MicroRNA-223 coordinates cholesterol homeostasis

Vickers et al
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Cholesterol homeostasis is a tightly regulated process. Previous work has been dominated by sterol-response element–related mechanisms, but the influence of micro-RNAs (miRs) is increasingly recognized, with miR-223 becoming a recent member of this group.

Several micro-RNAs (miRNAs or miR-) have been reported to be involved in cholesterol homeostasis.\(^1\)\(^-\)\(^4\) In a recent study, Vickers et al\(^5\) presented miR-223 as an important new player in this arena, one that they propose to be a coordinator of cholesterol homeostasis.\(^5\) This miRNA had been previously found to be rich in myeloid cells, where it regulates differentiation and inflammation,\(^6\)\(^-\)\(^8\) but the authors found that its abundance in hepatic cells is at least comparable with that of many functionally validated miRNAs. A relationship between miR-223 and cholesterol metabolism was suggested by finding its expression in human hepatoma (Huh7) cells was positively associated with intracellular cholesterol levels. In a series of studies in Huh7 cells, it was then shown that miR-223 (1) inhibited high-density lipoprotein cholesterol (HDL-C) uptake, (2) promoted cholesterol efflux, and (3) suppressed cholesterol biosynthesis. The authors also showed increased cholesterol levels in liver and plasma of miR-223 knockout mice, extending its involvement in cholesterol homeostasis in vitro to the in vivo setting.

The experimental approaches taken are well established in the miRNA field and were coupled to a productive use of bioinformatics. This included investigating whether the effects of miR-223 on a particular RNA were direct, by cloning into a luciferase reporter plasmid the putative target sequence in the 3’-UTR of that mRNA (or this sequence deleted in nucleotides required for miR-223 binding). The effect of exogenously supplied miR-223 on luciferase activity in transfected HEK293 cells was then measured. Thus, they determined that the molecular mechanisms for the functional reductions in HDL-C uptake and cholesterol biosynthesis after miR-223 treatment included its direct effect on the miRNAs encoding SR-BI (SCARB1) for the former process and HMG CoA synthase 1 (HMGC51) and methylsterol monoxygenase 1 (SC4MOL) for the latter.

In contrast to HDL-C uptake and cholesterol synthesis, the promotion of cholesterol efflux from Huh7 cells to apoA1, which is mediated by ABCA1, was shown to be indirect. Transcription factor analyses led them to consider Sp1 and Sp3 as the effectors; Sp1 is a known transcriptional activator of ABCA1 and Sp3 is an antagonist of Sp1. Both miRNAs harbor putative miR-223 target sites, but only Sp3 mRNA levels were reduced by miR-223 overexpression, and in luciferase reporter assays, the putative target site was confirmed as functional. Thus, the proposed scenario is that the reduction in Sp3 by miR-223 allows Sp1 greater positive sway over the ABCA1 promoter, with more efflux-promoting ABCA1 protein resulting. Consistent with this scenario was that when Sp3 itself was knocked down by siRNA, there was significant upregulation of ABCA1 gene expression, and with miR-223 overexpression, Sp3 nuclear binding activity decreased.

A major insight into the coordinated regulation of cholesterol homeostasis alluded to in the title of the article and referred to above also came from the Sp3 studies. The transcription factor analyses also revealed that there were a total of 24 Sp3 target genes significantly altered by miR-223 overexpression. This led the authors to predict that there are feedback loops. In silico probing of the promoter of miR-223 found one candidate Sp3 binding site and one dual Sp1/Sp3 site. When Sp3 was knocked down in Huh7 cells, there was a 6x increase in miR-223 levels. Thus, miR-223 and Sp3 regulate each other, fulfilling the authors’ prediction of a feedback loop.

As noted above, the in vitro methodologies that the authors used are standard to the field and the data presented are largely supportive of their interpretations. Nonetheless, there are some issues to consider before going on to the in vivo results. First, they relied on a single-cell model, the Huh7 line, which we have shown to significantly differ from primary hepatocytes in many aspects of lipoprotein metabolism.\(^9\) Future work should also use primary human hepatocytes, which are available from many sources. They also do not investigate cholesterol homeostasis in macrophages, despite the acknowledged roles of miR-223 in these cells, and the importance of macrophage ABCA1-mediated cholesterol efflux in atherosclerosis. For completeness, another major cholesterol efflux pathway in macrophages, which is regulated by ABCG1, should also be investigated.
It is also notable that the mature form of miR-223 is downregulated to well <50% within 8 hours after cholesterol depletion in Huh7 cells. Although primary miR-223 transcription was substantially suppressed, considering that the average half-life of miRNA is 5 days, the response seems to be too rapid for exclusive regulation by transcription. Given that miR-223 has been shown by the authors to be in the circulation, perhaps its secretion is also regulated by cholesterol depletion, which can be experimentally determined. Another interesting aspect of the levels of miR-223 is that despite its abundance being comparable with other validated hepatic miRNAs, many more significant metabolic effects in the article were observed by overexpressing miR-223 than by repressing it, suggesting that under normal conditions, it is still relatively low in abundance. This may point to a role of miR-223 more as a fine tuner of cholesterol homeostasis rather than as a master regulator. It is also possible that the overexpression results were confounded by promiscuous and artifactual targeting of nonmiR-223 regulated mRNAs or by overloading RISC and causing additional effects not related to direct targeting of the miR-223 regulated mRNAs.

