# Micro-RNAs in cardiac regeneration

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## Abstract

**Heart diseases are the leading cause of death in the developed world. Novel therapies are required for improving cardiac remodelling and function after injury, since adult mammalian hearts do not completely regenerate after injury, either due to an evolutionary loss of or inactivated regeneration mechanism (1).**

## Introduction

Micro-RNAs (miRNAs) are short, noncoding RNA molecules that act as negative regulators of gene expression by inhibiting protein translation. Through mRNA, miRNAs regulate signalling pathways and affect cell–cell communication (2, 3, 4). Micro-RNAs control the expression of several genes and influence the function of other miRNAs. Micro-RNAs regulate embryogenesis, cell proliferation and apoptosis. Recent studies have revealed important roles for miRNAs in diseases of adult tissues in mammals, including humans, especially in cancer and cardiovascular diseases (4).

Micro-RNAs control cardiomyocyte growth, contractility and angiogenesis through cardiomyocytes, endothelial cells, smooth muscle cells and fibroblasts. The study of miRNAs thus plays an important role in the pathophysiology of cardiovascular disorders, such as heart failure, myocardial infarction, hypertrophy, fibrosis, arrhythmia and atherosclerosis. The regulation of miRNAs possibly protects against ischaemia/reperfusion (I/R) injury and heart failure (4, 5). Altered miRNA expression could be seen in samples of cardiovascular disease patients, and miRNAs could thereby be sensitive biomarkers for acute myocardial infarction and other cardiac diseases (5).

## miRNAs in regeneration

The adult mammalian heart shows a regenerative response upon injury; however, it is insufficient to completely heal the heart (6–9). A robust regeneration mechanism has been observed in neonatal mice. Hearts of 1-day-old neonatal mice regenerate after partial surgical resection by means of a proliferation of pre-existing cardiomyocytes. No scar formation or hypertrophy was observed, and echocardiography revealed normal systolic function. However, the ability to regenerate is lost when neonatal mice are seven days old (10).

After ischaemic myocardial infarction, 1-day-old neonatal mice show myocardial necrosis and systolic dysfunction. However, when miRNA-15 was inhibited, the heart regenerated completely by 21 days through the proliferation of pre-existing cardiomyocytes. Micro-RNA-15 plays a significant role by inhibiting postnatal cardiomyocyte proliferation. Hence, inhibiting miRNA-15 from the postnatal age to adulthood promotes cardiomyocyte proliferation and improves cardiac function after myocardial infarction. These observations show that regeneration mechanisms can be re-awakened in mammals (11, 12). Micro-RNA-590 and miRNA-199a initiate the cell cycle re-entry and the proliferation of cardiomyocytes in neonatal and adult animals. In mice, both miRNA-590 and miRNA-199a promoted cardiac regeneration and a significant improvement of cardiac functional parameters (13).

In lower vertebrates, such as zebrafish, the heart regenerates completely by 4–6 weeks post-injury as a result of an endogenous activation of the regeneration process and cardiomyocyte de-differentiation and the proliferation of pre-existing cardiomyocytes (14–16). Epicardium-derived cells undergo epithelial-mesenchymal transition to form coronary smooth muscle cells and fibroblasts (15, 17). Epicardial markers, such as ***tbx18, tcf21*** and ***raldh2,*** are re-expressed after cryoinjury, suggesting that the epicardium is crucial for heart regeneration in zebrafish (15, 17). Tissue injury reactivates developmental genes in zebrafish: this is the basis for complete regeneration without scar formation (17).

