Embracing Bias
β1-Adrenergic Receptor–Biased Ligands and Nuclear miRNA Processing

Fadia A. Kamal, Joshua G. Travers, Burns C. Blaxall

Cardiovascular disease remains the leading cause of mortality and morbidity in the developed world, despite recent advances in therapeutic interventions, with an estimated annual cost in the United States alone of $312 billion. This emphasizes the demand for a greater understanding of the molecular mechanisms involved in cardiovascular pathophysiology and demonstrates the desperate need for innovative strategies for both treatment and prevention.2

Recent reports suggest an emerging role for microRNAs (miRs), small noncoding nucleic acid regulators of mRNA, in the development and progression of cardiovascular disease. miRs, a large family of highly conserved RNAs 18–25 nucleotides in length, are essential in the post-transcriptional regulation of gene expression. Typically, miRs are encoded within the introns of protein-coding genes; they also exist intergenically under the control of their own promoters.2,3 Transcription of these regions by RNA polymerase II generates miR precursors known as primary-miRs (pri-miRs), which are converted to mature miRs through the activities of 2 members of the RNase III family of enzymes. Cleavage of pri-miR by the enzyme Drosophila forms a ~70 nucleotide sequence, termed pre-miR, that is subsequently exported to the cytoplasm. Final processing by Dicer creates the ~20 nucleotide mature miR that incorporates into the miR-induced silencing complex, forming the active enzyme capable of inducing mRNA translational repression or degradation.2,4 Hundreds of distinct miRNAs can be influenced by an individual miR, allowing a single miR or family of miRs to coordinate substantial alterations in physiology and function collectively. It is increasingly clear that appropriate miR expression is crucial in cardiac development, function, and disease. Numerous cardiac complications, including myocardial infarction, hypertrophy, and remodeling, can be exacerbated by improper miR regulation.2,5 Intriguingly, pharmacological modulation of several miRs has been shown to reduce cardiac pathophysiology in animal models of disease.2,3 As miRs are implicated in multiple comorbidities associated with cardiovascular disease, the proteins that regulate their expression or activity may also represent important candidate drug targets.2 Many auxiliary proteins help to maintain the specificity and fidelity of Drosha and Dicer, the 2 enzymes principally responsible for miR processing. However, the identification of these factors remains incomplete, and further investigation is required to fully understand the mechanisms by which these proteins influence the generation and function of individual miRs.4,5

In this issue of Circulation Research, Kim et al6 investigate a new role for β-arrestin in the regulation of miR processing through a novel interaction with the pri-miR cleavage enzyme Drosha. The 2 β-arrestin isoforms (1 and 2) are traditionally recognized for their critical roles in G-protein–coupled receptor (GPCR) internalization and desensitization in response to sustained agonist stimulation. Conventionally, the binding of an agonist modifies receptor conformation, leading to the activation of G-protein–mediated pathways and a parallel recruitment of GPCR-kinases (GRKs). Phosphorylation of the agonist-bound receptor by these kinases stimulates β-arrestin–mediated internalization and termination of GPCR signaling. However, recent reports have identified a new pharmacological paradigm of GPCR signaling, in which β-arrestin–mediated, G-protein–independent signaling pathways are activated after the stimulation by certain biased ligands.7,8 Conventional antagonists compete with endogenous ligands for binding to the GPCR, resulting in an inactive receptor conformation and inhibition of further G-protein signaling. However, a biased antagonist, or a biased ligand, can cause analogous GPCR inactivation, while simultaneously activating β-arrestin–dependent, G-protein–independent signaling pathways. This concept of biased agonism involves a phenomenon by which specific GRKs create a distinct phosphorylation pattern within the receptor C terminus after ligand binding, creating a specific receptor bar-code that selects particular β-arrestin functions. Specifically, GRK2/3-mediated GPCR phosphorylation leads to receptor internalization and signal termination, whereas GRK5/6-mediated GPCR phosphorylation may promote G-protein–independent β-arrestin–mediated signaling.7 The biased ligand carvedilol, a β-blocker used extensively for the treatment of cardiovascular disease, is reported to exert β1, β2, and α1 adrenergic receptor blockade, in addition to its putative antioxidant effects. This concept of functional selectivity or biased agonism through β-arrestin–mediated pathways provides a potential explanation for the enhanced therapeutic properties of particular β-blockers, such as carvedilol. This is thought to be a consequence of β-arrestin1–mediated activation of cardioprotective epidermal growth factor receptor-extracellular-regulated kinase signaling.8,9 However, the current study by Kim et al6 suggests a potential therapeutic role for β-arrestin within the nucleus. Importantly, β-arrestin1 has been
shown to possess a nuclear localization signal, and unlike β-arrestin2, is retained within the nucleus, in part, because of the lack of a nuclear export signal. Recent findings support a nuclear role for β-arrestin1 in both the activation and the inhibition of gene transcription, revealing important functions in cell growth, apoptosis, and the immune response. Specifically, it was recently reported in cultured cells that β-arrestin1 translocates to the nucleus after β opioid receptor activation, leading to the recruitment of histone acetyltransferase p300 and the activation of CAMP response element binding protein mediated transcription. In a similar manner, β-arrestin1 can inhibit signal transducer and activator of transcription 1-mediated transcription through the recruitment of the tyrosine phosphatase TC45 after interferon-γ stimulation, also in cultured cells. β-arrestins are also capable of indirectly regulating gene transcription by interacting with the transcriptional regulators IκBα and MDM2 in the cytoplasm; however, little is known about their effects on microRNAs.

