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Editorial

Manipulating myocyte cell cycle control for cardiac repair

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This editorial refers to 'Cardiomyocyte proliferation and protection against post-myocardial infarction heart failure by cyclin D1 and Skp2 ubiquitin ligase' by Tamamori-Adachi *et al.*,[11] pp. 181–190, this issue.

A fundamental tenet in cardiac biology, namely that the heart is a postmitotic organ incapable of regeneration, has recently been challenged. According to the traditional belief, the number of cardiac myocytes we are born with is all we will have for the rest of our lives. If myocytes die (e.g. as the result of infarction), they cannot be renewed. This is why myocardial infarction (MI) and its consequences, such as congestive heart failure, continue to be a major cause of death worldwide despite the considerable therapeutic advances that have been made over the past decades.

In one attempt to evade this dilemma, cell replacement strategies are currently being investigated for their therapeutic potential to restore cardiac function. Indeed, a growing body of evidence from both basic animal studies and clinical trials indicates that cell-based strategies using different stem cell populations such as embryonic stem cells, skeletal myoblasts, haematopoietic stem cells, endothelial progenitor cells, and mesenchymal stem cells might be promising.[1,2] According to these studies, improvements in clinical symptoms, left-ventricular function, and myocardial perfusion are feasible with cell therapy. However, there is an ongoing controversy about the underlying mechanisms and, particularly, the capability of the different implanted progenitor cells to transdifferentiate and what the essential prerequisites for this transdifferentiation might be. Only on understanding these mechanisms and, likely, through the appropriate priming and engineering of the cells to be implanted regeneration by this approach might be achieved.

Another approach to induce cardiac regeneration for repair after injury would be to reverse the terminal differentiation, as many mammalian tissues respond to injury by activating committed progenitor cells or by triggering proliferation of differentiated cells that re-enter cell cycle. Although several groups have described the presence of progenitor or stem cells that can differentiate into cardiac myocytes,[3,4] there is still much debate and confusion in the field regarding their potential for cardiac myocyte differentiation and myocardial repair.[5,6] Limited induction of DNA synthesis in the heart has been described in response to various stimuli.[7,8] Yet, there has been little evidence for cytoplasmic division or cytokinesis in mammals. In contrast to cardiomyocytes from lower vertebrates, which are well endowed to divide postnatally,[9,10] proliferation ceases in the perinatal mammalian heart in conjunction with the downregulation of cyclin-dependent kinase (cdk) activities. Myocytes undergo an additional round of DNA synthesis and nuclear mitosis without cytokinesis (acytokinetic mitosis) that leaves the majority of adult cardiac myocytes binucleated in most species. Beyond this stage of differentiation, further increases in cardiac mass are possible only through an increase in cell size or hypertrophy.

The report by Tamamori-Adachi *et al.*[11] in the current issue of *Cardiovascular Research* provides encouraging evidence that manipulation of positive and negative regulators of cell cycle control mechanisms successfully promotes cardiomyocyte division and, hence, might yield new strategies for the treatment of ischaemic heart failure. The study continues previous work[12] and shows that adenoviral delivery of S-phase kinase-associated protein 2 (Skp2), an ubiquitin ligase that mediates degradation of the cell cycle inhibitor p27, together with nuclear-targeted cyclin D1 (D1NLS) and Cdk4 results in increased proliferation (BrdU-positive cells and expression of Ki67) and increased mitosis (phosphorylation of histone H3, Aurora B and Survivin) after coronary artery ligation in rats. Furthermore, gene expression profiling by microarray analysis of rat neonatal cardiomyocytes expressing D1NLS and Cdk4 in culture showed gene activation for a subset of G1/S, S phase, G2/M, and M phase checkpoints, thereby further suggesting the feasibility of profound and effective manipulation of cell cycle control in heart cells. Indeed, the authors demonstrated cytokinesis in cardiomyocytes *in vivo* with daughter cells staining positive for tropomyosin. To note, cytokinesis is important since in normal adult hearts a number of cardiomyocytes are polyploid and/or multinucleated. Moreover, nuclear hyperplasia and multinucleation have been associated with cell hypertrophy as mechanism of growth in the postnatal heart. The current study complements the previous attempts to overcome the proliferation arrest in adult cardiomyocytes by manipulation of cell cycle control. In these earlier studies, transgenic mice overexpressing cyclin D2 showed higher rates of DNA synthesis

