Mechanisms of Cardiac Repair and Regeneration

Kathleen M. Broughton,* Bingyan J. Wang,* Fareheh Firouzi, Farid Khalafalla, Stefanie Dimmeler, Francisco Fernandez-Aviles, Mark A. Sussman

Abstract: **Cardiovascular regenerative therapies are pursued on both basic and translational levels. Although efficacy and value of cell therapy for myocardial regeneration can be debated, there is a consensus that profound deficits in mechanistic understanding limit advances, optimization, and implementation. In collaboration with the TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes), this review overviews several pivotal aspects of biological processes impinging on cardiac maintenance, repair, and regeneration. The goal of summarizing current mechanistic understanding is to prompt innovative directions for fundamental studies delineating cellular reparative and regenerative processes. Empowering myocardial regenerative interventions, whether dependent on endogenous processes or exogenously delivered repair agents, ultimately depends on mastering mechanisms and novel strategies that take advantage of rather than being limited by inherent myocardial biology. (*Circ Res*. 2018;122:1151-1163. DOI: 10.1161/CIRCRESAHA.117.312586.)**

Key Words: consensus ■ heart ■ mitochondrial permeability transition pore ■ regeneration ■ stem cells

Myocardial repair and regeneration is an important area of research motivated by increasing occurrence and expanding distribution of heart failure and cardiac-related diseases. Desperate unmet need for novel interventional strategies to treat cardiovascular disease prompted the rapid implementation of clinical approaches to promote myocardial regeneration and repair, but outcomes thus far have been modest at best. To promote comprehensive discussion of cardiovascular regenerative medicinal products, an international research collaboration group was formed known as TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes). TACTICS recently produced a consensus statement prelude to topic-specific articles asserting need for a thorough understanding of mechanisms involved in myocardial repair and regeneration.[1] Serving this purpose, we offer an overview of key mechanisms involved in cardiac repair and regeneration including (1) survival and protection, (2) inflammation reduction, (3) cell–cell communication, (4) angiogenesis/vascularization, (5) cardiomyogenesis, (6) molecular regulation of proliferation and cell cycle, and (7) cardiac aging associated with impairment of reparative and regenerative potential. These mechanisms act both independently and collectively to influence cardiac regeneration (Figure). Clearly, there is still much that we do not understand, significant obstacles to be overcome, and boundless room for improvement. Nevertheless, amazing progress has been made in a relatively short time, and the excitement that myocardial regenerative research has created will undoubtedly continue.

Survival and Protection

Myocardial preservation postinjury and throughout remodeling mitigate long-term damage and cardiac dysfunction. Myocardial infarction (MI) affects left ventricular structure associated with loss of functional tissue leading to complications of ischemic (angina, additional infarctions, and infarct extension), mechanical (heart failure, cardiac hypertrophy, valve dysfunction, aneurysms and cardiac rupture),[1] or arrhythmic (ectopic heart rhythm, sinus node dysfunction, and atrioventricular node dysfunction[2–5]) nature. Preservation of tissue and cellular function is imperative to maintain functionality of the heart, with multiple signaling pathways involved that influence survival.

Cardiomyocyte survival is critical for the preservation of myocardium for ongoing hemodynamic performance and limitation of remodeling with concomitant fibrosis and scarring. Multiple signaling pathways are involved in cardiomyocyte survival[6–8] that operate through a mechanism associated with inhibition of senescence, reduction of inflammation, allowing for generation of new myocytes, and angiogenesis and vascularization of healthy cardiac tissue. One of the most extensively studied survival signaling pathways focuses on Akt, a Ser/Thr kinase with 3 known isoforms in mammals (Akt-1/PKB [protein kinase B]-α), Akt-2/PKB-β, and Akt-3/

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From the Department of Biology, San Diego State University Heart Institute and the Integrated Regenerative Research Institute, CA (K.M.B., B.J.W., F.F., F.K., M.A.S.); Institute for Cardiovascular Regeneration, Center of Molecular Medicine, Frankfurt, Germany (S.D.); and Department of Cardiology, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), CIBERCV and Universidad Complutense de Madrid, Spain (F.F.-A.).

*These authors contributed equally to this article.

