Extra- and intracellular factors regulating cardiomyocyte proliferation in postnatal life

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One of the striking differences that distinguish the adult from the embryonic heart in mammals and set it apart from the heart in urodeles and teleosts is the incapacity of cardiomyocytes to respond to damage by proliferation. While the molecular reasons underlying these characteristics still await elucidation, mounting evidence collected over the last several years indicates that cardiomyocyte proliferation can be modulated by different extracellular molecules. The exogenous administration of selected growth factors is capable of inducing neonatal and, in some instances, also adult cardiomyocyte proliferation. Other diffusible factors can regulate the proliferation and cardiac commitment of endogenous or implanted stem cells. While the individual role of these factors in the paracrine control of normal heart homeostasis still needs to be defined, this information is relevant for the development of novel therapeutic strategies for cardiac regeneration. In addition, recent evidence indicates that postnatal cardiomyocyte proliferation is controlled by genetically defined pathways, such as the Hippo pathway, and can be modulated by perturbing the endogenous cardiomyocyte microRNA network; the identification of the cytokines that activate these molecular circuits holds great potential for clinical translation.

Keywords Cardiomyocyte proliferation • Heart regeneration • Secreted factors • Paracrine control • MicroRNAs

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1. Introduction

Innovative biotechnological approaches to induce cardiac regeneration in patients with myocardial infarction or heart failure, which are based on the delivery of nucleic acids, recombinant proteins, or cells, are needed in light of the breadth of these diseases and the lack of curative treatments. Despite the benefit of current pharmacological therapies and remarkable, recent progress in the use of devices assisting the failing myocardium, the prognosis of heart failure remains poor, with mortality estimated at 40% of patients at 4 years from diagnosis. Of note, no new classes of drugs have been introduced in the management of patients with this condition because of angiotensin II receptor blockers, which date back to the mid-1990s, and several have failed in Phase III clinical trials.

Understanding the molecular correlates of cardiac myocyte proliferation during development and the reasons for the inability of these cells to replicate in adult mammals is prerequisite to developing innovative approaches that might support the sudden or chronic lack of a functional myocardium by promoting the formation of new contractile tissue.

While cardiac regeneration is a complex biological process requiring the structural and functional recreation of myocardial tissue, including stroma, vessels, and the conduction system, likely the most limiting step is the de novo generation of cardiomyocytes. Several exciting results have emerged in the last few years to indicate that cardiac regeneration might be achieved by directly stimulating the proliferation of already committed cardiac progenitors or even of fully differentiated cardiomyocytes. Here, we review the most recent information concerning the capacity of cardiomyocytes to proliferate and how various extra- and intracellular mechanisms regulate this process in postnatal life.

2. Cardiomyocyte replication: lessons from development and from other organisms

One of the most striking and still little understood observations in mammalian myocardial biology is the sudden exit of cardiomyocytes from the cell cycle after birth. It is well established that the heart is the first organ to form during embryonic development and that, during that period, as
Regulation of cardiomyocyte proliferation

at birth, cardiomyocytes have the ability to undergo DNA synthesis and cytokinesis. During development, there are waves of proliferation of already differentiated cardiomyocytes. For example, the tubular, primitive heart that is formed in the early stages of cardiac specification mostly consists of non-dividing cells. As development progresses, this tube starts looping and cardiomyocytes resume proliferation at the outer curvatures to form the future chambers. On the contrary, cardiomyocytes at the inner curvature remain resting and will form the conduction system. Thus, proliferation appears to be a common characteristic of the cardiomyocyte, which depends on developmental signals imparted to the cell.

Cardiomyocyte division stops at birth and is often accompanied by the uncoupling of DNA synthesis and mitosis from cytokinesis, by which several cells become multinucleated between P4 and P14 in the mouse. Binucleation is ~80% in adult mouse, rat, rabbit, and guinea pig and 45% in dog and cow; in pigs, cardiomyocytes can contain up to 16 nuclei. In humans, 75–90% of adult cardiomyocytes remain mononucleated. This observation might be relevant in light of the notion that mononucleated cardiomyocytes hold greater potential for cell division upon stimulation. The withdrawal of cardiomyocytes from the cell cycle after birth impacts profoundly on the capacity of the mammalian heart to repair after damage: loss of myocardial tissue in the foetal or neonatal life up to ~1 week of age is healed through the generation of new contractile tissue, while, later, fibrosis and scarring predominate.

