Heinrich *et al. J Cardiovasc Aging* 2023;3:36
DOI: 10.20517/jca.2023.27

The Journal of Cardiovascular Aging

Commentary

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Unraveling the mechanisms of cardiomyocyte proliferation and maturation in regenerative cardiac medicine

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How to cite this article: Heinrich P, Wu SM. Unraveling the mechanisms of cardiomyocyte proliferation and maturation in regenerative cardiac medicine. *J Cardiovasc Aging* 2023;3:36. https://dx.doi.org/10.20517/jca.2023.27

Received: 28 Jul 2023 **Accepted:** 8 Aug 2023 **Published:** 11 Aug 2023

Academic Editor: Ali J. Marian **Copy Editor:** Fangling Lan **Production Editor:** Fangling Lan

The article "Interplay between calcium and sarcomeres directs cardiomyocyte maturation during regeneration" by Nguyen *et al.* presents a significant advancement in the field of regenerative cardiac medicine by investigating the control of cardiomyocyte (CM) proliferation and maturation during heart regeneration[1]. After a myocardial infarction (MI), the mammalian heart loses a large number of CMs, resulting in the formation of a permanent fibrotic scar. Inducing endogenous CM proliferation to replace the lost CMs holds promise for regenerative therapies[2]. However, uncontrolled CM proliferation can lead to cardiomegaly and tumor formation and hinder successful functional integration into the heart. Nguyen *et al.* focus on understanding the mechanisms that regulate CM proliferation and maturation using zebrafish, mice, and induced pluripotent stem cell-derived CMs as model organisms[1].

The authors examined the role of Leucine-rich repeat containing 10 (LRRC10), a highly conserved protein exclusively expressed in CMs[3,4]. As a target gene of Nkx2.5 and GATA4, *Lrrc10* is expressed during cardiac development with elevated protein levels maintained in adulthood[5]. The knockdown of *lrrc10* in zebrafish resulted in developmental defects, including cardiac looping failure leading to reduced cardiac function and

death[3]. These findings were further confirmed by *Lrrc10* Knockout (KO) studies in mice that showed perinatal cardiomyopathy and progressive dilated cardiomyopathy (DCM) in adulthood[6]. More recent studies were able to provide additional validation of these findings that mutations in *LRRC10* are associated with the development of DCM in humans[7,8]. Mechanistically, LRRC10 has been shown to be involved in the control of excitation-contraction coupling as an auxiliary subunit of the cardiac-specific L-type-Ca^{2+} channels (LTCC). Malfunction of LRRC10 leads to a decrease in peak Ca^{2+} current with a concurrent increase in the delayed current, potentially resulting in a Ca^{2+} overload as a cause for DCM[8]. The precise molecular interaction network of LRRC10 in CMs, however, remains elusive. Apart from its involvement in calcium handling, evidence suggests it might participate in cytoskeletal dynamics as it has been shown to interact with α-actinin and α-sarcomeric actin at the Z-disc[6]. More recently, an interpopulation study of Mexican cavefish reported lrrc10 as a factor upregulated after cardiac injury only in a cavefish subpopulation that can exclusively regenerate the myocardium. The findings were confirmed in a zebrafish model, where *lrrc10*-KO leads to scar formation after injury, interestingly without alteration of the initial CM proliferation rates in response to injury[9].

To investigate the link between calcium handling and cardiac regeneration, Nguyen *et al.* provide a comprehensive study that focuses on LRRC10 as a key mediator and regulator of CM proliferation and maturation[1]. The investigators developed an *ex vivo* imaging system to track the dynamics of Ca^{2+} handling in transgenic zebrafish CMs during regeneration. They observed differences in Ca^{2+} influx and efflux between the border zone (BZ) CMs adjacent to the infarcted area and CMs from the uninjured remote zone. These differences were transient and resolved by 21 days post-injury, indicating a restoration of normal Ca^{2+} handling in regenerated CMs. The BZ-CMs functionally reverted to an embryonic-like state with an alteration in Ca^{2+} handling during early regeneration [Figure 1]. Through single-cell RNA sequencing (scRNA-seq) analysis, the investigators then characterized the process of CM redifferentiation and maturation during regeneration. Here, they found that lrrc10 was downregulated during early regeneration but upregulated at the onset of the late regeneration phase. *lrrc10* KO zebrafish failed to recover normal Ca^{2+} handling and remained in an early regeneration phase, indicating its critical role in CM maturation. The expression of lrrc10 negatively correlated with the expression of dedifferentiation markers, suggesting its inhibitory effect on early regeneration hallmarks and concomitant active contribution to CM maturation after injury.

To explore LRRC10's role in human CM maturation, the researchers used human induced pluripotent stem cell-derived CMs (hiPSC-CMs). They found that LRRC10 overexpression in hiPSC-CMs significantly improved sarcomere organization, increased LTCC and connexin-43 expression, as well as altered Ca^{2+} handling dynamics. scRNA-seq analysis revealed that hiPSC-CM maturation correlated with LRRC10 expression levels, further validating LRRC10's role in promoting CM maturation.