Correspondence to Mark A. Sussman, PhD, Department of Biology, SDSU Heart Institute, San Diego State University, 5500 Campanile Dr, San Diego, CA 92182. E-mail heartman4ever@icloud.com

Nonstandard Abbreviations and Acronyms

AMPK	AMP-activated protein kinase
BAX	Bcl-2-associated-X protein
CPC	cardiac progenitor cell
CREB	CAMP response element binding protein
CSC	cardiac stem cell
CTGF	connective tissue growth factor
ECM	extracellular matrix
EPC	endothelial progenitor cells
ET	endothelin
FAK	focal adhesion kinase
FoxO	forkhead box O
HMGB-1	high mobility box 1 protein
ICAM	intercellular adhesion molecule
IGF-1	insulin-like growth factor 1
IL	interleukin
M-CSF	macrophage colony-stimulating factor
MI	myocardial infarction
MMPs	matrix metalloproteinases
mTOR	mammalian target of rapamycin
Nrg	neuregulin
p21CIP1	cyclin-dependent kinase inhibitor p21
PIM-1	pro-to-oncogene proviral integration site for Moloney murine leukemia virus (PIM) kinase 1
PKB	protein kinase B
PKC	protein kinase C
Raf1	v-Raf1 murine leukemia viral oncogene homolog-1
sCR1	soluble complement receptor type 1
SERCA2	sarcoplasmic reticulum Ca2+-ATPase
Sfrp-2	secreted frizzled-related protein 2
TACTICS	Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes
TAK-1	TGF-β–activated kinase 1
TAZ	transcriptional coactivator with PDZ-binding motif
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinase
TNF	tumor necrosis factor
YAP	yes-associated protein

PKB-sγ) activated by several well-characterized growth factors, cytokines, mitogens, and hormones.[9] Akt signaling pathways promote cardiomyocyte survival in zebrafish,[10] mice,[11] rat,[12] rabbit,[9] canine,[13] swine,[14] and in human cells.[15] Akt induces multiple downstream targets in cardiomyocytes including phosphoinositol 3-kinase via Ras or ERK1/2 (extracellular signal-related kinase-1/2),[16] mTOR (mammalian target of rapamycin),[17] Raf1 (v-Raf1 murine leukemia viral oncogene homolog-1),[18] CREB (CAMP response element binding protein),[19] p21CIP1 (cyclin dependent kinase inhibitor p21),[20] FoxO (forkhead box O),[21] PIM-1 (pro-to-oncogene proviral integration site for Moloney murine leukemia virus [PIM] kinase 1),[22] IGF-1 (insulin-like growth factor 1),[23] but even this accounting falls short of a comprehensive list as reviewed previously.[24–26] Clearly, Akt serves as a nodal master regulator on multiple levels of cell biological activity including survival.

Cardiomyocyte survival after initial injury is also enhanced through autophagy by activation of BNIP-3 (BCL2 interacting protein 3), regulating opening of the MPTP (mitochondrial permeability transition pore).[27,28] Ischemia initially increases the ratio of AMP/ATP, resulting in activation of AMPK (AMP-activated protein kinase) along with inhibition of mTOR activity[8] to stimulate autophagy, whereas beclin-1 initiates autophagy during reperfusion.[29,30] Beclin-1, autophagosome formation, and autophagic flux are increased by ischemia/reperfusion and oxidative stress in mice.[31] Beclin-1 is inhibited by the antiapoptotic protein Bcl-2, which contributes to regulation of cell survival:cell death signaling ratio after initial injury.[32]