The failure of mammals to regenerate the myocardium after injury is a fact in sharp contrast with the high proliferative potential of cardiomyocytes in urodeles and fish, which maintain the capacity to regenerate the myocardium in adulthood. Recent evidence indicates that the damaged zebrafish heart reactivates a developmental programme involving the expansion of Gata4-positive cardiomyocytes to achieve regeneration. This observation has suggested that cardiac regeneration in the adult mammalian heart might also be achieved by reactivating the cardiac developmental programme. However, this does not necessarily have to be the case. For instance, the satellite cell transcription factor Pax7 is required for skeletal muscle regeneration in neonatal mice, but is dispensable for regeneration during juvenile and adult stages. In the mammalian heart, we and others have shown that the Notch pathway sustains cardiomyocyte proliferation at birth, Reactivation of this pathway in adult mice hearts, however, is ineffective in inducing cardiac regeneration after myocardial infarction (unpublished data), despite the fact that Notch is essential for adult heart repair in zebrafish. Thus, cardiac regeneration in adult mammalian hearts does not necessarily recapitulate the events that occur during development.

It has been argued that cardiomyocyte differentiation after birth might be incompatible with cell division due to molecular and mechanical impossibility of assembly and disassembly of the contractile apparatus. However, embryonic and neonatal cardiomyocytes are capable of synthesizing, assembling, and disassembling their cellular contractile proteins in a coordinated manner through the assembly of contractile structures for chromosome segregation and cytokinesis while they actively proliferate. Thus, proliferation and differentiation are not mutually exclusive in the developing heart, precisely as in the adult zebrafish and other species that regenerate the heart. Genetic fate-mapping and multi-isotope imaging mass spectrometry studies have in fact shown, also in mice, that slow-rate cardiomyocyte renewal in the adult heart is sustained by the division of pre-formed cardiomyocytes.

3. Why do mammalian cardiomyocytes stop replicating early after birth?

Little information is available on the molecular mechanism by which cardiomyocytes stop dividing after birth. It is also unclear whether the signals that regulate the exit from the cell cycle are cell autonomous or extracellular. Interestingly, embryonic cardiac myocytes proliferate in culture according to a schedule that closely resembles that which occurs in vivo, suggesting the existence of an intrinsic timer which might rely, at least in part, on intracellular activation of the cyclin-dependent kinase inhibitors p18 and p27.

On the other hand, various environmental events take place at birth when cardiomyocytes withdraw from the cell cycle. One of the major changes occurring as a consequence of breathing is a sudden and marked increase in oxygenation. While oxygen tension in the heart is estimated between 18 and 28 mmHg during the foetal life, when arterial blood enters the heart after delivery, it rises rapidly in the whole organism to 100 mmHg. A reasonable hypothesis for this is that the sudden increase in oxygenation, which is expected to foster oxygen free radicals (reactive oxygen species, ROS) experienced by heart cells at birth, might stop cardiomyocyte proliferation and favour their terminal differentiation. Consistent with this possibility, the epicardium has been recognized as a cardiac stem cell niche, characterized by relative hypoxia and proving permissive to the proliferation of a population of cardiac progenitor cells. Epicardial progenitor cells are similarly activated during embryonic development when they contribute to the formation of cardiomyocytes, vascular, endothelial, and smooth muscle cells, and in adulthood after injury, when their contribution to the cardiomyocyte pool can occur after priming with thymosin beta-4. Interestingly, this factor is able to activate epicardial progenitors by up-regulating anti-oxidative enzymes, thereby reducing oxidative stress in these cells. In addition, low oxygen tension positively influences the proliferation of human cardiomyocyte progenitors isolated from human hearts. Overall, these results support the notion of a role for hyperoxia in stopping cardiomyocyte proliferation at birth, and point towards the existence of a hypoxic stem cell niche as a source of cardiac progenitor cells in the adult heart. However, full understanding of the mechanism governing the activation of these cells and their capacity to contribute to cardiac regeneration after injury is still missing.