Recent studies indicate that β-arrestin1 may potentially interact with 2 components of the miR microprocessor complex: Drosha and hnRNPA1, an RNA-binding protein involved in RNA helicase-independent miR processing. Kim et al show a novel role for β-arrestin1 in promoting the post-transcriptional processing of a subset of miRs (β1-miRs) in human cells and murine hearts after stimulation by the β1-AR–biased ligand carvedilol. They demonstrate that carvedilol stimulation triggers GRK5/6 phosphorylation of the β1-adrenergic receptor (β1-AR) and mediates the recruitment of β-arrestin1 to the ligand bound receptor. This results in the translocation of β-arrestin1 to the nucleus where it activates Drosha-mediated miR processing by forming a nuclear complex of β-arrestin1-Drosha-hnRNPA1 that assembles specifically on pri–β1-miRs (Figure).

The authors first use genetically altered human embryonic kidney 293 (HEK293) cells to show that carvedilol stimulation upregulates the expression of human miR-190 in a β1-AR but not in a β2-AR or an α1,2-AR–dependent mechanism. Consistent with the bar-code phosphorylation hypothesis, carvedilol-mediated miR-190 upregulation was attenuated by knockdown of GRK 5 or 6 and β-arrestin1. The authors suggest that the carvedilol-mediated effect on miR regulation is post-transcriptional because there was elevated expression of mature and pre-miR but not pri-miR after stimulation with carvedilol.

To complement their in vitro findings, the authors transitioned their study to the murine heart and identified miR expression changes in response to 7-day treatment with carvedilol using a miR microarray profiling assay. Of 1040 individual miRs tested, 21 were found to be upregulated, and only 5 of which were confirmed by quantitative reverse transcriptase-polymerase chain reaction: 125a-5p, 125b-5p, 150, 199a-3p, and 214. Several of these miRs have previously been reported for their myriad roles in cardiovascular disease. It will be of significant future interest to determine whether a similar cadre of miRs are regulated by carvedilol in the setting of a myocardial insult, reflecting the condition of patients with heart failure in which carvedilol is generally used. Interestingly, miR-190, the main target of their in vitro studies in HEK293 cells, was not detectable in murine hearts. Using β-arrestin1 and β-arrestin2 knockout animals, the authors determined that β-arrestin1 was required for the carvedilol-mediated upregulation in these 5 miRs. Consistent with results observed in HEK cells, carvedilol could promote the expression of pre- and mature miRs but not of pri-miRs. Furthermore, upregulation in the expression of pre-miRs was dependent on the β1-AR-GRK5/6-β-arrestin1 signaling pathway. Various knockout mouse lines (Table) were used to explore the in vivo mechanisms involved in the carvedilol-mediated signaling pathways that modulate miR expression. The authors then performed RNA-Chip analysis in HEK293 cells cotransfected with pCMV-β1-miRs, tagged β-arrestin1, hnRNPA1 or Drosha, along with siRNAs targeting β-arrestin1 to suggest that the interaction between β-arrestin1 and hnRNPA1 is pri-miRNA dependent and is sensitive to RNase treatment. The knockout mouse models used by Kim et al in their article not only served as valuable tools in the identification of subtype-specific functions of β-ARs but also in appreciating adaptive, alternative mechanisms that maintain critical physiological processes in the cardiovascular system when cellular signaling mechanisms are disturbed. Although these conclusions are provocative, the variable cardiac phenotypes reported for these knockout models, especially the high pre-natal lethality of the β1-AR knockout mice, may affect experimental outcomes and complicate the conclusions that can be drawn (Table).