Along with autophagy, antiapoptotic signaling promotes cardiomyocyte survival. Apoptosis peaks within hours after an ischemic event and is induced by both extrinsic and intrinsic pathways.[33] For example, TNF-α (tumor necrosis factor α) is an extrinsic molecule increased by congestive heart failure[34] as part of the inflammatory response (discussed below). TNF-α activity increases by immune cell release of the stress protein high-mobility group box 1[35] that amplifies apoptotic effects in cardiomyocytes. Intrinsically, proapoptotic BAX (Bcl-2-associated-X protein) and BH-3–only proteins may amplify the apoptotic cascade to destabilize the outer mitochondrial membrane and release additional proapoptotic molecules to activate caspases.[36] Suppression of BAX and p38 mitogen-activated protein kinase phosphorylation increases Akt-Bcl-2 and reduces apoptosis in the ischemic myocardium.[37] Another important regulator of cardiomyocyte apoptosis is p53, such that reduction in p53 levels decreases apoptosis and promotes myocyte survival during ischemia[38] and complete deletion of p53 also decreases apoptosis in cardiomyocytes after MI.[39] Despite decades of cumulative, compelling, and often elegant detailed studies the topic of antiapoptotic signaling as related to cardiomyocyte survival and preservation of function continues to be an actively studied and central theme of myocardial biology.[6–8,40,41]

Inflammation Reduction

Inflammation naturally occurs in response to injury and is prerequisite for remodeling, regeneration, and scar formation. Infarction injury triggers inflammation extending myocardial damage, triggering healing, and remodeling responses. Inflammatory responses in injured myocardium include mononuclear cell infiltration, interleukins that modulate inflammation, mast cell accumulation in the healing scar, fibroblast and extracellular matrix (ECM) remodeling, and temporal regulation of angiogenesis in the evolving and subsequently healing infarct. Cardiac healing involves multiple waves of interstitial cell types, initiated with infiltration of mononuclear cells and mast cells responding to multiple cytokine and growth factors including C5a, TGF (transforming growth factor)-β1, MCP-1 (methyl-accepting chemotaxis protein), IL (interleukin)-8, histamine, TNF-α, IL-6, and ICAM (intercellular adhesion molecule)-1.[42] After monocyte recruitment to infarcted regions, these cells mature and differentiate into macrophages driven by cytokines including M-CSF (macrophage colony-stimulating factor).[43] Lymphocytes in ischemic and reperfused myocardium express IL-10, contributing to the healing process by inducing TIMP (tissue inhibitor of metalloproteinase)-1 expression.[44] IL-10 simultaneously suppresses inflammation

