To proliferate or not to proliferate

Youngsook Lee*

Department of Anatomy, School of Medicine and Public Health, University of Wisconsin, 1300 University Avenue, Madison, WI 53706, USA

Online publish-ahead-of-print 7 April 2010

This editorial refers to ‘Cardiomyocyte cell cycle control and growth estimation *in vivo*—an analysis based on cardiomyocyte nuclei’ by S. Walsh *et al.*, pp. 365–373, this issue.

Cardiomyocytes in mammalian hearts undergo significant reduction in their cell proliferation capacity soon after birth, which is not disputed. However, it remains uncertain whether the adult heart contains a limited capacity for cell proliferation. Through the use of immunohistochemical staining methods, it has been reported that adult mouse cardiomyocytes can re-enter the cell cycle and duplicate.^{1,2} However, the origin of the cell-cycle-positive cells was not determined. Others reported permanent inhibition of cell-cycle progression in adult cardiomyocytes in mice.³ With advances in lineage tracing technology, a recent genetic labelling study demonstrated that stem or precursor cells did not contribute significantly to the formation of cardiomyocytes during normal ageing up to 1 year in the mouse. In contrast, precursor cells may participate in the formation of new cardiomyocytes after cardiac injury.⁴ In humans, high myocyte proliferation rates that could result in the exchange of all cardiomyocytes within 5 years have been reported by employing immunostaining technology on sections of human patients who died of acute myocardial infarction.⁵ In contrast, using ¹⁴C dating analyses in humans, Bergmann *et al.*⁶ reported that cardiomyocytes are renewed with a gradual decrease from 1% turning over annually at the age of 25 to 0.45% at the age of 75. According to the best-fit models that allow turnover rates to change with age, at the age of 50, 55% of the cardiomyocytes remain from the time around birth and 45% have been generated later.

To resolve these conflicting results, it is necessary to employ comprehensive approaches that complement each other and to analyse the data quantitatively. The study performed by Walsh *et al.*⁷ appearing in this issue describes thorough analyses of cell-cycle control to address this conflicting problem. Walsh *et al.* provide strong evidence that cardiomyocytes in mice withdraw from the cell cycle soon after birth, and, therefore, cardiomyocytes in the murine adult heart do not proliferate under normal conditions. To investigate the proliferative capacity of cardiomyocytes, Walsh *et al.* performed purification of cardiomyocyte by FACS sorting of cTroponinT (cTnT)-positive cells. The authors then extracted nuclei from sorted cells to measure DNA content in order to accurately measure DNA synthesis of cardiomyocytes by considering the binuclear nature of cardiomyocytes. FACS sorting and immunostaining for BrdU uptake or Ki-67

indicated that a range of 12–23% (depending on different methods) of embryonic cardiomyocytes were positive and 1–8% by neonatal day 7, which became almost non-detectable by day 14. These results demonstrate a complete block of cell-cycle entry to G1 and S phase, indicating a permanent cell-cycle withdrawal. This study also demonstrated that polyploidization of murine myocytes occurred predominantly in the first week of postnatal life. Mouse cardiomyocytes began to be binucleated soon after birth and reached nearly 98% of binucleation between postnatal days 5 and 8. These data indicate a dramatic increase in uncoupling of karyokinesis and cytokinesis shortly after birth, which supports the notion of complete postnatal cell-cycle arrest. Gene profiling experiments were performed to compare embryonic, neonatal, and adult hearts using purified cardiomyocytes isolated from α -MHC-eGFP transgenic mice. Multiple genes responsible for cell-cycle regulation were enriched in the neonatal hearts, which seems to correlate well with cell-cycle withdrawal.

The strengths of this study are (i) the use of multiple methods for cell-cycle detection such as immunohistochemistry involving BrdU uptake and Ki-67 antibodies, (ii) the use of flow cytometry for better quantification, (iii) the use of α -MHC-eGFP transgenic mice to mark cardiomyocytes, (iv) the comparison of gene expression levels among embryonic, neonatal, and adult cardiomyocytes by gene profiling experiments, and (v) the use of isolated cardiomyocyte nuclei for accurate quantification, since the high percentage of adult mouse cardiomyocytes are binucleated. Moreover, to pave a clear path for cardiac regeneration, one should define whether adult cardiomyocytes are capable of proliferating. In this regards, it is advantageous to use cTnT-positive cells as a cardiac marker, which selects differentiated cardiomyocytes and eliminates precursor or stem cells that may not express cTnT. Gene profiling experiments comparing gene expression patterns among purified cardiomyocytes from embryonic, neonatal, and adult hearts would provide crucial information on cell-cycle regulation during transitions from a proliferative to a non-proliferative period.

