Cardiovascular Genetics: A News Round-Up

Cardiac Regeneration
Harnessing the Power Within

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Study Hypothesis

The ability of the heart to regenerate to a meaningful extent after injury has been demonstrated in animal models, such as zebrafish; however, higher organisms seem to have lost this advantageous capacity, leading to cell loss and subsequent heart failure after insult. Recent studies found a narrow window of regenerative opportunity during the first 7 days after birth in mice, but this capacity fades away by the second week of life. In this study, Aguirre et al hypothesize that molecular mechanisms underlying cardiomyocyte regeneration in lower organisms are present, although dormant, in mammals creating a powerful prospect for regenerative interventions.

How Was the Hypothesis Tested?

The authors concentrate on the study of miRNAs as regulators of multiple simultaneous processes potentially involved in regeneration. Leveraging the established zebrafish model of cardiac regeneration after amputation of the ventricular apex, they identify changes in miRNA expression during regeneration, focusing on entities evolutionary conserved across vertebrates. Predictive algorithms are used to pinpoint downstream targets of miRNAs of interest, which are validated through luciferase reporter assays before subsequent experiments.

Levels of candidate miRNAs, as well as their targets of interest, are assessed in zebrafish during heart development and regeneration using quantitative RT-PCR and immunofluorescence. With these readouts, direct functional correlation between miRNAs and target proteins is tested by modulation of miRNA/target levels using mimics, antago miRs, morpholinos, as well as rescue miRNAs. Additionally, bromodeoxyuridine incorporation is also monitored to identify cell proliferation.

Levels of miR-99/100 and Let-7a/c together with target genes FNTβ (beta subunit of farnesytransferase) and SMARCA5 (SWI/SNF-related matrix–associated actin-dependent regulator of chromatin subfamily a, member 5) are also examined in various samples representative of human and murine cardiomyocyte differentiation and heart development, including murine embryonic, neonatal, and adult cardiomyocytes, as well as human embryonic stem cell–derived cardiomyocytes, adult, and embryonic hearts. Furthermore, expression changes were assessed in injured samples (postmortem human heart tissue and experimentally induced infarcted hearts from adult mice).

Next, dedifferentiation and proliferation (reminiscent of the regenerative process) are tested in vitro after manipulation of candidate miRNA levels by treatment with anti-miRs. Specifically, expression of GATA4 and PCNA is monitored in anti-miR-treated adult murine cardiomyocytes, as well as human fibroblasts and vascular cells. At the global level, gene expression changes occurring during cardiomyocyte dedifferentiation are studied on RNA-sequencing and transcriptomic analysis of the expression profiles in anti-miR-treated cardiomyocytes and control counterparts. Additionally, proteomic changes associated with cardiac regeneration are characterized by semiquantitative mass spectrometry on samples in which dedifferentiation had been verified, including regenerating zebrafish hearts, neonatal mouse hearts, and anti-miR-treated adult myocardial mouse tissue. As a result, additional metabolic-related parameters, such as glycolysis/ox-phos ratio and mitochondrial fusion, were investigated in dedifferentiated cells. Also, organotypic slices from mouse hearts are used as a model to test dedifferentiation characterized by sarcomeric disassembly and downregulation of markers, such as Cx43, MyHC, H3 phosphorylation, and upregulation of GATA4.

To establish whether levels of target proteins FNTβ and SMARCA5 are sufficient to recapitulate miRNA effects, they are lentivirally overexpressed in primary mouse cardiomyocytes in vitro. GATA4 expression as well as cell proliferation are characterized as hallmarks of the regenerative process. Furthermore, to demonstrate mechanistic specificity, FNTβ/SMARCA5 are knocked down using siRNA in cells simultaneously treated with anti-miRs. Implications of candidate miRNAs and downstream targets in cardiomyocyte specification during stem cell differentiation are also studied through expression level modulation and monitoring of maturation markers, such as Cx43 and MYL7.

Finally, regenerative potential of an anti-miR therapy was tested in vivo in a mouse model of myocardial infarction. After an initial verification of the functional effect of lentiviral delivery of the anti-miR treatment, a more targeted adenoviral approach is used for intracardiac distribution of anti-miR-99/100 and anti-Let-7a/c.

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Functional outcome is measured by echocardiography, including parameters, such as fractional shortening, ejection fraction, and ventricular wall thickness. Structural changes are determined using Masson’s Trichrome staining, as well as immunostaining for dedifferentiation, proliferation, and cytokinesis markers.

