An Unexpected Switch
Regulation of Cardiomyocyte Proliferation by the Homeobox Gene Meis1

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Meis1 Regulates Postnatal Cardiomyocyte Cell Cycle Arrest
Mahmoud et al

The mammalian heart has a limited capacity for regeneration after damage. In contrast, teleost fish and urodelian amphibians readily regenerate even large cardiac defects. One of the central differences between species capable and incapable of efficient heart regeneration is the ability to reinitiate cardiomyocyte proliferation. The importance of cardiomyocyte proliferation for heart regeneration is underscored by the finding that fetal and neonatal mouse hearts efficiently regenerate until efficient proliferation of cardiomyocytes ends during the first week after birth.1,2 In a recent article in *Nature*, Mahmoud et al3 now reported that constitutive and inducible cardiomyocyte-specific deletion of the homeobox gene *Meis1* lifts the ban on cardiomyocyte proliferation in adult hearts of mice. *Meis1* deficiency increases cardiomyocyte numbers and heart/body weight ratio while maintaining cardiomyocyte size and heart function. Overexpression of *Meis1*, however, blocks cardiomyocyte proliferation and heart regeneration after myocardial infarction in newborn mice, suggesting that Meis1 acts as a critical driver of cell cycle arrest in the postnatal myocardium.

The traditional view of the adult heart as an essentially postmitotic organ has changed substantially in the past few years. It is now widely acknowledged that the heart has the ability to undergo a certain degree of renewal, although the extent and the cellular origin(s) of renewal are still debated. Newly generated cardiomyocytes have been claimed to be primarily derived from resident cardiac stem cells4 or bone marrow–derived stem cells5 or bone marrow–derived stem cells.6 More recently, the older idea of low-level proliferation of preexisting cardiomyocytes in the mammalian heart has gained new appeal because of the use of novel genetic fate-mapping and isotope labeling technologies.6 Further support for heart regeneration from pre-existing cardiomyocytes comes from recent observations in teleost fish and urodelians.7–9

New cardiomyocytes might originate from fully differentiated mononucleated cardiomyocytes or from cardiomyocytes that have undergone dedifferentiation.10,11 Given that adult cardiomyocytes exhibit diversity of ploidy and number of nuclei, it seems unlikely that all adult cardiomyocytes harbor identical proliferative potential. Interestingly, a strong positive correlation exists between the number of mononucleated, diploid cardiomyocytes, and the regenerative capacity of the heart in different species, such as newt,12 zebrafish,13 and rodent fetal and P1 neonates,14–16 suggesting a higher proliferation capacity for mononucleated/diploid cardiomyocytes. Mahmoud et al17 made the intriguing observation that deletion of *Meis1* not only increases the total number of adult cardiomyocytes, but also increases the ratio of mononucleated/diploid cardiomyocytes. Meis1 might thus promote generation of mononucleated cardiomyocytes or block proliferation of mononucleated cardiomyocytes. Although further studies will have to unravel the exact regulatory mechanisms of Meis1 action during cardiomyocyte proliferation, it will also be interesting to see whether manipulation of cardiomyocyte proliferation (ie, by overexpression or inactivation of *Meis1* in mature cardiomyocytes) alters cardiomyocyte renewal during aging. Such experiments might help to answer the question whether cardiomyocytes are replenished by already existing cardiomyocytes or by cardiac stem cells.

Several studies suggest that dedifferentiation of fully differentiated adult cardiomyocytes after injury increases the proliferative potential of cardiomyocytes.6,8,10–12 Cardiomyocyte dedifferentiation in mice does not only lead to reexpression of so-called fetal genes, such as ANP and α-smooth muscle actin, but also activates stem cell marker genes, including c-Kit and Runx1.10,12 It is of importance to determine whether adult cardiomyocytes undergo dedifferentiation after *Meis1* deletion before cell cycle reentry or whether cardiomyocytes need to traverse through a partially dedifferentiated state to escape the proliferation block imposed by Meis1. Alternatively, dedifferentiation might not be involved at all in cell proliferation events caused by *Meis1* deficiency.