However, a potential cardiac regenerative capacity cannot be addressed in this study. By collecting cells that express cTnT prior to examining BrdU incorporation or Ki-67 labelling, potential cardiac progenitor/stem cells that do not express cTnT have been excluded. Although this study strongly supports permanent withdrawal of postnatal cardiomyocytes from the cell cycle, the next step would be to investigate whether this phenomenon is altered under pathological conditions using the same methods employed in the current study. In addition, further analysis of gene profiling data would be necessary to

determine the molecular pathways of cell-cycle regulation. To unravel the secret of transitions of cell-cycle regulation soon after birth, these in-depth analyses would be essential and may reveal unexpected players critical in cell-cycle withdrawal in postnatal hearts.

Cardiac regenerative capacity seems to differ in different species. In humans, adult myocardium displays proliferating cardiomyocytes^{5,6} and less binucleation compared with adult mouse myocytes,^{7,8} suggesting a prolonged coupling of karyokinesis and cytokinesis that leads to cell renewal capability. The neonatal phase of binucleation and polyploidization in humans lasts for the first decade of life. In this regard, it will be important to learn how nature repairs the broken heart in other species.⁹ In urodele, the myocardium is regenerated by dedifferentiation of existing cardiomyocytes. Zebrafish can regenerate its heart after the bottom fifth has been removed¹⁰ by recruitment of progenitor cells.¹¹ However, two recent reports indicate that mature cardiomyocytes are the principle source for regenerating the zebrafish heart.^{12,13} Therefore, it would be important to investigate whether there is an increase in proliferating cardiomyocytes after injury. If so, whether they arise from (i) cardiac precursor cells that give rise to multiple lineages of cells within the heart,^{14–16} (ii) stem cell populations of cardiac or non-cardiac origin, or (iii) existing cardiomyocytes by re-entering the cell cycle by dedifferentiation must be determined. Lineage tracing technology that allows permanent marking is crucial to distinguish these possibilities.

Acknowledgements

The author thanks Matt J. Brody and J. Amanda Kim for critical reading of the article.

Conflict of interest: none declared.

Funding

The author is supported by a grant from the National Institute of Health (HL067050).

References

1. Anversa P, Nadal-Ginard B. Myocyte renewal and ventricular remodeling. *Nature* 2002;**415**:240–243.
2. Anversa P, Kajstura J. Ventricular myocytes are not terminally differentiated in the adult mammalian heart. *Cir Res* 1998;**83**:1–14.
3. Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Cir Res* 1998;**83**:15–26.
4. Hsieh PCH, Segers VFM, Davis ME, MacGillivray C, Gannon J, Molkentin JD *et al*. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat Med* 2007;**8**:970–974.
5. Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R *et al*. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med* 2001;**344**:1750–1757.
6. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S *et al*. Evidence for cardiomyocyte renewal in humans. *Science* 2009;**324**:98–102.
7. Walsh S, Pontén A, Fleischmann BK, Jovinge S. Cardiomyocyte cell cycle control and growth estimation *in vivo*—an analysis based on cardiomyocyte nuclei. *Cardiovasc Res* 2010;**86**:365–373.
8. Brodsky V, Sarkisov DS, Arefyeva AM, Panova NW, Gvasava IG. Polyploidy in cardiac myocytes of normal and hypertrophic human hearts: range of values. *Virchows Arch* 1994;**424**:429–435.
9. Yi BA, Wernet O, Chien KR. Regenerative medicine: developmental paradigms in the biology of cardiovascular regeneration. *J Clin Invest* 2010;**120**:20–28.
10. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. *Science* 2002;**298**:2188–2190.
11. Lepilina A, Coon AN, Kikuchi K, Holdway JE, Roberts RW, Burns CG *et al*. A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell* 2006;**127**:607–619.
12. Kikuchi K, Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF *et al*. Primary contribution to zebrafish heart regeneration by *gata4*⁺ cardiomyocytes. *Nature* 2010;**464**:601–605.
13. Jopling C, Sleep E, Raya M, Martí M, Raya A, Belmonte JCI. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* 2010;**464**:606–609.
14. Wu S, Fujiwara Y, Cibulsky SM, Clapham DE, Lien CL, Schultheiss TM *et al*. Developmental origin of a bipotential myocardial and smooth muscle cell precursor in the mammalian heart. *Cell* 2006;**127**:1137–1150.
15. Kattman S, Huber TL, Keller GM. Multipotent FLK-1⁺ cardiovascular progenitor cells give rise to the cardiomyocytes, endothelial, and vascular smooth muscle lineages. *Dev Cell* 2006;**11**:723–732.
16. Moretti A, Caron L, Nakano A, Lam JT, Bernshausen A, Chen Y *et al*. Multipotent embryonic *Isl1*⁺ progenitor cells lead to cardiac smooth muscle and endothelial cell diversification. *Cell* 2006;**127**:1151–1165.