The Let-7 family of miRNAs is expressed during embryonic development (18). Let-7c is upregulated in human cardiac maturation ***in vitro***. Overexpression of the Let-7 family of miRNAs matures human embryonic stem-cell-derived cardiomyocytes and increases cell size, sarcomere length and contraction (1, 19). Let-7 miRNAs are upregulated in murine and human heart disease samples (20). Inhibition of Let-7c improves cardiac remodelling and function after infarction in zebrafish and mice (18, 21, 22). In zebrafish, the inhibition of Let-7c increases the rate of fibrinolysis, the number of proliferating cell nuclear antigen (PCNA) –positive cardiomyocytes at 7 dpi, and the expression of the epicardial marker ***raldh2*** at 7 dpi. Cardiac function measured with echocardiography recovered slightly more rapidly after the inhibition of Let-7c. These results reveal a beneficial role of Let-7c inhibition in adult zebrafish heart regeneration (22).

Micro-RNA-99/100 is highly expressed in an uninjured zebrafish heart and downregulated after injury. Fntβand Smarca5, required for zebrafish heart development and regeneration, are upregulated as a result of lowered expression of miRNA-99/100. A similar pattern is observed in higher mammals. However, mammals fail to downregulate miRNA-99/100 after injury (1). In a murine model, the blocking of both miRNA-99/100 and Let-7c resulted in efficient cardiomyocyte de-differentiation and regeneration. Both miRNA-99/100 and Let-7c were blocked by anti-mirs. The blocking of both miRNA-99/100 and Let-7c also resulted in improved cardiac function (1).

In zebrafish, miRNA-144 is upregulated until 21 days after injury, indicating that it could play an important role in the regeneration process (23). MicroRNA-19 is downregulated during zebrafish heart regeneration (24). MicroRNA-101a expression is depleted three days after amputation (dpa) in adult zebrafish. However, it is again upregulated by 7–14 dpa, until regeneration is completed. Upregulation of miRNA-101a is essential for cardiomyocyte proliferation and scar clearance in adult zebrafish (24).

Micro-RNA-133 is required for cardiac development, and the expression of miRNA-133 is reduced during regeneration. The depletion of miRNA-133 increased cardiomyocyte proliferation and reduced scar formation in adult zebrafish (25). Genetic knockout of miRNA-133 elevated cardiomyocyte proliferation in neonatal mice (26). In adult mice, it has been observed that miRNA-133 levels decrease during cardiac hypertrophy, while an elevation of miRNA-133 inhibits hypertrophy (26).

## Conclusions

MiRNAs are fascinating candidates for studying cardiac regeneration. They regulate cardiac development, contractility, function and regeneration after myocardial infarction or ischaemic injury (12). Several miRNAs are regulated after cardiac injury. Here, the focus has been on miRNA-15, miRNA-199a, miRNA-590, miRNA-101a, Let-7c, miRNA-99/100, miRNA-144, miRNA-19, and miRNA-133, as well as their role in cardiac regeneration after infarction in adult zebrafish, neonatal mice and adult mice. Regulating miRNAs has potential effects on cardiomyocyte proliferation and regeneration, hence making miRNAs probable targets for improving the regenerative capacity of the injured mammalian heart.