Cardiomyocyte proliferation is governed by distinct signaling pathways and involves cell cycle, transcriptional, and epigenetic regulation, as well as metabolic reprogramming.18 The cell cycle arrest of adult cardiomyocytes might also be an adaptive response to emerging new energy demands after transition from fetal to postnatal stages. Data from the same group around Hesham Sadek suggested that Meis1 not only directs transcription of cell cycle inhibitors, but also regulates metabolic pathways in hematopoietic stem cells (Figure [A]).19 More specifically, deletion of *Meis1* in hematopoietic stem cells induces a shift of cellular metabolism toward...
increased oxygen consumption resulting in enhanced reactive oxygen species production. Interestingly, reactive oxygen species stimulate differentiation of embryonic stem (ES) cells to cardiomyocytes, but also enhance the proliferative capacity of ES cell–derived cardiac cells and neonatal cardiomyocytes.20 Hence, it is possible that Meis1 contributes to the quiescent state of adult cardiomyocytes by regulating redox homeostasis. Notably, metabolic cues also have a strong impact on epigenetic modifying enzymes, which control the cardiac lineage by regulating DNA modifications, histone modifications, and ATP-dependent chromatin remodeling.21 Thus, it will be rewarding to investigate the metabolic state of Meis1+ cardiac myocytes, the potential impact of Meis1 on reactive oxygen species production, the influence of Meis1 on the epigenetic landscape, and the possible link to cardiomyocyte proliferation.

Inhibition of Meis1 is not the only route to enhance cardiomyocyte proliferation. In a recent study, Eulalio et al22 identified 2 miRNAs (hsa-miR-590 and hsa-miR-199a), which promoted cell cycle reentry of adult cardiomyocytes ex vivo and stimulated cardiomyocyte proliferation concomitantly in neonatal and in adult animals. So far, the relevant proliferation-promoting targets of hsa-miR-590 and hsa-miR-199a remain mostly enigmatic. Interestingly, however, the authors detected a downregulation of Hopx, a small, divergent homeodomain protein that suppresses embryonic cardiomyocyte proliferation.23 Absence of Hopx leads to hyperacetylation of Gata4, probably by failing to stabilize the interaction of Hdac2 and Gata4 and is associated with a marked increase in cardiac myocyte proliferation.24 Although the primary mode of action of Meis1 seems to be different (see below), the parallel is intriguing and highlights the role of homeobox proteins in the control of cardiomyocyte proliferation.

Meis1 belongs to the TALE (3-amino-acid loop extension) family of homeodomain transcription factors. Members of the Pbx and Meis homeobox gene families enhance the otherwise relatively nonspecific DNA binding properties of a subset of Hox transcription factors and regulate gene expression as heterooligomeric complexes with Hox proteins.25 Meis1 is highly expressed in mixed-lineage leukemia (MLL) leukemias26 and serves a major role in establishing leukemic stem cell potential and frequency by quantitatively regulating the extent of self-renewal, differentiation arrest, and cycling.27 Expression of Meis1 in leukemia cells is correlated with substantially lower transcript level of the cycling-dependent kinase inhibitor Cdkn2a (also known as p16Ink4a) and increased expression of Bmi-1, a negative regulator of the Ink4a locus (Figure [B]).27 Hence, it is surprising that Mahmoud et al3 demonstrated direct transcriptional activation of the Ink4b-Arf-Ink4a and Cdkn1a (also known as p21) genes by Meis1 in the adult mouse heart using chromatin immunoprecipitation and reporter gene assays. Although speculative at the moment, it is possible that the presence of additional transcriptional cofactors, such as the oncogenic version of the MLL histone methyltransferase, contributes to the cell type–specific regulation of Ink4b-Arf-Ink4a and Cdkn1a. MLL is instrumental to transform self-renewing myeloid progenitors into leukemia stem cells and might thus lead to repression rather than activation of these negative cell cycle regulators. The full characterization of molecular processes controlled by Meis1 in cardiomyocytes and other cell types will hopefully resolve these mysteries. The findings of Mahmoud et al3 are also noteworthy in several other respects: (1) the expression of cell cycle regulatory proteins in the heart undergoes complex expression changes during the first 2 postnatal weeks, suggesting involvement of a similarly complex, elaborate network of upstream regulatory molecules.18 The relatively modest (40%) increase in the expression level of a single homeobox gene during early postnatal heart development is difficult to reconcile with such complex alterations, although the lack of Meis1 might initiate a cascade of events leading to dramatic changes in cell cycle regulation. (2) Attempts to induce robust proliferation of adult cardiomyocytes by expression of positively acting cell cycle regulators, such as cyclinD2 and E2Fs, have been only partially successful and resulted in a relatively small percentage of cycling cardiomyocytes. Increased proliferation of adult cardiomyocytes was assumed to result primarily from continuous proliferation of fetal cardiomyocytes but not from de novo initiation of cell cycle entry.18,28