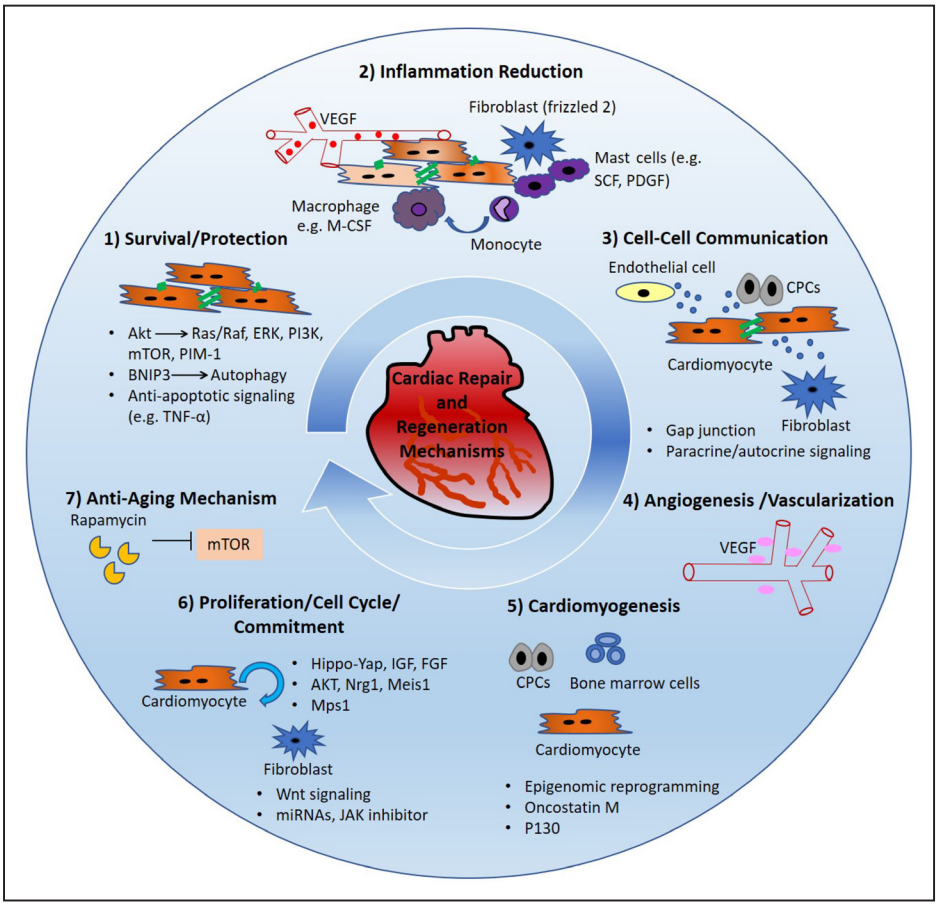

Figure. Cardiovascular repair and regeneration involve multiple mechanisms. Representative categories and selected examples of processes to enhance heart repair and regeneration covered in this review. Mechanisms work independently on a molecular level to collectively mediate concurrent cellular actions of survival, repair and regenerative responses. AKT indicates protein kinase B; BNIP3, BCL2 interacting protein 3; CPC, cardiac progenitor cell; ERK1/2, extracellular signal-related kinase-1/2; FGF, fibroblast growth factor; IGF, insulin-like growth factor; JAK, janus kinase; M-CSF, macrophage colony-stimulating factor; Meis-1, Meis homobox 1; Mps-1, monopolar spindle 1; mTOR, mammalian target of rapamycin; Nrg-1, neuregulin; PDGF, platelet-derived growth factor; PIM-1, pro-to-oncogene proviral integration site for Moloney murine leukemia virus (PIM) kinase 1; PI3K, phosphoinositide 3-kinase; SCF, stem cell factor; TNF, tumor necrosis factor; and VEGF, vascular endothelial growth factor.

through inhibition of multiple cytokines (IL-6, IL-8, IL-12, TNF-α, IL-1α, and IL-1β). Mast cell accumulation in fibrotic regions of infarction[45] is stimulated by chemoattractants including SCF (stem cell factor), PDGF (platelet-derived growth factor), VEGF (vascular endothelial growth factor), and βFGF (basic fibroblast growth factor).[45–49] Myofibroblasts accumulate within a week after infarction in the ischemic and border zone with structural and phenotypic characteristics of smooth muscle cells.[50] Myofibroblasts express a homologue of frizzled 2 and are involved with spatial control of cardiac healing to thereafter undergo apoptosis in the mature scar.[51] Inflammatory response factors, such as VEGF, IL-8, and βFGF promote angiogenesis in the healing myocardium.[52]

Cellular mediated inflammatory response includes neutrophil infiltration in the reperfused myocardial infarct region, neutrophil–endothelial interactions, neutrophil rolling and activation of selectins, leukocyte β2 integrins, chemotaxis, and chemokines. Neutrophils release oxidants, proteases, and autacoids that collectively trigger additional cell recruitment.[53,54] The large and stiff morphology of neutrophils is thought to promote adherence to capillary endothelium and impair reperfusion. Neutrophil rolling leads to upregulation of selectin family members including cell-surface molecules L-selectin, E-selectin, and P-selectin.[55–57] L-selectin is expressed in various circulating cell types (eg, neutrophils, monocytes, T cells, and B cells) and is released from the surface of these activated cells.[55] E-selection is expressed following activation of endothelial cells by cytokines such TNF-α or IL-1β.[56,58] P-selectin is induced by agents such as thrombin, histamine, complement fragments, and oxygen-derived free radicals.[57] Integrins mediate selectin-dependent adhesion of neutrophils, particularly through activation of β2 integrins.[59] Neutrophils are also attracted to IL-8[52] and sequential release of chemoattractants such as C5a in the interstitial fluid after IL-8 production.[60]

Although neutrophils are attracted to and activated in the ischemic and reperfused myocardium to assist in recovery, neutrophils can perpetuate damage in the injured myocardium. Neutrophil-induced myocardial injury, opposed to neutrophil assisted repair, occurs from adhesion-dependent cytotoxicity and ICAM-1 activation.[61] ICAM-1 mRNA circulates in both normal as well as ischemic regions in the heart; however, ICAM-1 protein resides in ischemic tissue areas ≈3 to 6 hours after injury.[62,63] High levels of ICAM-1 mRNA in the viable border zone region of an infarction is also where

highest amounts of neutrophil infiltration occur. Likewise, in the ischemic region where ICAM-1 mRNA expression is elevated, IL-6 mRNA expression is also elevated. IL-6 serves as a nitric oxide-dependent cardiac depressant associated with stunned myocardium.[64] As inflammation and damage progresses with cellular infiltrates, the stage is being set for transition toward myocardial healing.