Besides hyperoxia, cardiomyocytes are subjected to increased mechanical stress after birth, due to the sudden increase in workload of the left ventricle. Cells at the inner surface of the developing heart have reduced proliferation relative to those at the outer layer because of greater strain, and are the first to exit cell cycle. Cardiomyocytes are equipped with complex stress–strain sensors embedded in Z disks and titin filaments that are able to modify the transcriptional activity in response to mechanical stress. Experiments performed using an isovolumic rat heart Langendorff model showed that mechanical stretch induces the expression of various cytokines. Among these, interleukin 6 (IL6) and insulin-like growth factor 1 (IGF1) have been recognized as paracrine controllers of cardiomyocyte proliferation. The hypothesis that mechanical stretch can directly affect the proliferative potential of cardiac myocytes is consistent with recent results obtained by apical resection in 1-day-old mice, which induced massive cardiomyocyte proliferation and full heart...
regeneration, while this proliferative response was lost in 7-day-old mice, coinciding with increased cardiac workload. Fish, amphibians, and reptiles have a low-pressure heart, which may enable their higher cardiac regenerative capacity.

Of interest, is the fact that the two major environmental changes that occur after birth, namely hyperoxia with increased ROS formation and mechanical stretch, are strictly connected and interdependent. Mechanical strain increases the levels of intracellular ROS, which in turn promote cardiomyocyte differentiation and block their division.

In addition to these environmental events, changes in the composition of the extracellular matrix (ECM) are emerging as key regulatory events controlling the rate of cardiomyocyte proliferation before and after birth. In particular, cardiomyocyte growth in the developing heart correlates with regulated shifts in the expression of ECM proteins and integrin receptors. In the developing myocardium, the ECM is more effective in promoting growth due to a high concentration of fibronectin, whereas an increase in collagen I inhibits growth after birth. Consistently, focal adhesion kinase (FAK), a critical mediator of fibronectin binding to integrin receptors, functions to facilitate cardiomyocyte proliferation during heart development. whereas FAK-related non-kinase (FRNK), which corresponds to the non-catalytic C-terminal portion of FAK, is transiently expressed in the postnatal heart with peak levels occurring just prior to the withdrawal of cardiomyocytes from the cell cycle. Thus, it is possible that FRNK dampens FAK-dependent cardiomyocyte cell-cycle progression, facilitates cell-cycle exit, and regulates the uncoupling between DNA synthesis and cytokinesis in the postnatal myocardium.

4. Stimulation of cardiomyocyte proliferation by genetic manipulation

Over the last several years, there have been various attempts to stimulate cardiomyocyte proliferation by overexpressing various cell-cycle regulators (reviewed in refs [33–40]). In particular, transgenic overexpression of cyclin A2, which drives cell-cycle progression over the G2/M checkpoint, was reported to stimulate cardiomyocyte proliferation and to provide benefit after myocardial infarction. Transgenic mice overexpressing the cdk2 kinase also showed a transient increase in cardiomyocyte proliferation and the conspicuous presence of less-differentiated, mononucleated cardiomyocytes.

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5. The Hippo pathway as an essential regulator of cardiomyocyte proliferation

In multicellular organisms, appropriate cell number and organ size are determined by coordination of cell proliferation, cell size, and apoptosis. The Hippo signalling pathway, originally discovered by genetic screens in the Drosophila eye, is a broad and essential regulator of cell proliferation and organ size. In mammalian cells, the main effector of the pathway is the transcriptional co-activator Yap (Yki in Drosophila). Mst1/2 (Hippo) interacts with Ww45 (Salvador) to phosphorylate and activate the Lats1/2 (Warts) and Mob1 (Mats) complex, which in turn phosphorylates and inactivates Yap. Genetic deletion of the upstream inactivating kinases (Mst1, WW45, and Lats) cause marked cardiac hyperplasia and knockout mice die postnatally with cardiomegaly. On the contrary, transgenic mice overexpressing Mst1 or Lats2 die postnatally with dilated cardiomyopathy without compensatory ventricular myocyte hypertrophy. Consistent with these phenotypes, deletion of floxed Yap alleles using Nixc2.5-Cre, or Tmrt2-Cre, expressed during early heart development, resulted in embryonic lethality due to myocardial hypoplasia. Deletion at a later developmental stage caused reduced cardiac function and death after 10 weeks of age due to dilated cardiomyopathy, while transgenic overexpression of a constitutively active, nuclear Yap mutant (Yap S112A) caused cardiomyocyte hyperproliferation and thickened myocardium.