Principal Findings

By studying previously demonstrated cardiac regeneration in zebrafish, the authors identified 2 conserved miRNA families (miR-99/100 and let-7a/c) undergoing substantial downregulation during the healing process. This downregulation was in agreement with a significant increase in expression of confirmed target genes fntb and smarca5 during regeneration. Conversely, miR-99/100 levels increase during development, whereas expression of fntb and smarca5 is downregulated with cardiac differentiation.

After demonstrating developmental involvement of the miR-99/100-Fntb/Smarca5 axis through miR/protein level modulation, the authors moved on to dissecting the role of miR-99/100 in zebrafish heart regeneration. Specifically, they found that increasing miR-99/100 levels had a negative effect on the regenerative response of the adult zebrafish heart, including decreased cardiomyocyte proliferation. This effect could be replicated by chemical inhibition of one of the target proteins (Fnt), suggesting that the identified targets play a role in the regenerative response to miRNA modulation.

When interrogated in mammalian systems (human and mouse), similar results were obtained, including higher miRNA levels in older cardiomyocytes. However, downregulation of miRNA or upregulation of target proteins required for regeneration was absent in injured adult heart samples, revealing a failure to activate endogenous mechanisms or repair. In this context, Aguirre and colleagues set out to manipulate miRNA and target protein levels in vitro to circumvent the regenerative roadblock apparent in higher organisms. Experimental silencing of candidate miRNAs resulted in upregulation of target proteins in adult murine cardiomyocytes, leading to dedifferentiation and proliferation features. Of note, these effects did specifically affect cardiomyocytes, but were not observed in other cardiac cytotypes, including fibroblasts and vascular cells.

At the transcriptomic level, epigenetic remodeling, cardiac development, proliferation, and metabolic pathways were found to be implicated in mammalian dedifferentiation. Proteomic verification of gene expression changes highlighted metabolic processes as important players, which was further supported by increased glycolysis and mitochondrial network fragmentation observed in dedifferentiating cardiomyocytes. Beyond in vitro cultures, exploiting the 3-dimensional microenvironment provided by ex vivo organotypic heart slices allowed the authors to show salient features of dedifferentiation (such as sarcomeric disarray, downregulation of cardiac markers, and reexpression of early transcription factors) in a more complex system upon silencing of miR-99/100 and miR-Let7a/c, additionally finding reduced sensitivity to hypoxia in slices in which candidate miRNAs had been inactivated. FNTβ and SMARCA5 were shown to be effectors of the regenerative response controlled by miR-99/100 and miR-Let7a/c, with cellular dedifferentiation and proliferation recapitulated by protein overexpression. Moreover, manipulation of this miRNA/protein axis was shown to have inverse effects on cardiomyocyte specification, with increased levels of miRNA or knockdown of their target proteins resulting in maturation of human cardiomyocytes derived from embryonic stem cells.

Finally, Aguirre et al sought to demonstrate the therapeutic potential of restoring a functional miRNA regulation in vivo. To this aim, they delivered anti-miRs (either through lentiviral or adenoviral vectors) in a mouse model of myocardial infarction and evaluated changes in heart performance. Therapeutic silencing of miR-99/100 and Let-7a/c resulted in improvement of cardiac function as determined by ejection fraction and fractional shortening and partial restoration of structural integrity, including wall thickness, infarct size, and fibrotic scar. At the cellular level, upregulation in expression of FNTβ and SMARCA5 was noted, correlating with increased markers of dedifferentiation, DNA synthesis, and cell division.

Implications

Over the past few years, miRNAs have emerged as powerful regulators of physiological processes, thanks in part to their unique target promiscuity affecting cellular pathways at multiple levels. Additionally, miRNAs are easily modulated by mimics or antagonirs, further increasing their translational value. In this study, Aguirre and colleagues identify 2 families of miRNAs differentially regulated in the process of heart regeneration naturally occurring in the zebrafish model. Although both miRNAs and target proteins were evolutionary conserved in mammals and dedifferentiating/proliferating phenotypes could be recapitulated in vitro after manipulation of miRNA/protein levels, spontaneous regulation of these pathways was altered in vivo failing to reproduce the regenerative response in mice and humans. Experimental manipulation of miRNA levels restored proliferative and dedifferentiation capacity previously observed in zebrafish bypassing evolutionary limitations and resulting in significant improvement of heart structure and function after myocardial infarction in mice. By leveraging existing (although dormant) endogenous capacities, the authors highlight the possibility of reactivating regenerative machinery, bringing us closer to the treatment of heart disease.

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Disclosures

None.

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