Humoral inflammatory responses include complement activation, increased reactive oxygen species, and multiple cytokines. Complement cascade activation (C1, C2, C3, and C4) occurs through cardiomyocyte necrosis and release of cardiolipin.[65] Interference with the complement system, such as infusion of human sCR1 (soluble complement receptor type 1) decreases infarction size[66] and may reduce myocardial injury. Reactive oxygen species inhibit cardiomyocyte function,[67] activate the complement cascade,[68] induce P-selectin expression,[69] chemokine upregulation,[70] and increase the ability of ICAM-1 to bind to neutrophils.[71] Cytokines, such as TNF-α, IL-1β and IL-6 trigger inflammatory responses after myocardial ischemia.[58,72] Together with these aforementioned humoral agents, antibody-mediated immune responses represent another facet of coordinated inflammation reaction.

Cell–Cell Communication

Efficient cellular cross-talk ensures proper myocardial development, homeostasis, and initiation of adaptive responses to injury and stress. Cell–cell communication within or between organs allows exchange of electric and chemical signals through gap junctions and secretome signaling. Transfer of secreted factors is mediated by apoptotic bodies (1–5 μm in diameter), microvesicles (100–1000 nm in diameter), and exosomes (40–100 nm in diameter).[73] Additional outside-in and inside-out signaling within tissue occurs between cells and the ECM to adjust myocardial mechanical force, thereby preserving cardiac function during homeostasis and in response to myocardial injury. Critical players in cell–ECM communication primarily produced by fibroblasts include integrins, metalloproteinases, and ECM proteins such as collagen, laminin, fibronectin, osteopontin, thrombospondins, tenascins, and periostin. Myocardial tissue stiffness triggers FAKs (focal adhesion kinases) as well as YAP (yes-associated protein)/TAZ (transcriptional coactivator with PDZ-binding motif) leading to interstitial cell activation.[74]

Cardiomyocytes communicate through multiple routes including gap junctions, adhesion complexes, and secretome factors. The cardiomyocyte secretome influences hemodynamics by regulating vascular tone and blood flow through vasoconstriction ET (endothelin)-1, vasodilation (urocortin),[75] or development and long-term growth of coronary vessels (VEGF, angiopoietins, HGF [hepatocyte growth factor], FGF [fibroblast growth factor], PDGF, and TGF-β)[76,77] as adaptation to stress.[78] Additionally, endothelium influences cardiomyocyte response via paracrine signals including Nrg (neuregulin) to protect from ischemia/reperfusion damage.[79] Direct interaction via connexin family gap junction proteins, allows passage of ions and small solutes controlling impulse conduction. Gap junctions undergo remodeling during myocardial hypertrophy characterized by phosphorylation changes and lateralization from intercalated discs.[80]