Taken together, these results clearly indicate that the Hippo pathway controls cardiomyocyte proliferation during development as well as during the postnatal phase. Of interest, the Yap transcriptional co-activator appears to de-repress the genes activated by the Wnt/β-catenin pathway during development. The induction of cardiomyocyte proliferation involves the activation of IGFl receptor signalling, consequent activation of Akt, and inactivation of GSK-3β, resulting in the eventual stabilization of β-catenin, a positive regulator of cardiac growth.

The extent to which reactivation of Yap in the adult life might induce cardiac regeneration remains to be seen. In transgenic mice that overexpress Yap S112A, myocardial infarction is repaired with reduced fibrosis and increased myocardial tissue, compared with control mice. In a consistent manner, Hippo deficiency has recently been shown to enhance cardiomyocyte generation with functional recovery after adult myocardial infarction. It will now be interesting to test whether the exogenous delivery of activated Yap in wild-type animals by gene transfer might induce cardiac repair after myocardial infarction by promoting cardiac regeneration.

Given the apparent relevance of the Hippo pathway in the regulation of cardiomyocyte proliferation in various instances, the understanding of the upstream stimuli regulating this pathway also bears paramount interest. Hippo signalling in mammalian cells can be modulated by cellular DNA damage response, contact inhibition, and the dynamics of the cytoskeleton, in particular by F-actin depolymerization (reviewed in ref. [65]). Conceivably, all these stimuli might be operational during both myocardial development and after damage.

6. Control of cardiomyocyte proliferation by microRNAs

Work performed by different laboratories has indicated that heart development and disease is controlled by several different microRNAs...
(miRNAs), a class of small, non-coding, evolutionarily conserved RNAs that negatively regulate gene expression by repressing protein translation, or by promoting miRNA degradation (reviewed in ref. 68,69). Each miRNA can target hundreds of different mRNAs, whereas one miRNA is interfered with multiple miRNAs.

The essential role of RNA interference during cardiac development was originally shown in the effect of the heart-specific conditional knock-out of the miRNA-processing enzyme Dicer. This resulted in embryonic, or early postnatal, lethality due to heart failure. 70,71 Notably, the disruption of the miRNA-processing machinery at different postnatal ages also determines pathological ventricular remodelling and contractile dysfunction, 72,73 indicating that miRNAs are not only required for normal embryonic and neonatal heart development, but are crucial for cardiac homeostasis in adulthood.

Recent evidence indicates that specific miRNAs are involved in postnatal cardiomyocyte mitotic arrest. By comparing the miRNA expression profile of the mouse ventricle at Day 1 and at Day 10, miR-195 (a member of the miR-15 family) was found induced at Day 10, when cardiomyocytes have exited the cell cycle. 74 Overexpression of this miRNA during development caused premature cardiomyocyte cell-cycle arrest, leading to congenital heart hypoplasia, and prevented regeneration after myocardial infarction in P1 neonatal hearts. 75 Consistently, a chemically modified RNA oligonucleotide blocking the seed sequence of the miR-15 family members increased cardiomyocyte mitosis in neonatal mice, promoted adult cardiomyocyte proliferation, and preserved cardiac contractile function after injury. 75,76 Additional studies in zebrafish revealed a similar role for miR-133 in blocking cardiomyocyte proliferation and regeneration following apical resection injury. 77 Finally, miR-29a also suppressed cardiac cell proliferation, whereas its inhibition promoted cardiomyocyte division possibly by targeting cyclin D2. 78

