Great Expectations
MicroRNA-30d and Cardiac Resynchronization Therapy

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The broken heart. You think you will die, but you just keep living, day after day after terrible day.

Charles Dickens’ Great Expectations

There seem to be as many approaches to managing heart failure as there are causal factors. Heart failure management is evolving from a one-size-fits-all approach centered around therapy with neurohormonal antagonists toward strategies tailored to provide the optimal clinical benefit based on the individual patient profile. Thus, a major collective enterprise of the translational research community has been to identify subsets of patients that will derive greater benefit from one or another management scheme. The initial observation that, like cancer and other pathologies, microRNAs are regulated in heart disease was coupled with observations that microRNAs are found circulating in stable form in the blood to raise expectations that microRNAs would prove useful as disease biomarkers, providing insights into the aspects of heart disease not revealed through traditional clinical testing. In the current issue of Circulation, Melman et al propose miR-30d as a biomarker for heart failure responsiveness to cardiac resynchronization therapy. This article aptly illustrates the promise, problems, and pitfalls with the current state of evaluating microRNAs as biomarkers of cardiovascular disease. To quote Pip’s sister in Great Expectations: “Answer him one question, and he’ll ask you a dozen directly.”

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Melman et al initially identified miR-30d as one of several microRNAs exhibiting statistically significant differences or a strong trend toward being differentially abundant in the plasma of patients who had undergone cardiac resynchronization therapy. The pilot microarray study of 12 male patients with nonischemic cardiomyopathy reflected the 2 extremes of response: 6 nonresponder subjects had no change or a decrease in left ventricular ejection fraction (LVEF), and 6 responder subjects exhibited marked functional improvement (increase in LVEF averaging 24%). In an expanded cohort of 40 subjects, 3 of the microRNAs were confirmed as having significantly different circulating levels between categorical responders (here defined as an increase in LVEF of at least 10%) and categorical nonresponders (all others). The correlation data between log2 relative [miR-30d] and the change in LVEF after synchronized biventricular pacing are depicted in Figure 2B of the article, and are redrawn here and modified for the purposes of illustration (Figure). The correlation coefficient is reported as 0.39, P=0.01. These data reveal a 10 log2 range in relative miR-30d concentration. After censoring 2 outliers in which miR-30d levels are 2 and 4 logs greater than the highest values reported for the other 40 subjects (Figure), the authors indicate that the correlation between miR-30d plasma concentration and change in %LVEF after resynchronization no longer achieved significance. However, binary probabilistic modeling of categorical discrimination between responder and nonresponder using circulating miR-30d levels as the variable (Figure 2C of Melman et al) remained significant after removing the outliers.

What is currently known about miR-30d in the heart? Interrogation of extensive human and mouse heart miR-Seq data deposited in public databases reveals that miR-30d is 1 member of a family of identical or nearly identical microRNAs (miR-30a, b, c, d, and e) that are abundant and present at remarkably similar levels in normal hearts of humans and mice (Tables 1 and 2, Table I in the online-only Data Supplement). In both human and mouse hearts, 4 of the 5 miR-30 family members are among the top 20 most abundant cardiac microRNAs: miR-30a is the most abundant miR-30 family member, and the 9th most abundant cardiac microRNA in both species. miR-30d is the 11th most abundant miR in normal human hearts and the 12th most abundant miR in mouse hearts. Although the sequencing read data are reported differently in another small RNA sequencing study, miR-30e, -30d, -30a, -30c, and -30b were ranked the 14th, 15th, 17th, 26th, and 35th most abundant myocardial microRNAs. In failing human hearts, cardiac levels of miR-30 family members are either similar to reference or modestly lower than reference nonfailing human myocardium (Table 1, Table I in the online-only Data Supplement). The latter study also assayed plasma samples and found that the most abundant miR-30 family member in plasma (assayed by small RNA sequencing) was miR-30d, which ranked as 12th most abundant and was present at =10% of the level of the most abundant circulating microRNA, miR-21. Neither cardiac nor circulating blood microRNA sequencing data are available for experimental murine heart failure, but the entire miR-30 family is downregulated =20% in the myocardium of mice early after experimental pressure overload induced by surgical transverse...
Previous reports have suggested that plasma miR-30d levels are a biomarker for type 2 diabetes mellitus, whereas whole blood levels of miR-30c are part of a panel of regulated circulating microRNAs associated with myocardial infarction. miR-30d was recently observed to mediate programmed cardiomyocyte death in diabetic cardiomyopathy, which contrasts with the antiapoptotic actions reported by Melman et al.6

