The Role of MicroRNA-133 in Cardiac Hypertrophy Uncovered

Maha Abdellatif

Studies of microRNAs (miRNAs) have recently uncovered a new level of intricacy in the regulation of gene expression during organogenesis and pathogenesis. miR-133, which is enriched in cardiac and skeletal muscle, is involved in cell specification, differentiation, and development. It is also downregulated during cardiac hypertrophy, which suggested that it may play a role in the underlying pathogenesis. Studies that included targeted deletion, overexpression, and antisense-specific knockdown of miR-133 have unraveled many aspects of its function and targets but have not directly revealed its role in the development of pressure overload–induced hypertrophy. In particular, targeted deletion of miR-133a-1 or miR-133a-2, each of which resulted in a 50% reduction in cardiac miR-133, equivalent to the decrease associated with cardiac hypertrophy, exhibited no cardiac growth or functional abnormalities under normal or pressure overload conditions. On the other hand, complete ablation of miR-133 via double knockout resulted in aberrant proliferation and apoptosis of myocytes, cardiac defects, and prevalent embryonic lethality, whereas those that escaped the lethal phenotype ended with severe cardiac dilatation, but no myocyte hypertrophy. In contrast, knockdown of miR-133 via antisense targeting was sufficient for inducing cardiac hypertrophy and reinduction of the fetal gene program in the adult mouse heart. The discrepancy between the outcomes could be explained by the different onsets of miR-133 knockdown in the heart. However, none of the studies investigated the relevance of the decrease in miR-133 in the context of pressure overload hypertrophy.

It should be noted that whereas overexpression or knockout of a gene in a normal cell can provide invaluable insight regarding its function, normalizing its levels during a specific pathological condition is requisite for obtaining direct evidence regarding its role in the underlying pathogenesis. Indeed, adopting this approach was one of the advantages of the study by Matkovich et al that is reported in this issue, in which they generated a transgenic mouse model overexpressing an α-myosin heavy chain–regulated miR-133 transgene compensated for the reduction in endogenous miR-133 in the transgenic heart, a situation in which the overexpressed transgene compensated for the reduction in endogenous miR-133. Although cardiac weight was not normalized as a result of this intervention, other aspects of hypertrophy (including apoptosis, fibrosis, and the downregulation of \( I_{\text{Ca}} \)) were restored to baseline levels, whereas the decline in \( I_{\text{Ca,f}} \) and prolongation of QT interval were partly reversed. The decrease in fibrosis in the transgenic mouse could not be explained by suppression of connective tissue growth factor, which is a validated miR-133 target in cardiac myocytes and fibroblasts; it is most likely a consequence of reduced myocyte dropout, infiltration, fibroblast proliferation, and contrast to a β-myosin heavy chain–driven miR-133, these animals survived with no abnormalities other than a modest prolongation of the QT interval. The lack of more severe aberrations, which may have been expected from the suppression of some of the validated targets of miR-133 (Table), could be explained by the possibility that miR-133 under normal conditions is saturating with respect to some of its target mRNAs. However, the function of miR-133 was exposed after applying pressure overload to the miR-133 transgenic heart, a situation in which the overexpressed transgene compensated for the reduction in endogenous miR-133. Although cardiac weight was not normalized as a result of this intervention, other aspects of hypertrophy (including apoptosis, fibrosis, and the downregulation of \( I_{\text{Ca}} \)) were restored to baseline levels, whereas the decline in \( I_{\text{Ca,f}} \) and prolongation of QT interval were partly reversed. The decrease in fibrosis in the transgenic mouse could not be explained by suppression of connective tissue growth factor, which is a validated miR-133 target in cardiac myocytes and fibroblasts; it is most likely a consequence of reduced myocyte dropout, infiltration, fibroblast proliferation, and