It is reasonable to consider that if some miRNAs can force cardiomyocyte exit from the cell cycle, others could promote their proliferation. To tackle this issue on an experimental platform, we have performed high-throughput screening using a whole genome, human miRNA library (composed of over 800 miRNA mimics) and identified 40 miRNAs able to stimulate DNA duplication and cytokinesis of cultured mouse and rat neonatal cardiomyocytes. 79 Of these 40, the most effective hits, namely miR-199a-3p and miR-590-3p, were also able to induce cell-cycle re-entry and cytokinesis of cultured adult cardiomyocytes. In vivo, these miRNAs specifically promoted cardiomyocyte proliferation and not fibroblast proliferation, in both neonatal hearts and in the adult following myocardial infarction. 79

Alternative approaches have identified a few additional miRNAs that regulate cardiomyocyte proliferation. For example, miR-199a was found to promote the proliferation of neonatal cardiomyocytes via its effect on Sox6 and cyclin D1. 80 miR-17–92, an oncogenic miRNA cluster, 81,82 proved to be essential for cardiomyocyte proliferation because the cardiac-specific knockout of this cluster caused cardiac hypoplasia, while its transgenic overexpression induced cardiomyocyte proliferation in embryonic, postnatal, and adult hearts. 83

Overall, these studies support the potential utility of miRNA-based therapeutics for the induction of cardiomyocyte proliferation and cardiac regeneration. miRNA mimics can be delivered as naked nucleic acids to the cardiomyocytes; alternatively, miRNA precursor genes can be embedded into viral vectors having high tropism for cardiomyocytes, such as those derived from the adeno-associated virus (AAV). In both cases, the double-stranded miRNAs intercept the cellular RNAi machinery at different levels (Figure 1). Since a potential risk of inducing undesired proliferation in non-target tissues has to be considered for any miRNA (or miRNA inhibitor) inducing cardiomyocyte proliferation, additional studies assessing the safety of intracardiac and systemic administration of pro-regenerative molecules in animal models are required before proceeding to undertake clinical trials.

### 7. Extracellular control of cardiomyocyte proliferation by cytokines and growth factors

The adult heart is not composed exclusively of cardiomyocytes, but also hosts a variety of cell types, including fibroblasts, adipocytes, endothelial, smooth muscle, and inflammatory cells. Most of these cell types are likely to contribute to the regulation of cardiomyocyte biology, including their capacity to proliferate, through the release of diffusible molecules. While this network of paracrine interactions still needs to be fully deciphered, over the last few years a number of cytokines and growth factors have been shown to be capable of inducing cardiomyocyte proliferation when administered exogenously. These are schematically represented in Figure 2, in the context of the experimental settings used to demonstrate their activity.

The existence of diffusible factors exerting a mitogenic effect on cardiomyocytes has been obtained mostly in rat or mouse neonatal cardiomyocytes, since these cells maintain a transitory capacity to proliferate after isolation. 84,85 Fibroblast growth factor 2 (FGF2) was one of the first growth factors shown to promote neonatal cardiomyocyte DNA synthesis via protein kinase C activation. 86 In the adult myocardium, activity of this cytokine is potently inhibited by FGF16, an FGF family member that is preferentially expressed in the postnatal heart and is thus possibly involved in cardiomyocyte withdrawal from the cell cycle. 87 Similar to FGF2, platelet-derived growth factor (PDGF) was also reported to induce significant proliferation in neonatal cardiomyocyte cultures, through the simultaneous down-regulation of the cell-cycle G1 phase inhibitor p27, activation of AKT, and inhibition of GSK-3β activity. 88,89 Finally, another secreted molecule reported to augment the proliferation rate of slow-dividing neonatal cardiomyocytes in culture was IL6. This is abundantly secreted in the heart by both the epicardium 90 and the hypoxic adipose stromal cells. 88

Apart from the release of cytokines, fibroblasts might also control cardiomyocyte proliferation through the modulation of ECM composition, as is consistent with the important role of the extracellular environment during development. In a co-culture system, embryonic cardiac fibroblasts, but not adult fibroblasts, were found to sustain cardiomyocyte proliferation through the secretion of fibronectin, collagen, and heparin-binding epidermal growth factor (EGF)-like growth factors, requiring beta-1 integrin expressed on the myocardial cell membrane for their function. 88

