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Muscling up the heart: a preadolescent cardiomyocyte proliferation spurt contributes to heart growth

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Summary

A recent study from Naqvi et al in *Cell* shows that in mice during preadolescence, cardiomyocytes undergo a burst of proliferation that is related to a thyroid hormone surge. This study challenges the conventional model of the timing of terminal differentiation and provides a direction for further mechanistic research on cardiomyocyte proliferation and differentiation.

In mice and rats, cardiomyocytes transition from the mono- to the binucleated phenotype in the first week of life^{1, 2}. It is commonly thought that this indicates the transition from the proliferative state to permanent cell cycle exit. However, the precise molecular-genetic mechanisms of terminal differentiation are unknown. Reversing this process and stimulating the proliferation of terminally differentiated cardiomyocytes is a century-old challenge and considered by many scientists as a Holy Grail. It has been thought that heart growth after birth is exclusively through enlargement (hypertrophy) but not proliferation of cardiomyocytes³. However, several lines of evidence challenge the absoluteness of the notion that new cardiomyocytes are not generated after birth. First, Poss et al found that adult zebrafish can completely regenerate heart defects by increasing cardiomyocyte proliferation, indicating that differentiated adult cardiomyocyte phenotypes exist that can re-enter the cell cycle⁴. Second, we have demonstrated that human hearts show cardiomyocyte proliferation until the second decade of life⁵. Third, it was demonstrated that some adult mammalian cardiomyocytes can be stimulated to reenter the cell cycle^{6–10}. Fourth, we have demonstrated that neuregulin-stimulated cell cycle re-entry happens predominantly in the mononucleated portion⁹. The causative connection between endogenous cardiomyocyte proliferation and myocardial regeneration was established in zebrafish⁴ and neonatal mice¹¹, although part of the latter has recently been challenged¹². A number of reports indicate that stimulating cardiomyocyte proliferation in adult mammals improves myocardial repair^{9, 10}. Thus, controlling post-natal cardiomyocyte proliferation holds great promise for heart regeneration, but requires a mechanistic understanding. The report by Naqvi in the May 8,

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Disclosures

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2014 issue of *Cell* offers a new cellular mechanism of post-natal cardiomyocyte proliferation.¹³

Naqvi et al reported that mice show a burst of cardiomyocyte proliferation during preadolescent development, between 13 and 18 days of life. The authors demonstrate that the final cardiomyocyte number present in adult mice is established by this burst of proliferation, which is regulated by the thyroid hormone/IGF-1/IGF-1-receptor/Akt pathway. Furthermore, the authors show that this burst is associated with improved myocardial repair and heart function after cardiac injury.

Naqvi et al first compared heart weight and cardiomyocyte cell size between early preadolescent (~P10) and young adult (~P35) mice. They found that cardiomyocyte cell volume increased 2-fold between P10 and P35, which was largely driven by an increase of cell length. This cardiomyocyte enlargement alone cannot account for the 3.5-fold increase of heart weight between P10 and P35, and over-proliferation of non-cardiomyocytes seems unlikely. The discrepancy prompted the authors to quantify the number of cardiomyocytes. By enzymatic disaggregation and counting with a hemocytometer, they identified two post-natal periods of rapid cardiomyocyte proliferation: a ~40% increase between P1 and P4, and a further 40% increase (~500,000 cardiomyocytes) between P14 and P18. The first period of increase of cardiomyocyte numbers is consistent with the findings of Li et al., 1996². The discovery of the second window is unanticipated and extends the period of endogenous cardiomyocyte proliferation and thereby represents an opportunity for advancing the cellular model of post-natal heart growth. The cardiomyocyte numbers in mice from P18 to one year old did not change, which means that the final number of cardiomyocytes present in the adult heart is established in mice by 18 days of age.

To define the timing of the proliferative burst, the authors checked cell cycle markers and performed BrdU injections. Their results showed that the burst begins with many cardiomyocytes re-entering the cell cycle late on P14. By 9 o'clock in the morning of P15, approximately 14–34% of cardiomyocyte nuclei were positive for Aurora B kinase, a chromosomal passenger protein that is present from M-phase to cytokinesis. This represents a 36-fold increase of cardiomyocyte mitosis and cytokinesis in the left ventricle. This proliferative burst has not only a specific temporal pattern; it was also spatially restricted to the left ventricle and much more pronounced in the sub-endocardial zone.

What is the cellular origin of this proliferative burst? In P15 mouse hearts, approximately 10% of cardiomyocytes are mono-nucleated and ~90% are bi-nucleated¹, which raises the question whether both cardiomyocyte phenotypes contribute to the proliferative burst. On the afternoon of P15, there was a 2-fold increase of the percentage of mononucleated cardiomyocytes, pointing to the possibility that this phenotype shows preferential proliferation. However, when Naqvi et al. compared the cell cycle activity between the mono- and binucleated fraction, they found in both phenotypes ~30% mitotic cardiomyocytes, detected by nuclear Aurora B kinase staining. This led to the interpretation that, due to their high prevalence, binucleated cardiomyocytes make a major contribution to the proliferative burst. Based on their imaging results, the authors propose a model in which binucleated cardiomyocytes, previously thought to be a terminally differentiated and non-

proliferative phenotype, can re-enter the cell cycle, duplicate their DNA, divide their nuclei to become to become quadruplinucleated, then assemble two cleavage furrows around the two spindles, and finally divide. This unique cell cycle produces two mononucleated and one binucleated daughter. Since this cell cycle requires the coordinated formation of two cleavage furrows in a binucleated cardiomyocyte, it is different from the cell cycle of binucleated hepatocytes, which assemble the condensed chromosomes from two nuclei in *one* metaphase plate¹⁴. A complementary interpretation is also offered, in which the binucleated cardiomyocytes found in M-phase are on the way to becoming multinucleated. Future work should be directed at characterizing these unique cell cycles further.