It is not clear how increased circulating plasma miR-30d levels might identify patients with heart failure more likely to respond to ventricular resynchronization. A recent microarray microRNA profiling study detected a decrease in circulating miR-30d in human heart failure, which contrasts with the observation by Melman et al6 that circulating miR-30d and the other miR-30 family members are more abundant in cardiac myocytes than in nonmyocytes.

Table 1. Cardiac microRNA Sequence Data for miR-30 Family Members in Human Hearts (GSE46224)

<table>
<thead>
<tr>
<th>Rank</th>
<th>miR</th>
<th>Nonfailing</th>
<th>Non-isch CM</th>
<th>ich CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>hsa-miR-30b-5p</td>
<td>32 767</td>
<td>33 489</td>
<td>32 073</td>
</tr>
<tr>
<td>10</td>
<td>hsa-miR-30d-5p</td>
<td>26 362</td>
<td>26 716</td>
<td>25 995</td>
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<tr>
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<td>hsa-miR-30e-5p</td>
<td>12 557</td>
<td>13 241</td>
<td>13 022</td>
</tr>
<tr>
<td>19</td>
<td>hsa-miR-30b-3p</td>
<td>11 292</td>
<td>8603</td>
<td>8623</td>
</tr>
<tr>
<td>27</td>
<td>hsa-miR-30c-5p</td>
<td>6777</td>
<td>5761</td>
<td>5380</td>
</tr>
</tbody>
</table>

Sequence data are expressed as reads per million aligned microRNA reads. GSE numbers are NCBI Gene Expression Omnibus subseries files for the original sequence data from references. CM indicates cardiomyopathy; and isch, ischemic.

or preparation complicate direct comparative analyses of these types of data.

Because all of the miR-30 family members are highly abundant in hearts, miR-30d may be passively released by injured or dying cardiomyocytes. Importantly however, Melman et al6 detected an increase in vesicular miR-30d released from cultured neonatal rat cardiac myocytes subjected to repetitive mechanical deformation, suggesting that miR-30d can be actively secreted. Nevertheless, stretching cultured neonatal cardiomyocytes is an imperfect model of ventricular dys-synchrony, and it is important to consider that increases in steady-state plasma levels of a given microRNA may reflect greater miR secretion, enhanced miR biosynthesis, increased miR stability, or a combination of these mechanisms.

The promise of a new biomarker is that it will improve clinical discrimination for the condition that it marks, in comparison with the existing gold standard. The current gold standard for predicting a favorable response to ventricular resynchronization in heart failure is electrocardiographic QRS duration. As a categorical variable, QRS duration was part of the inclusion criteria for the study by Melman et al6. However, likely because of limited cohort sizes, QRS duration did not predict resynchronization response. Thus, it is not possible to compare the new biomarker with the existing gold standard here because the gold standard failed. It will be interesting to see if larger studies might in the future discover that the lack of exact predictive concordance between circulating miR-30d and QRS duration is advantageous, reflecting different aspects of heart failure that considered in combination constitute a superior response biomarker to either factor alone.

As noted by the authors, additional work is necessary before miR-30d can be accepted in either its proposed clinical role as a biomarker for predicting the success of ventricular resynchronization therapy for heart failure, or mechanistically as a factor that is dynamically regulated in heart failure and favors cell growth over cell death. These uncertainties are certainly not unique to miR-30d, and seemingly reflect the current state of the field. Here, I will follow Mr. Jaggers’ advice to Pip in Great Expectations, “Take nothing on its looks; take everything on evidence” and suggest ways in which research in this rapidly evolving area might evolve to fully meet our expectations:

1. When possible, initial biomarker discovery studies of circulating microRNAs should use next-generation sequencing. Hypothesis-generating research too often has to compromise breadth of data coverage to ensure the accuracy of the read-out, or vice versa. Fortunately, next-generation sequencing is both the most comprehensive and most accurate method to profile RNA species of all sizes. Coverage is essentially complete; if a microRNA is present in even miniscule amounts, it will be detected provided that the sequencing depth is sufficient. miR-Seq provides better accuracy for microRNAs that undergo alternative processing (known as isomiRs) and that may not be readily detected by standard arrays or quantitative reverse transcription polymerase chain reaction. Furthermore, RNA sequencing is inherently quantitative, whereas “spike-in” protocols, such as the one used by Melman et al6 to normalize quantitative...
reverse transcription polymerase chain reaction results, have limitations that include differing stabilities of the endogenous and exogenous microRNAs. miR-Seq results are not adversely impacted by annotation errors in miR sequence that can impair the efficacy of array probes and polymerase chain reaction primers designed around erroneous sequences. A recent comparison of quantitative microRNA expression platforms concluded that the standard analytic filters used for comprehensive sequencing may reduce the sensitivity of this technique for extremely rare RNAs. However, in comparison with dedicated quantitative polymerase chain reaction, sequencing was unparalleled for capturing expression differences.

2. Case-control associations should be validated in independent populations. The essence of experimental validation is independent replication. In the current era of comprehensive genomics, it is standard practice for an initial report of a given genotype-phenotype association to demonstrate independent replication of the index finding in ≥1 separate populations, as our group did some years ago with a common heart failure–associated polymorphism at 1p36.13. Of course, true validation requires that separate groups confirm the findings in even more independent cohorts, as was accomplished with 1p36.13. Adding additional subjects to an index cohort, while helping to increase statistical confidence, is not sufficient for independent validation.

3. The predictive value of a biomarker, either for at-risk populations or individuals, must ultimately be demonstrated prospectively. Even when a statistical association is demonstrated in multiple independent case-control populations, correlation data are not sufficient to demonstrate the efficacy for a given metric to stratify individual risk or response. Indeed, there are many valid associations between the levels of a marker and the prevalence of a condition that, when applied to individuals, are poor clinical stratifiers or predictors because the correlation is not sufficiently tight, the variance is too great, or the marker is confounded by extraneous environmental or comorbid factors. It can be argued that including actual predictive data for individuals is too high a bar to set for initial biomarker discovery and characterization, in favor of binary probabilistic modeling. Fortunately, it is not necessary to actually perform a prospective trial to assess the predictive value of a novel biomarker. Existing outcomes data for the condition of interest can be analyzed blindly to ascertain the predictive efficacy of the prospective biomarker according to models developed from the discovery data. Such analyses performed for individuals within the discovery cohort can help gauge real-world clinical utility of the biomarker and are useful to optimize the design of larger studies with longer follow-up, as proposed by Melman and colleagues.

Drs Melman, Das, and colleagues are to be congratulated for attempting such a challenging project. A biomarker that improves the selection of heart failure cases for ventricular resynchronization has the potential to improve patient care, lower medical costs, and prevent ineffective device implantation in nonresponders. Circulating microRNAs seem to be the next big thing in biomarkers, and for good reason. The current study adds to accumulating data supporting the concept that circulating microRNAs will become useful as biomarkers of heart disease, and extends this notion to a biomarker predicting the specific therapeutic response to a form of targeted disease management. Circulating microRNAs would be readily obtainable from patients via simple venipuncture, and a limited panel of dozens or hundreds of validated microRNA biomarkers could be assayed in whole blood, serum, or plasma by using standardized arrays; such data would be comparable across different centers. Because release or secretion of microRNAs is likely modulated by features of cardiovascular diseases that differ from those that promote the release of circulating protein biomarkers assayed by standard clinical tests, one of the expectations is that circulating microRNA biomarkers will add to our understanding of disease pathogenesis and refine clinical decision making. For this purpose, whether a circulating microRNA is actively or passively released, and the extent to which it induces a biological effect (as via cell-to-cell communication), does not impact its utility as a biomarker. Both issues are interesting and important, but are capable of being addressed independently.

Disclosures

None.

References

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Circulation. 2015;131:2172-2175; originally published online May 20, 2015;
doi: 10.1161/CIRCULATIONAHA.115.017176

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