An additional source of mitogenic cytokines is the pool of mononuclear leucocytes normally residing in the myocardium, which is massively expanded after damage. Inflammatory white blood cells secrete the cytokine TWEAK [tumour necrosis factor (TNF)-like, weak inducer of apoptosis], a member of the TNF superfamily, which was reported to promote cardiomyocyte proliferation. 91 This factor acts upon binding to the Fn14 (FGF-inducible molecule 14) receptor, which is expressed by neonatal cardiomyocytes and is subsequently down-regulated in adulthood. Unfortunately, however, the receptor is also expressed in pathological conditions, where it drives cardiomyocyte hypertrophy and dysfunction. 91,92

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*Note: The text continues with further details and references.*
In contrast to the relatively large number of proteins able to extend the proliferative potential of neonatal rodent cardiomyocytes, relatively fewer factors appear capable of also acting on adult cells, which are intrinsically more refractory to stimulation. One inducer of adult cardiomyocyte proliferation is Neuregulin-1 (NRG1), a member of the EGF family. It is first expressed by the endocardial endothelium of neonatal hearts, where it mediates trabeculation and cushion formation, and later by endothelial cells. Consistent with previous work on neonatal cardiomyocytes, Kuhn and collaborators have shown that NRG1 is also capable of stimulating DNA synthesis, sarcomere disassembly, and...
cytokinesis in adult, differentiated rat cardiomyocytes, by activating ErbB4 signalling. The administration of NRG1 \textit{in vivo} after myocardial infarction sustained a significant pro-regenerative effect, as assessed by different tracing techniques, including fate-mapping and dynamic genetic labelling experiments.\textsuperscript{7} This finding has potentially important translational value, since the factor can be administered systemically. In addition, the beneficial effects of NRG1 might extend beyond the induction of cardiomyocyte proliferation since differently spliced variants of the protein exert an important function in cardiac tissue homeostasis in the adult life.\textsuperscript{95} The administration of recombinant NRG1 is currently under investigation as a therapeutic molecule in patients with heart failure and for its properties as a protective agent to overcome the cardiac toxicity of anticancer molecules targeting the ErbB receptors.\textsuperscript{96}

Another secreted molecule reported to increase proliferation of both neonatal and differentiated, adult cardiomyocytes is periostin.\textsuperscript{97} This ECM factor, which plays an important role during cardiac development,\textsuperscript{98} is fundamental to scar formation following myocardial infarction.\textsuperscript{99} Periostin was reported to induce the re-entry of differentiated cardiomyocytes into the cell cycle through its interaction with integrins and the activation of the phosphatidylinositol-3-OH (PI3K) pathway; delivery of this factor to the myocardium improved cardiac function after infarction and decreased scar formation.\textsuperscript{97} The pro-proliferative activity of periostin on myocardial cells, however, remains controversial, since these findings were not subsequently reproduced.\textsuperscript{100} More recently, the delivery of a recombinant periostin peptide into the pericardial space in swine after myocardial infarction was reported to improve function and stimulate regeneration, but also to markedly increase myocardial fibrosis.\textsuperscript{101} Up-regulation of periostin was also recently associated with myocardial fibrosis in failing, human hearts.\textsuperscript{102} Besides the controversy on the actual effect of periostin on myocardial proliferation, these observations nonetheless cast serious doubt on the actual therapeutic potential of this molecule.

## 8. Cytokines and growth factors regulating cardiac progenitor cell proliferation in the adult heart

Over the last decade, considerable effort has been invested in the development of stem cell-based therapies for cardiac repair.\textsuperscript{103} Various types of adult progenitor cells have been injected into the damaged heart or into coronary circulation, in both pre- and clinical trials, with the ambitious goal of triggering cardiac regeneration. Among these cell types are unfraccionated bone marrow cells, mononuclear cells, mesenchymal stromal cells (MSCs, originally derived from the bone marrow, but, more recently, also from other tissues), haematopoietic stem cells, endothelial progenitor cells, skeletal myoblasts, and cardiac progenitor cells.\textsuperscript{104}