Overall, the findings presented by Kim et al suggest that β-arrestin1 mediates the post-transcriptional processing of miRs through its interaction with both pri-miRs and the Drosha

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Phenotype of Targeted Gene Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1-AR KO</td>
<td>High prenatal lethality</td>
</tr>
<tr>
<td>β1-AR KO</td>
<td>Lack of cardiac chronotropic and inotropic responses to isoproterenol</td>
</tr>
<tr>
<td>β1-AR/β2-AR DKO</td>
<td>Compensatory alterations in cardiac muscarinic receptor density and vascular β1-AR responsiveness</td>
</tr>
<tr>
<td>β-AR KO</td>
<td>Enhanced contractility and vessel reactivity in response to sodium nitroprusside</td>
</tr>
<tr>
<td>β-arrestin1 KO</td>
<td>Impaired contractility in response to sodium nitroprusside</td>
</tr>
<tr>
<td>β-arrestin2 KO</td>
<td>Impaired lymphocyte chemotaxis after CXCR4 stimulation</td>
</tr>
<tr>
<td>GRK5 KO</td>
<td>Attenuated hypertrophy and hypertrophic gene transcription after TAC and chronic phenylephrine infusion</td>
</tr>
<tr>
<td>GRK6 KO</td>
<td>Impaired lymphocyte chemotaxis after CXCR4 stimulation</td>
</tr>
</tbody>
</table>

AR indicates adrenergic receptor; CXCR, C-X-C chemokine receptor; DKO, double knockout; GRK, G-protein–coupled receptor kinase; and TAC, transverse aortic constriction.
microprocessor complex after biased ligand binding of the \( \beta_1 \)-AR with carvedilol. Further studies are warranted to confirm the effect of \( \beta_1 \)-AR–mediated miRNA processing on the expression of proteins involved in cardiac physiology. Importantly, it will be crucial to study activation of this putatively salutary pathway in the clinical setting of heart failure, where carvedilol is commonly used to attenuate excess adrenergic stimulation of the heart. Moreover, similar to previous data from the Rockman laboratory that demonstrated \( \beta_1 \)-AR–mediated \( \beta \)-arrestin-epidermal growth factor receptor transactivation, the mechanism suggested by Kim et al for the interaction between \( \beta \)-arrestin1, Drosha, pri-miR, and hnRNPA1 was determined largely in HEK293 cells. Although this elucidates a possible mechanism, it may not entirely represent in vivo mechanism and thus may limit the implications of the findings because HEK293 cells do not necessarily recapitulate cardiovascular signaling pathways. Future studies using primary cardiac cell culture from animals with normal and injured myocardium will provide valuable, confirmatory evidence for this regulatory mechanism within the cardiovascular system.

Finally, because of the complexity of miR regulation and processing, treatment of chronic conditions, such as cardiovascular disease by manipulating miR expression, provides a formidable yet not insurmountable challenge. Although inhibition of miRs was successfully and safely achieved and reported in the first phase 2a clinical trial of its kind in patients with hepatitis C, overexpression of miRs as a therapeutic strategy may be far more challenging. To this end, the development and study of high-affinity \( \beta_1 \)-AR–biased ligands that activate \( \beta \)-arrestin1–mediated miR regulation, as outlined in the current work by Kim et al, provide a promising novel therapeutic strategy for cardiovascular disease.

**Disclosures**

None.
References


Key Words: Editorials • beta1-adrenergic receptor • beta arrestin 1 • biased ligands • G-protein coupled receptor • microRNA
Embracing Bias: β1-Adrenergic Receptor–Biased Ligands and Nuclear miRNA Processing
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Circ Res. 2014;114:742-745
doi: 10.1161/CIRCRESAHA.114.303237
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/114/5/742

An erratum has been published regarding this article. Please see the attached page for:
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In the *Circulation Research* article by Kamal et al (Embracing Bias: β₁-Adrenergic Receptor–Biased Ligands and Nuclear miRNA Processing. *Circ Res.* 2014;114:742–745. DOI: 10.1161/CIRCRESAHA.114.303237), corrections were needed.

In many instances, “β₁-AR” was incorrectly expanded during copyediting to “β₁-arrestin”. Also, in the Table footnote abbreviations, “TAR” has been corrected to “TAC”.

The errors have been corrected in the online version of the article, which is available at http://circres.ahajournals.org/content/114/5/742.
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