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## References

1. Aguirre A, Montserrat N, Zacchigna S, Nivet E, Hishida T, Krause MN, et al. In vivo activation of a conserved microRNA program induces mammalian heart regeneration. *Cell Stem Cell* 2014 Nov 6;15(5):589–604.
2. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004 Jan 23;116(2):281–97.
3. van Rooij E, Kauppinen S. Development of microRNA therapeutics is coming of age. *EMBO Mol Med* 2014 Jul;6(7):851–64.
4. Wojciechowska A, Braniewska A, Kozar-Kaminska K. MicroRNA in cardiovascular biology and disease. *Adv Clin Exp Med* 2017 Aug;26(5):865–74.
5. Kukreja RC, Yin C, Salloum FN. MicroRNAs: new players in cardiac injury and protection. *Mol Pharmacol* 2011 Oct;80(4):558–64.
6. Addis RC, Epstein JA. Induced regeneration--the progress and promise of direct reprogramming for heart repair. *Nat Med* 2013 Jul;19(7):829–36.
7. Aguirre A, Sancho-Martinez I, Izpisua Belmonte JC. Heart regeneration: a tale of cell reprogramming. *Circ Res* 2013 Oct 25;113(10):1109–11.
8. Laflamme MA, Murry CE. Heart regeneration. *Nature* 2011 May ;473(7347):326–35.
9. Bersell K, Arab S, Haring B, Kuhn B. Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell* 2009 Jul 23;138(2):257–70.
10. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, et al. Transient regenerative potential of the neonatal mouse heart. *Science* 2011 Feb 25;331(6020):1078–80.
11. Porrello ER. microRNAs in cardiac development and regeneration. *Clin Sci (Lond)* 2013 Aug;125(4):151–66.
12. van Rooij E, Olson EN. MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. *Nat Rev Drug Discov* 2012 Nov;11(11):860–72.
13. Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, et al. Functional screening identifies miRNAs inducing cardiac regeneration. *Nature* 2012 Dec 20;492(7429):376–81.
14. Jopling C, Sleep E, Raya M, Marti M, Raya A, Izpisua Belmonte JC. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* 2010 Mar 25;464(7288):606–9.
15. Kikuchi K. Dedifferentiation, Transdifferentiation, and Proliferation: Mechanisms Underlying Cardiac Muscle Regeneration in Zebrafish. *Curr Pathobiol Rep* 2015;3(1):81–8.
16. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. *Science* 2002 Dec 13;298(5601):2188–90.
17. Gonzalez-Rosa JM, Peralta M, Mercader N. Pan-epicardial lineage tracing reveals that epicardium derived cells give rise to myofibroblasts and perivascular cells during zebrafish heart regeneration. *Dev Biol* 2012 Oct 15;370(2):173–86.
18. Seeger T, Xu QF, Muhly-Reinholz M, Fischer A, Kremp EM, Zeiher AM, et al. Inhibition of let-7 augments the recruitment of epicardial cells and improves cardiac function after myocardial infarction. *J Mol Cell Cardiol* 2016 May;94:145–52.

19. Kuppusamy KT, Jones DC, Sperber H, Madan A, Fischer KA, Rodriguez ML, et al. Let-7 family of microRNA is required for maturation and adult-like metabolism in stem cell-derived cardiomyocytes. *Proc Natl Acad Sci USA* 2015 May 26;112(21):E2785–94.
20. Bao MH, Feng X, Zhang YW, Lou XY, Cheng Y, Zhou HH. Let-7 in cardiovascular diseases, heart development and cardiovascular differentiation from stem cells. *Int J Mol Sci* 2013 Nov 21;14(11):23086–102.
21. Tolonen AM, Magga J, Szabo Z, Viitala P, Gao E, Moilanen AM, et al. Inhibition of Let-7 microRNA attenuates myocardial remodeling and improves cardiac function postinfarction in mice. *Pharmacol Res Perspect* 2014 Aug;2(4):e00056.
22. Narumanchi S, Kalervo K, Perttunen S, Wang H, Immonen K, Kosonen R, et al. Inhibition of let-7c Regulates Cardiac Regeneration after Cryoinjury in Adult Zebrafish. *J Cardiovasc Dev Dis* 2019 Apr 4;6(2):10.3390/jcdd6020016.
23. Klett H, Jurgensen L, Most P, Busch M, Gunther F, Dobreva G, et al. Delineating the Dynamic Transcriptome Response of mRNA and microRNA during Zebrafish Heart Regeneration. *Biomolecules* 2018 Dec 28;9(1):10.3390/biom9010011.
24. Beauchemin M., Smith A., Yin V.P. Dynamic microRNA-101a and Fosab Expression Controls Zebrafish Heart Regeneration. *Development (Cambridge, England)* 2015;142:4026–4037. doi: 10.1242/dev.126649.
25. Yin VP, Lepilina A, Smith A, Poss KD. Regulation of zebrafish heart regeneration by miR-133. *Dev Biol* 2012 May 15;365(2):319–27
26. Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, et al. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev* 2008 Dec 1;22(23):3242–54. ■

**Conflicts of Interest**

- There are no conflicts of interest.