Interstitial cells residing between cardiomyocytes are highly contributory to intercellular communication, response to environment, ECM, and remodeling stimuli. A primary example are cardiac fibroblasts that sense and respond to biomechanical stress with autocrine/paracrine factors including ET-1, TNF-α, IL-1β, ATP, IGF-1 FGF-2, Ang-II (and downstream target TGF-β), collectively leading to fibrosis.[81–83] Additional fibroblasts are derived from the endothelial cell pool via TGF-β–mediated endothelial-mesenchymal transformation consequential to fibrosis.[84] TGF-β–induced fibroblast proliferation also promotes ECM production and downstream CTGF (connective tissue growth factor) action.[85] CTGF is negatively regulated by miRNAs produced by fibroblasts (miR-30) and cardiomyocytes (miR-133) acting through neighboring fibroblasts.[86] In return, fibrosis promotes cardiomyocyte hypertrophy through TGF-β–mediated activation of Smad and TAK-1 (TGF-β–activated kinase 1).[87] Fibroblasts are also a major source of Sfrp-2 (secreted frizzled-related protein 2) that may blunt myocardial injury responses.[88,89] Fibroblast-produced IL-33 is another putative cardiomyocyte-protective paracrine signal in pressure overload and MI mouse models.[90] Interestingly, in contrast to the adult myocardium where cardiomyocyte-fibroblast communication generally prompts hypertrophy, fibroblasts mediate cardiomyocyte proliferation via ECM-mediated β1-integrin signaling during embryonic development.[87] Communication from cardiac fibroblasts to activate resident c-Kit$^+$ cardiac progenitor cells (CPCs) is mediated by a secreted factor called HMGB-1 (high mobility box 1 protein).[91] CPCs also contribute to intercellular communication and significantly expand in heart failure patients.[92] Exosomes derived from resident c-Kit$^+$ CPCs or cardiosphere-derived cells reduce cardiomyocyte apoptosis, increase viable mass and improve cardiac function in vivo[93] mediated in part by miR-146a, miR-210, and miR-132.[93–95]

Angiogenesis/Vascularization

Vessels deliver oxygen and metabolites and export waste products and new blood vessel formation is essential for recovery of tissue function after ischemia or tissue injury. Impaired circulatory flow correlates with a poor prognosis in patients cause depressed coronary flow reserve after acute MI[96] as well as compromised microcirculatory response with dilated cardiomyopathy.[97] Similarly, infarct expansion in mice is determined by capillary density in the border zone region.[98] Disruption of angiogenic response and angiocrine signaling from endothelial cells promotes heart failure in mice after transaortic constriction,[99,100] whereas overexpression of angiogenic VEGF rescued hibernating myocardium.[101] As such, vasculogenic action is inextricably linked to preservation and repair of injured myocardium. Ischemic damage in rodent hindlimb models promotes tissue revascularization by arteriogenesis through collateral formation wherein remodeling of existing arterioles to larger conduction arteries re-establishes blood flow.[102] Unfortunately, myocardial arteriogenesis is inefficient with huge heterogeneity of collateral vessel formation in myocardial ischemia patients.[103]

Endothelial progenitor cells (EPCs) incorporate into newly formed capillaries and promote revascularization of ischemic tissues. EPCs translocate from bone marrow to the vascular

injury sites playing an important role in vascular restitution and functional repair after MI.[104,105] Angiogenic incorporation and maturation of EPCs into functionally active endothelial cells has been debated, but lineage tracing experiments show bone marrow-derived cells expressing the EPC marker endothelial NO-synthase contribute to recovery after acute MI.[106] Benefits from EPC activity may derive from physical formation of new capillaries that subsequently regress during resolution of inflammation and wound healing or paracrine action regulating angiogenesis and vessel maturation.[107] Surely, paracrine mechanisms augment proangiogenic activity of EPCs as well as other progenitor and inflammatory cells such as adipose tissue-derived hematopoietic progenitor cells, bone marrow- and tissue-derived mesenchymal cells, monocytes and T-cell subpopulations (reviewed[108]). Paracrine factors such as VEGF alone efficiently foster angiogenesis, but of questionable therapeutic value for long-term vascularization because mature vessels fail to materialize post-treatment. Cellular cues for angiogenesis inferred from zebrafish as well as postnatal retina models indicate initiation by an endothelial tip cell followed by migrating and proliferating stalk cells.[109] Subsequent pericyte recruitment together with mechanical activation of endothelial cells by blood flow promotes vessel maturation. Thus, it seems that multiple concurrent processes might be essential for neoangiogenic activity to be persistent and functional.

