaauUUGGGAgA 3' P2rx7

A

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>8 week MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egr2</td>
<td>1.0 ± 0.03</td>
<td>2.0 ± 0.15**</td>
<td>1.0 ± 0.05</td>
</tr>
<tr>
<td>P2x7r</td>
<td>1.0 ± 0.05</td>
<td>1.9 ± 0.17**</td>
<td>1.3 ± 0.25</td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

**B. NRVC**

<table>
<thead>
<tr>
<th></th>
<th>NRVC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative Fold Induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti-miR control</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti-miR-150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egr2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C

<table>
<thead>
<tr>
<th></th>
<th>HL-1</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>anti-miR control</td>
<td>anti-miR-150</td>
<td></td>
</tr>
<tr>
<td>Relative Fold Induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-150</td>
<td>***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D

<table>
<thead>
<tr>
<th></th>
<th>HL-1</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>miR overexpression</td>
<td></td>
</tr>
<tr>
<td>Relative Fold Induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-150</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>anti-miR-150</td>
<td>miR-150 mimic</td>
</tr>
<tr>
<td>Relative Fold Induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egr2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2x7r</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 manuscipt in preparation
β-arrestin1-biased β1AR signaling-mediated miR regulatory network: A new player in cardiac protection

A: Noma T et al, JCI. 2007; 117(9):2445-58

B: Kim IM et al. PNAS. 2008;105(38):14555-60

C: Circ Res. 2014, Feb 28

D: Aims for AHA GIA & SDG grants
Acknowledgments

Kim Lab
Yongchao Wang
Kyoung-mi Park
Yaoping Tang
Jian-peng Teoh
Qiuping Hu

GRU
VBC Metabolic Group
Huabo Su
Yaoliang Tang
David Fulton
Neal Weintraub
John Johnson

Duke University
Howard Rockman
Robert Lefkowitz

Temple University
Walter Koch

American Heart Association
Learn and Live
12GRNT12100048
14SDG18970040
GRK-mediated phosphorylation “bar code” into biased 7TMR signaling

β-arrestins as cytoplasm-nucleus messengers

Domain structure of β-arrestins