At P15, $14.5 \pm 2.6\%$ of cardiomyocyte nuclei in the LV were Aurora B kinase-positive, indicating that they were in prophase or advanced phases of the cell cycle. It is known that cardiomyocyte mitosis is accompanied by sarcomere disassembly¹⁵, and Naqvi et al. appropriately tested whether global heart function may be affected during this phase of sarcomere disassembly. Since they found that myocardial function was not reduced, this raises the interesting cell biological question of how a large portion of cardiomyocytes can disassemble their sarcomeres without affecting global myocardial contractility.

Besides advancing the cellular model of post-natal myocardial growth, what are the implications of this study? Due to the aforementioned connection between the activation of cardiomyocyte proliferation and myocardial regeneration, Naqvi et al appropriately tested to what degree the response to myocardial injury may differ before and after the proliferative burst. They used as injury model ligation of the left anterior descending coronary artery^{16, 17} to induce myocardial injury at P2, P15, and P21. Although the initial injury size was similar at all 3 time points, injury at P15 and P21 induced transmural scars, but not at P2. The relative scar size was significantly smaller at P15 compared with P21. Cardiomyocyte cell cycle activity was highest at P2 and decreased with age. Myocardial injury did not induce an additional increase compared with sham-operated mice, in contrast to an increase of mitotic cardiomyocytes at 7 dpi as previously reported¹⁷. Recovery of myocardial function was complete after P2 injury, partial after P15, and absent after P21 injury. In summary, the extent of repair after myocardial injury induced at P15, i.e. *during* the cardiomyocyte proliferative burst, was at an intermediate position between injury at P2 and injury at P21. The interpretation of the results involving relative scar sizes is challenging in this situation because myocardial growth happens at the same time as scar formation and compaction. Future research should examine the causative relationship between the burst of cardiomyocyte proliferation and myocardial repair more deeply. For this, the molecular mechanisms controlling the proliferative burst have to be elucidated to an extent that enables disruption of the burst with molecular-genetic tools.

What are the molecular mechanisms controlling the proliferative burst? Naqvi et al noticed an accelerated growth of the heart between P11 and P18, which was accompanied by an increase of the ratio of mRNA encoding the sarcomeric proteins α - and β -myosin heavy chain. The control of these genes by the thyroid hormone T3 is well established¹⁸, and serum T3 levels increased 5.6-fold between P4 and P12. To examine a potential causative relationship, Naqvi et al applied the clinically used drug propylthiouracil (PTU) to inhibit T3 biosynthesis. Treatment with PTU beginning at P7 inhibited the accelerated heart growth

and lowered the number of cardiomyocytes. These results implied thyroid hormone in triggering the proliferative burst, but raised the question of whether this is a direct or indirect effect. Insulin-like growth factor 1 (IGF-1) is a well-established molecular control mechanism of cardiomyocyte size and proliferation¹⁹. IGF-1 mRNA and myocardial tissue levels increased between P10 and P15. This was paralleled by an increase of the expression of the IGF-1 receptor and activation of the downstream kinase Akt, and addition of PTU abolished this effect. In summary, the Naqvi et al study offers a molecular model involving thyroid hormone and the IGF-1/Akt pathway. The proposed molecular mechanisms prompt the question about the observed local regulation of cardiomyocyte proliferation during the burst in the sub-endocardial zone and in the left ventricle.

How does the Naqvi et al model of a preadolescent burst of cardiomyocyte proliferation compare with prior reports? We have reported that cardiomyocyte proliferation contributes to myocardial growth in humans during the first 20 years of life⁵. Although our measurements showed a 3.4-fold increase of the number of cardiomyocytes during this period, we did not detect a distinct burst of cardiomyocyte proliferation in pre-adolescent humans. This maybe due to the higher variation of human data and the inherent limitations of analyzing a larger number of appropriate human hearts. Another recent paper proposed that the exposure to relatively higher ambient oxygen upon birth induces DNA damage and terminal differentiation of cardiomyocytes in mice²⁰. This model is seemingly at odds with the model of Naqvi et al., however, these two models may be reconciled if mechanistic analyses of specific cardiomyocyte phenotypes, which may undergo permanent cell cycle withdrawal at different times, reveal different mechanisms.

In conclusion, this study represents an important advance in the elucidation of postnatal myocardial growth mechanisms as it raises the possibility to regenerate myocardium in young humans through manipulating this burst. Further elucidation of the controlling molecular-genetic mechanisms should provide a handle to achieve this and to address the associated mechanistic questions using mouse genetics. Future research should define the precise cellular mechanisms of division of binucleated cardiomyocytes.

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