Cardiomyogenesis

Two primary hypotheses of cell source that contribute to new cardiomyocyte formation are from the resident stem and progenitor cell pool differentiation and from preexisting cardiomyocytes dedifferentiation followed by cell-cycle reentry. By all accounts, resident cardiomyocyte mitotic activity remains a rare event in late postnatal and adult mice, and degree of myocardial regeneration from preexisting cardiomyocytes is functionally insignificant.[110,111] A fate-mapping study combining 2 different pulse-chase approaches concluded that preexisting cardiomyocytes are the primary source of cardiomyocyte replacement after injury.[112] Similar findings of αMHC (alpha myosin heavy chain)-expressing cardiomyocytes as the origin of rare postnatal cardiomyogenesis events were reported from in vivo clonal analysis and fate mapping at a single-cell level based on the mosaic analysis with double markers model.[113] An instant lineage tracing strategy based on tracking of c-kit antigen expression asserted that detection of fluorescent positive cardiomyocytes within a 48-hour time window implicates preexisting cardiomyocyte rather than derived from stem cells through lineage conversion, although transient expression of c-kit within cardiomyocytes was not ruled out by the analyses.[114] All these genetic lineage-tracing studies share common outcomes of severely limited cardiomyocyte formation from preexisting cardiomyocyte pool as a rare event. Cardiomyocyte mitosis during the first year of life in humans contributes to 0.04% of total cardiomyocytes present at birth and dropping to 0.009% in a 20-year-old young adult heart as assessed by phospho-Histone3 immunolabeling.[115] Annual human cardiomyocyte turnover rates over lifespan are calculated to be 1.9% (adolescent), 1% (middle age), and 0.45% (old age), and by age 50 the cardiomyocytes remaining from birth are ≈55%, whereas 45% are generated later in life.[116] There are clearly substantial limitations of endogenous regenerative mechanisms from cardiomyocytes in the adult mammalian heart. Although the adult mammalian heart possesses an extremely limited capacity for cardiomyocyte renewal, the understanding mechanism(s) of cardiomyocyte proliferation and cell-cycle arrest are fundamental to develop strategies to stimulate turnover and promote cardiac regeneration.

Cardiomyocyte dedifferentiation and proliferation is well-known in lower vertebrates (eg, zebrafish and newts[117–119]) as the primary mechanism of heart regeneration,[120] but the early postnatal mammalian cardiomyocytes also capable of considerable plasticity. Within the first week of postnatal life, neonatal mouse heart can fully regenerate after partial surgical resection, and this regenerative response was characterized by cardiomyocyte proliferation.[121,122] Neomyogenesis is unique to neonatal heart repair, as this critical feature is lost after postnatal day 7 and in the adult mouse heart.[122] Perinatal persistence of cardiomyocyte proliferation in mice may also occur, with a burst of cardiomyocyte mitosis at postnatal day 15.[121] In contrast, adult murine cardiomyocytes dedifferentiate, proliferate, and partially exhibit properties of CPCs including c-Kit expression and multipotency.[123] Cardiomyocyte dedifferentiation changes in structural and fetal protein expression levels[124] and is a primary cellular response in response to stresses such as atrial fibrillation and hibernating myocardium.[125] Molecular mechanism(s) of cardiomyocyte dedifferentiation and proliferation involve epigenomic reprogramming with downregulation of cardiac structure and function genes as opposed to induction of cell-cycle reentry and proliferation genes.[126] Dedifferentiation increases in human and mouse cardiomyocytes treated with osteopontin (OSM [oncostatin M], a member of the IL-6 inflammatory cytokines)[127] as well as P130 and retinoblastoma protein.[128] Despite these initial observations, cardiomyocyte dedifferentiation in the adult mammalian heart remains a rare and inefficient process, with poorly understood underlying molecular mechanisms requiring further characterization.

Self-renewing, clonogenic, and multipotent CSCs have been extensively studied as a potential source to promote myocardial repair and regeneration through direct engagement with tissue as well as indirect actions to activate endogenous myocardial cells in studies of mice, rats, dogs, swine, and humans.[129–135] CSC-derived from postnatal hearts expresses stem cell surface markers (c-Kit/Sca-1) and stem cell phenotypic function (clonogenicity/sphere formation ability).[130,136,137] Activation by environmental stimuli such as infarction injury prompts CSCs that normally reside in cardiac niches to divide, migrate, undergo lineage commitment, and mitigate pathological damage.[138] CSCs lineage determination in the adult mammalian heart demonstrate the presence of Ca^{2+} oscillations as well as Numb, α-adaptin, and Notch-1 signaling as regulators of symmetrical versus asymmetrical division that influence fate decisions.[139,140] Asymmetrical division of CSCs leading to lineage commitment is promoted by ATP released from dying cells that increases Ca^{2+} oscillations through the IP3 pathway in the endoplasmic reticulum.[141]