To investigate the mechanistic interplay of Ca^{2+} handling and LRRC10, patch-clamp measurements were carried out in wild-type and *lrrc10* mutant zebrafish. These studies revealed an altered Ca^{2+} transient behavior in *lrrc10* mutant zebrafish CMs, including decreased LTCC density and prolonged action potentials. Furthermore, the researchers identified the zebrafish equivalent of the cardiac dyad, a region important for efficient excitation-contraction coupling, which was disrupted after injury. It was demonstrated that lrrc10 is necessary for the reassembly of dyad components, such as LTCC complex and sarcomeres, during heart regeneration. Comparable disorganization of the cardiac dyad was observed in mouse and human hearts after MI.

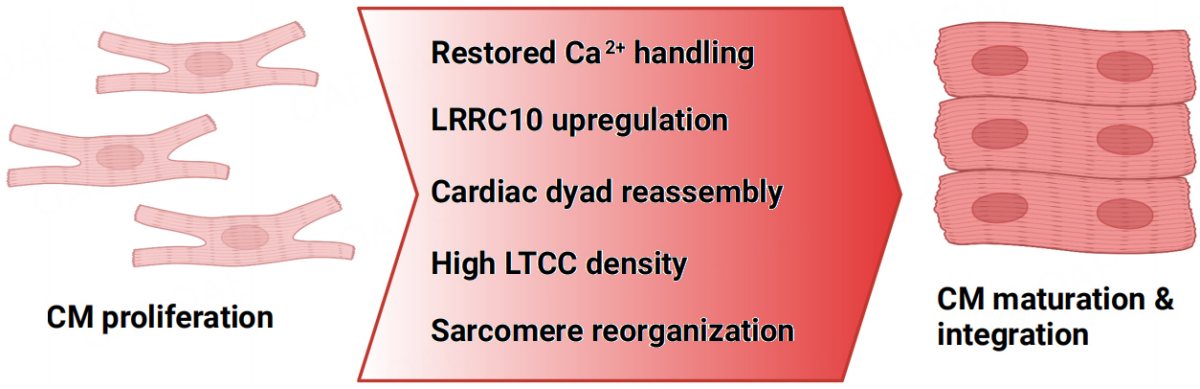

Figure 1. Processes involved in CM maturation & integration after injury.

Lastly, the researchers observed a peak in CM proliferation at 7 days post-injury (dpi), coinciding with transient peak expression of lrrc10. Double-pulse chase experiments showed that high lrrc10 expression correlated with completed proliferation and CM maturation. Consistent with this observation, lrrc10 overexpression decreased CM proliferation at the peak proliferation time point. These inhibitory effects on CM proliferation were conserved across mammalian and human-induced pluripotent stem cell-derived CMs. Finally, Nguyen *et al.* were able to show that co-expression of Lrrc10 with genes inducing CM proliferation prevented cardiomegaly in several different experimental models[1].

While the study presents very comprehensive findings that significantly advance our understanding of cardiac regeneration, some limitations and potential areas for further investigation should be considered. One limitation of the study is the predominant reliance on a zebrafish model for heart regeneration. Although zebrafish possess a natural regenerative capacity, there are inherent differences between zebrafish and mammalian heart regeneration processes. Further investigations using primary adult human cardiomyocytes would provide additional insights into the relevance of the findings to human cardiac regeneration.

Furthermore, although the authors focus on the role of LRRC10 in CM maturation and demonstrate its involvement in sarcomere organization, calcium handling, and inhibition of CM proliferation, the specific molecular mechanisms underlying these effects remain unclear. Future studies could investigate potential downstream signaling pathways, such as Hippo and Wnt, as well as molecular interactions through which LRRC10 modulates CM maturation and proliferation. Additionally, the functional significance of LRRC10's interaction with LTCC and sarcomeric components could be explored in more detail.

Apart from these few limitations, Nguyen *et al.* provide valuable insights into the control of CM proliferation and maturation during heart regeneration[1]. Understanding the molecular mechanisms underlying these processes is crucial for the development of effective regenerative therapies for heart diseases. The findings of this study should provide important implications for the field of regenerative cardiac medicine.

DECLARATIONS

Authors' contributions

Conceived and wrote the paper: Heinrich P, Wu SM

Availability of data and materials
Not applicable.

Financial support and sponsorship
American Heart Association - Established Investigator Award, Hoffmann/Schroepfer Foundation, Additional Venture Foundation, Joan and Sanford I. Weill Scholar Fund, and the NSF RECODE grant (to Wu SM) and American Heart Association Predoctoral Fellowship (23PRE1018158), and Boehringer Ingelheim Fonds MD Fellowship (to Heinrich P).

Conflicts of interest
Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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