The integration of the regulatory modules that were gleaned by the studies in vitro is shown in the Figure. The authors sought to extend this scheme to an in vivo model and turned to the miR-223−/− mouse. One major limitation in this aspect of the article is that SR-BI is not a target of miR-223 in mice. Thus, the effects in vivo of miR-223 on HDL-C uptake, an important component of the Huh7 studies, cannot be explicitly discerned. Nonetheless, there were still many interesting findings in the mouse studies. Most notably, there were increased levels of cholesterol in the plasma (mainly in large HDL particles) and liver (in the form of free cholesterol) of miR-223−/− mice, consistent with decreases in miR-223 suppression of cholesterol biosynthesis and promotion of cholesterol efflux. It was not surprising, then, that the expression of the cholesterol synthesis gene HMGCS1 was upregulated, as it was in vitro when miR-223 was inhibited, but it is perplexing that the expression of the efflux factor ABCA1 was not changed. This may have been because of the more complex, indirect, regulation of ABCA1 by miR-223, which also raises the issue that the hundreds of other genes found altered in the livers of these mice (>900) may be contributing many epistatic or modifying influences on the actions of miR-223 not just on ABCA1 expression, but also on multiple other metabolic factors and pathways.

In miR-223−/− mice, plasma triglyceride and phospholipid levels were also elevated, but liver levels were not significantly altered. There was some suggestion in Huh7 cells that inhibition of miR-223 increased triglyceride and fatty acid synthesis. If this were true at the level of the mouse liver, then one plausible explanation for the findings in vivo is that there is increased loading of very low-density lipoprotein with these lipids, thereby preventing any hepatic accumulation. This possibility can be explored in vivo using methods well established for this purpose.

The authors note the previous dominance of the sterol-response element mechanisms in the thinking about cholesterol homeostasis. Citing the results from the gene expression analysis in the miR-223−/− livers, in which many genes expected to be downregulated by the increased cholesterol content were, in fact, upregulated, they posit that this disconnect supports an important role of miR-223 and perhaps other factors in responding to sterol pressure in addition to the sterol-response element pathway. Undoubtedly this is true, given the accumulating and sobering results from systems biology that few pathways are immune to perturbations in others, but whether there will be the same translational potential for miR-223 as for the sterol-response element pathways (ie, statins) remains to be seen. One translational possibility suggested by the authors is based on their previous work that extracellular miR-223 is present in plasma complexed to HDL and its level was significantly increased in hypercholesterolemic humans (and mice). They now speculate whether the circulating miR-223 level may serve as a biomarker altered cholesterol homeostasis. This is an intriguing possibility that probably could be investigated by analysis of plasma samples already in storage from clinical studies.

Another translational possibility suggested was atherosclerosis. Here, the situation could be complex. For example, in addition to the unknown effects of the hundreds of genes altered by miR-223 status, the therapeutic direction taken suggested by the authors—that of increasing miR-223—may interfere significantly in SR-BI function in the liver. In mice deficient or low in SR-BI expression, it has previously been shown that this results in increased atherosclerosis, presumably because HDL-C uptake by the liver, required for effective reverse cholesterol transport, is impaired. Symmetrically, overexpression of SR-BI in mice is atheropreventive. Whether other effects of miR-223 treatment on cholesterol metabolism would outweigh decreased SR-BI expression, therefore, is an open question, even in mice, as the authors admit.
 Nonetheless, although still preliminary in terms of the full scope of its mechanistic effects and the translational implications, Vickers et al is an important step in extending our knowledge of the complex regulation of cholesterol metabolism and we look forward to subsequent reports with keen interest. We are nearing the 30th anniversary of the Nobel Prize to Michael Brown and Joe Goldstein for their groundbreaking discoveries on cholesterol homeostasis, and as great as that accomplishment is, the current report is an apt reminder that there is still much more to learn.

Disclosures

None.

References

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