A paracrine effect, based on the secretion of a broad variety of cytokines, chemokines, and growth factors, is under scrutiny to justify the modest, albeit existing, therapeutic benefit provided by these cells. Besides remarkable effects on cardiac protection, metabolism, contractility, remodelling, and neovascularization, accumulating evidence tends to indicate that this stem cell paracrine effect might also control endogenous cardiomyocyte generation and proliferation.\textsuperscript{107} – 109 Various cytokines and growth factors have been invoked to explain these effects. These include hepatocyte growth factor and IGF1, which can promote migration, proliferation, and differentiation of resident cardiac stem cells upon

\textbf{Figure 2} Venn diagram showing the experimental set-up used to demonstrate the capacity of the indicated secreted factors to promote cardiomyocyte proliferation. References are reported in the text.
secretion by implanted MSCs. MSCs also secrete C3orf58, a protein that induces neonatal cardiomyocyte proliferation; cardiac-
specific overexpression of this factor in transgenic mice resulted in
enhanced DNA synthesis and cytokinesis in both neonatal and adult
animals. Additional growth factors shown to promote cardiac pro-
genitor cell proliferation are NRG1 and FGF114 and the members of
the vascular endothelial growth factor (VEGF) family; the latter were
reported to promote cardiomyocyte proliferation,115,116 induce stem cell
recruitment and expansion,116 or drive differentiation of epicardial-
derived progenitors to form cardiomyocytes. Most of these factors,
however, exert pleiotropic activities; the specific weight of promoting
cardiac cell proliferation in explaining their beneficial effect on cardiac
function needs more in-depth investigation.

Consistent with its pivotal role in cardiac morphogenesis during de-
velopment, the epicardium constitutes a potential source of progenitor
cells and paracrine signals for cardiac repair. These progenitors, which
are characterized by the specific re-expression of the embryonic gene
Wilm’s tumour 1 (WT1), are very sensitive to thymosin β4, which is
expressed, in the adult heart, by differentiated cardiomyocytes118 and
drives their differentiation to form new cardiomyocytes. Again, the
epicardium also secretes a variety of factors, such as VEGF-A, angio-
poietin-1, FGF2, PDGF, stromal cell-derived growth factor 1, and IL6,87 all of which have been recognized to promote heart repair.

9. Final considerations and emerging concepts

When taking all the above-reported information collectively, it can safely
be concluded that cardiac myocytes are endowed with an intrinsic cap-
cacity to proliferate, which is suppressed to various extents by extra-
or intracellular factors in the postnatal life. The nature of these stimuli
and the way in which they impact on the cardiomyocyte cell cycle still needs
to be fully understood. Multiple evidence over the last couple of decades
has shown that, in the postnatal cardiomyocyte, re-activation of DNA
synthesis and karyokinesis is not an uncommon event under stress con-
ditions. Very few of the treatments that induce cardiomyocyte re-entry
into the cell cycle, however, are able to stimulate cell division and to
increase cell number. Thus, cytokinesis and actual proliferation still
appear to be the limiting steps that markedly distinguish the mammalian
heart from the heart in urodeles and teleosts.

A final, very interesting observation concerns the nature of the signals
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76. Cote GM, Sawyer DB, Chabner BA. ERBB2 inhibition and heart failure.
75. Odiete O, Hill MF, Sawyer DB. Neuregulin in cardiovascular development and disease.
74. Zhao YY, Sawyer DR, Baliga RR, Opel DJ, Han X, Marchionni MA, Kelly RA. Neuregulins
73. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
72. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
70. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S,
69. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
68. Cote GM, Sawyer DB, Chabner BA. ERBB2 inhibition and heart failure.
66. Zhao YY, Sawyer DR, Baliga RR, Opel DJ, Han X, Marchionni MA, Kelly RA. Neuregulins
65. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
64. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
62. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S,
61. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
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57. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
56. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
55. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
54. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
53. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
52. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
51. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
50. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
49. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
48. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
47. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
46. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
45. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
44. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
43. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
42. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
41. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
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39. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
38. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
37. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
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35. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
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31. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
30. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
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28. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
27. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
26. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
25. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
24. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
23. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
22. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
21. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
20. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
19. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
18. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
17. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
16. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
15. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
14. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
13. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
12. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
11. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
10. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
9. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
8. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
7. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
6. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
5. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
4. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
3. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,
2. Hinrichsen R, Haunso S, Busk PK. Different regulation of p27 and Akt during cardio-
1. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D,