To the Editor:

With interest, we read the recent article by Wilson et al1 in Circulation: Cardiovascular Genetics describing the role for miR-499 in dynamic microRNA (miRNA) expression programs during cardiac differentiation of human embryonic stem cells (hESCs). The authors demonstrated that a signature group of miRNAs is present in hESCs, whose expression is significantly altered after cardiomyogenic differentiation. We agree that improved understanding of cardiomyogenic differentiation is important, thereby including miRNA regulation. However, we believe that to understand general miRNA regulation and to interpret in silico prediction analysis, further studies and confirmations are needed, including other available cell sources.

In our previous study,2 we used undifferentiated human cardiomyocyte-derived progenitor cells (hCMPCs) and hCMPCs fully differentiated into cardiomyocytes (hCMPC-CM). We were pleased to read that the authors found several cardiac-related miRNAs, like miR-1, -133, -208, and -499, to be upregulated in hESC-CM (Figure 2f1), which supports earlier findings showing that these miRNAs are highly upregulated in hCMPC-CM2 and in cardiac-differentiated ESCs.3 Moreover, miR-125, -143/-145, -199a/-214, and -27b (Figure 2d) were also highly increased in hCMPC-CM, and miR-18a, -19, -20, -25, -663, -92, and -93 (Figure 2e) were significantly decreased in hCMPC-CM.1 Interestingly, the embryonic-associated miRNAs (Figure 2c1) were not observed in our undifferentiated hCMPCs,2 suggesting that these miRNAs are related to an ESC pluripotent state and not expressed in progenitor cells that are predestined, like the hCMPCs. We believe that by comparing different cell sources and the induction of cardiomyogenic differentiation, a more complete understanding and selection of potential interesting miRNAs can be made.

Wilson et al investigated whether predicted targets for miR-1, -208, and -499 were reduced on cardiac differentiation in hESC. Their data show that the target expression is actually higher in beating embryoid bodies and hESC-CM than in hESCs (Figure 3b1). Only in fetal heart the target expression is lower than in hESCs. We therefore believe that it is not justified to suggest that target gene expression is gradually reduced during cardiac differentiation. In the authors’ discussion, they stated to be surprised by the continued target expression in hESC-CM; however, it is important to understand that these targets are in silico predictions, of which the majority is not validated by reporter gene analysis.

Previously, we reported the cardiac-specific expression of miR-499 and its coexpression with and location within the MYH7B gene.2 As Wilson et al,1 we demonstrated that miR-1 and miR-499 overexpression greatly enhanced cardiomyogenic differentiation but, more importantly, that miR-1 or miR-499 inhibition could completely prevent cardiac differentiation of hCMPCs, thereby establishing the indispensable role for miR-1 and miR-499 in cardiomyogenic differentiation. Our group validated SOX6 as a target of miR-499, and by small interfering RNA inhibition of SOX6, we could greatly enhance hCMPC cardiac differentiation. We therefore believe that SOX6 is a crucial myogenic differentiation factor that deserves attention. By in silico prediction analysis, Wilson et al confirmed that the most significant pathway targeted by miR-499 is the Wnt/β-catenin pathway, including SOX6 (Table 1). It would be interesting, therefore, to investigate whether cardiomyogenic differentiation of hESCs is governed by targeting of SOX6 as well, thereby answering important mechanistic questions about cardiac differentiation.

Sources of Funding

This work was supported by the Netherlands Heart Foundation (2003B07304) (to Dr Sluijter and Dr Doevendans), Bsik program “Dutch Program for Tissue Engineering” Grant 6746 (to Dr Sluijter and Dr Doevendans), and a Bekalis price (to Dr Doevendans).

Disclosures

None.

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References


Letter by van Mil et al Regarding, “Dynamic MicroRNA Expression Programs During Cardiac Differentiation of Human Embryonic Stem Cells: Role for miR-499”

Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org

DOI: 10.1161/CIRCGENETICS.110.958595

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_Circ Cardiovasc Genet_ 2011;4:e3
doi: 10.1161/CIRCGENETICS.110.958595
_Circulation: Cardiovascular Genetics_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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