Mahmoud et al3 used different approaches (BrdU incorporation, pH3 and auroraB staining, counting of cell numbers) to

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**Figure.** Schematic illustration of the function of Meis1 in different cell types. A, Meis1 maintains the quiescence of hematopoietic stem cells by activating HIF1α and HIF2α to prevent accumulation of reactive oxygen species (ROS). B, The Meis1/Pbx/Hoxa9 complex activates oncogenic genes, such as Flt3, c-Myb, Bmi1/p16Ink4a (inhibitor) or the cell cycle regulator Cdk6 in myeloid leukemia cells causing cellular hyperproliferation. C, In neonatal and adult cardiomyocytes, Meis1 (presumably in complex with Hoxa10, Hoxa11, and Hoxa12) transcriptionally activates cycling-dependent kinase inhibitors (CDKIs) Ink4b-ARF-, Ink4a locus and Cdkn1a, which leads to cell cycle arrest because of inhibition of CDKs. CF indicates cofactor.
determine the increased proliferative activity of Meis1-deficient cardiomyocytes. However, most data demonstrating increased cell cycle activity of cardiomyocytes were obtained using myocardial tissue sections. Several conditions have been identified that affect identification of cardiomyocyte nuclei in sections. In fact, a recent study concluded that the chance of misidentifying a myocyte nucleus with current methodologies resides in ranges from 1 in 3 to 1 in 10, thereby advising care in the analysis of rare events, such as the initiation of cardiomyocyte proliferation. It would be reassuring if results from the Nature study would be repeated using mice with genetically labeled cardiomyocyte nuclei allowing unequivocal identification of proliferating cardiomyocytes. Finally, it should not be forgotten that adult cardiomyocytes retain capacity to synthesize DNA, which contributes to increased nuclear ploidy in severely hypertrophic hearts but does not necessarily lead to cell division. Hence, not all Meis1-deficient cardiomyocytes that stain positive for pH3 or BrdU might undergo cytokinesis. Although Mahmoud et al described a significant increase in the number of cardiomyocytes and a higher number of mononucleated cardiomyocytes in both, the constitutive as well as in induced Meis1 mutants, the extent of the proliferative potential of individual Meis1-deficient cardiomyocytes needs to be analyzed in more detail in future studies. Specifically, one would like to know whether proliferating adult cardiomyocytes undergo only a single round of cell division or are able to divide several times. Because only a subset of Meis1-deficient cardiomyocytes enters the cell cycle, additional processes need to be in place that block cell division. At present, it is not clear whether such additional barriers are temporarily relieved in proliferating cardiomyocytes either by stochastic or by regulatory means or whether individual cardiomyocytes differ in their propensity to enter the cell cycle. The latter scenario would argue for distinct modes of cell cycle regulation in subpopulations of cardiomyocytes. Under any circumstances, a more detailed fluorescence activated cell sorting-based cell cycle analysis of cardiomyocytes at different stages of consecutive cell cycles including mitotic cell divisions (eg, by the BrdU-Hoechst method) will provide more accurate insights about the exact number of dividing cardiomyocytes after Meis1 inactivation.

In summary, the identification of a new regulator of cardiomyocyte proliferation and potential therapeutic target for cardiac regeneration enables new approaches to unlock the proliferative block of cardiomyocytes. Although it seems unlikely that increased proliferative capacity of adult cardiomyocytes alone suffices to mimic the regenerative virtues of zebrafish and urodoleans, the current findings represent a major step forward. Additional studies will have to show whether the increase in the proliferation rate of Meis1-deficient cardiomyocytes enables Meis1 mutant mice to regenerate damaged hearts efficiently, thereby restoring regenerating abilities that are normally observed only in fetal and newborn mammalian hearts. Yet, the relatively small number of cardiomyocytes expressing cell cycle markers and the limited proliferating response raises some concerns about the robustness of the proliferative potential of cardiomyocytes lacking Meis1. Under disease conditions, low numbers or slowly proliferating cardiomyocytes have to compete with rapidly proliferating fibroblasts to prevent myocardial scar formation. Concomitant inhibition of fibroblast proliferation might help in such a competition but would at the same time increase the risk of cardiac rupture. Further insights into molecular processes governed by Meis1 will also have to resolve the paradox of proliferative effects of Meis1 in leukemic cells (Figure [B]) and antiproliferative effects in cardiomyocytes (Figure [C]).

Disclosures

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