Table. Validated miR-133 Targets

<table>
<thead>
<tr>
<th>Tissue or Cell Type</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac myocytes</td>
<td>Ras homolog gene family, member A (RhoA)</td>
<td>6</td>
</tr>
<tr>
<td>Cardiac myocytes</td>
<td>Cell division cycle 42 (Cdc42)</td>
<td>6</td>
</tr>
<tr>
<td>Heart/293HEK cells*</td>
<td>Ether-a-go-go-related potassium channel (HERG)</td>
<td>12</td>
</tr>
<tr>
<td>Heart/293HEK cells*</td>
<td>Hyperpolarization activated cyclic nucleotide-gated potassium channel 2 (HCN2)</td>
<td>13</td>
</tr>
<tr>
<td>Heart/Cos-1 cells*</td>
<td>Cyclin D2</td>
<td>4</td>
</tr>
<tr>
<td>H9c2</td>
<td>Caspase-9</td>
<td>10</td>
</tr>
<tr>
<td>Cardiac myocytes/Cos*</td>
<td>Connective tissue growth factor (CTGF)</td>
<td>9</td>
</tr>
<tr>
<td>C2C12</td>
<td>Serum response factor (SRF)</td>
<td>3</td>
</tr>
<tr>
<td>MC3T3</td>
<td>Runt related transcription factor 2 (Runx2)</td>
<td>14</td>
</tr>
<tr>
<td>Dopaminergic neurons</td>
<td>Paired-like homeodomain 3 (Pitx3)</td>
<td>15</td>
</tr>
<tr>
<td>Zebrafish</td>
<td>TTK family protein kinase (Mps1)</td>
<td>16</td>
</tr>
<tr>
<td>Lung cancer cell line</td>
<td>BCL2-like (BCL2L)</td>
<td>17</td>
</tr>
<tr>
<td>Lung cancer cell line</td>
<td>Myeloid cell leukemia sequence 1 (MCL-1)</td>
<td>17</td>
</tr>
</tbody>
</table>

*The target was confirmed by miRNA-dependent inhibition of the endogenous protein in the heart or cardiac myocytes and by the luciferase reporter assay in 239 HEK or Cos cells.

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collagen deposition. Accordingly, this was associated with less ventricular stiffness and lower left ventricular end-diastolic pressure. One shortcoming of the study, though, is that the miR-133 targets that mediates its antiapoptotic function remain to be identified, because the authors excluded the previously validated caspase-9,10 serum response factor, and cyclin D2 targets in the heart.

One of the intriguing findings of this study is that whereas overexpression of miR-133 reduced the density of I<sub>o,h</sub> in the unstimulated heart, it prevented any further decline associated with increased pressure overload. This apparent paradox can be explained by the fact the I<sub>o,h</sub> accessory subunit, K<sub>cnip2</sub>, whose expression was inhibited in the unstimulated transgenic heart, is not a direct target of miR-133. Thus, the effect of miR-133 on this protein might have been reversed as a result of the reprogramming of gene expression in the hypertrophied myocytes. This emphasizes the need to explore the functions of miRNAs under different developmental, normal, stressed, and pathological conditions to identify the full range of their effects and their potential for therapeutic targeting. A similar argument applies to the verification of the targets of miRNAs in a particular cell type, because “one for many and many for one” characterizes the relation between miRNAs and their targets. This implies that under different conditions, the effect of a miRNA on a target gene is modulated by other coexisting differentially expressed miRNAs and/or RNA-binding proteins that may target the same gene. This explains why some validated targets cannot be confirmed under different experimental conditions. In addition, variations in a specific miRNA:target mRNA stoichiometry in different cellular backgrounds would inevitably impact the outcome of experimental manipulations of the miRNA level. For example, if miR-133 were saturating relative to any of its target mRNA during normal conditions, overexpressing it would be superfluous and, hence, ineffective. In contrast, a similar manipulation during cardiac hypertrophy, where its endogenous levels are downregulated, is apt to reveal its function, as indeed is demonstrated by the work of Matkovich et al.8 This would also argue for the need to reexamine the levels of caspase-9, serum response factor, and cyclin D2 in the miR-133 transgenic mouse during pressure overload.

In conclusion, although reducing miR-133 in a normal adult cell background was sufficient to induce cardiac hypertrophy,8 restoring its levels during pressure overload did not prevent it but did repress apoptosis, fibrosis, and K<sup>+</sup> channel remodeling.8 This leads to the conclusion that miR-133 does not play an obligatory role in the mechanism underlying the increase in myocyte size during pathological hypertrophy. However, its downregulation is a prerequisite for the development of apoptosis, fibrosis, and prolongation of the QT interval. The different outcomes that were observed following experimental modulation of miR-133 levels in different settings emphasize the fact that the transcriptome/proteome makeup of the cell influences miRNA functionality.

Finally, although we now have a good understanding of the role of miR-133 during cardiac hypertrophy, the full range of targets that mediate its effects remain to be identified and characterized. One of the reasons that miRNAs may potentially serve as more effective therapeutic targets is their capacity to regulate an array of genes, which collaborate in a particular cellular function, versus a single gene. Thus, to fully exploit miRNAs for therapeutic purposes, it is necessary not only to identify and validate all targets but also to examine their functional interactions.11

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Disclosures
None.

References

Non-standard Abbreviation

<table>
<thead>
<tr>
<th>miRNA</th>
<th>microRNA</th>
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Disclosures
None.


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