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and signs of mitosis (H3P) or newly formed myocardium after infarction,[13,14] likely by phosphorylation of cyclin D2-target retinoblastoma gene product (pRb) being prevented. Accordingly, a cyclin D2 knockout prevented the increase in the relative heart weight after transverse aortic constriction.[15] Furthermore, mice lacking Rb and p130 showed signs of persisting proliferation of cardiomyocytes during adulthood (BrdU, H3P), along with the expression of c-Myc, E2F, and CycE, and increased activity of Cdk2, Cdk4, and Cdk6.[16] There is also strong evidence that inhibition of p38 MAPK in combination with FGF1 stimulation, promotes hypertrophy and cytokinesis in adult cardiomyocytes.[17,18] Interestingly, inhibition of p38 MAPK augments growth factor-mediated induction of all phases of the cell cycle and substantially enhances the proliferative capacity of mammalian cardiomyocytes. Additionally, p38 activity prevented upregulation of factors required for karyokinesis and cytokinesis, suggesting a role for p38 in G2/M checkpoint control. Furthermore, cyclin A2 induced cardiomyocyte mitosis (H3P), increased myocardial proliferation, and improved heart function after MI,[19,20] while ectopic expression of cyclin B1 and Cdk2 induced mitosis-promoting factor (MPF) activity in adult cardiomyocytes, which resulted in a mitotic phenotype, but no complete cell division.[21] Taken together, the data in the current study further confirm that manipulation of cell cycle regulation might offer reasonable strategies for cardiac regeneration and treatment of ischaemic heart failure. The significance of the induced myocyte proliferation is strengthened by the observed protection against cardiac dysfunction and heart failure in an ischaemia/reperfusion-induced acute MI model.

There are, however, still unanswered questions that need to be addressed. First, the quantitative contribution of the observed myocyte proliferation *per se* to the observed protective effects is not clear. Besides technical limitations (assessment and variability of the initial MI size), it is quite possible that cardio-protective effects (i.e. smaller MI size and improved function) are mediated by multiple mechanisms as stated by the authors. These may include paracrine effects by induction or suppression of cytokines and growth factors, resulting in reduced rate of apoptosis and increased vascularization as also reported in this study. Also, the temporal correlation of these angiogenic and anti-apoptic effects to cell proliferation is not completely conclusive. Moreover, the role of other factors contributing to post-ischaemic heart failure (i.e. hypertrophy, remodelling) has not been studied, and the occurrence of transient dedifferentiation of the myofibrils in the proliferating adult cardiomyocytes has not been investigated. It may also be speculated that the observed enhanced proliferation occurred in young cardiomyocytes simply because the cells derive from the (limited) pool of cardiac progenitor cells. If so, then additional cell cycle regulators need to be targeted in adult cardiomyocytes for a more substantial onset of proliferation. Alternatively, this goal may also be achieved by the development of more efficient methods for gene delivery. Finally, the adenoviral delivery for gene transfer always leaves the possibility of a somewhat dysregulated proliferation. As suggested by the authors, this potential problem has to be considered in a clinical context. Alternative methods like FGF1 stimulation/p38 MAPK inhibition, periostin, or IGF1 are less specific but more easily adjustable.

Although the report by Tamamori-Adachi *et al.*[11] emphasizes the rationale for continuing research on promoting re-entry of myocytes into the cell cycle for cardiac regeneration, the fundamental shift in paradigm from the heart being strictly a postmitotic organ to one that carries some, although arrested, regenerative capacities clearly has a long way to go before such a therapeutic strategy can be applied in the clinical setting. In addition to taking into account safety considerations, it will be a key prerequisite to identify the appropriate master regulators of cell cycle control or the most advantageous combinations thereof, which was the aim of the current report.

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