Bone marrow-derived cells have also been intensively assessed for capacity to mediate myocardial repair and

regeneration as documented in many basic and clinical studies.[142–149] c-Kit+ bone marrow cells injected directly into the local injury site exhibit lineage commitment toward myocytes and vessels, mitigating damage from coronary artery disease.[143] Bone marrow-derived mesenchymal stromal cells function in myocardial regeneration and survival primarily by influencing resident cardiac cells. Mesenchymal stromal cell-mediated cytokine release regulates cardiac cells behavior through multiple signaling pathways.[150,151] Observation of male bone marrow–derived progenitor cells transplanted into female hearts and found as cardiomyocytes with Y-chromosomes raised the possibility of progenitor cells differentiated into cardiac cells.[152] Bone marrow cells migrate to the damaged tissue (facilitated through cytokines paracrine system) has led to a significant magnitude of myocardial remodeling and functional repair.[145,146] However, outcomes of multiple clinical trials involving treatment of heart failure patients with bone marrow-derived cells has settled into a consensus of safe, albeit very modest, improvements in myocardial structure and function. Overall, the poor retention and survival of these and other adoptively transferred stem cell types into damaged myocardium remains a major limitation for clinical implementation.

Molecular Regulation of Proliferation, Cell Cycle, and Commitment

Genetic triggers for cell-cycle reactivation to drive cardiomyocyte mitosis in the adult heart have been advanced as potential therapeutic targets for heart regeneration. For example, Hippo-Yap signaling is critical for cell intrinsic regulation of cardiomyocyte proliferation. Hippo-deficient mouse embryos develop cardiomegaly with robust cardiomyocyte proliferation and potentiated canonical Wnt (wingless-type mouse mammary tumor virus)/β-catenin signaling.[153,154] Loss of cardiac-specific Yap and Taz (cKO) impairs heart development, and knockout mice suffer progressive dilated cardiomyopathy.[155,156] Yap-cKO neonatal hearts failed to regenerate after MI at P2, displayed extensive fibrotic infarct scar and deleterious loss of healthy myocardium, whereas control mice effectively recovered from resection challenge.[156] Constitutive Yap activation in adult heart significantly enhanced cardiac regeneration, improved cardiac function, and promoted cardiomyocyte proliferation by 2.5-folds after infarction challenge.[155,156] Regenerative activity of Yap is partially associated with stimulating IGF, phosphorylation of Akt, and inhibition of GSK-3β (glycogen synthase kinase 3 beta) which could enhance β-catenin nuclear recruitment.[154,156]

Additional molecular candidates for regulation of cardiomyocyte proliferation include Meis-1 (Meis homobox 1) and Nrg-1. Meis-1 is a homeodomain transcription factor essential for normal cardiogenesis and embryonic hematopoiesis. Meis-1 deletion in adult hearts increased a number of cardiomyocytes prompted to enter cell cycle with concomitant increases in cytokinesis, whereas Meis-1 cardiomyocyte overexpression induced premature cell-cycle arrest in neonatal hearts.[157] Inhibitory actions of Meis-1 on proliferation are hypothetically mediated through transcriptional activation of CDK inhibitors p15, p16, and p21.[157,158] (Nrg1) purportedly induces mature mononucleated cardiomyocyte cell division through ErbB4/ErbB2 (erythroblastic leukemia) receptor leading to enhanced myocardial regeneration.[159,160] Transient induction of constitutively active ErbB2 may trigger cardiomyocyte dedifferentiation and proliferation on MI, followed by redifferentiation and regeneration.[159,161] Administration of recombinant Nrg-1 rescues cardiomyocyte depletion in pediatric heart disease and stimulates generation of the new myocardium in neonatal mice after injury.[162] Cardiomyocyte cell-cycle reentry stimulated by the rNrg-1 administration in human infants with congenital heart disease at <6 months of age could provide a therapeutic opportunity for treatment before surgical repair for developmental defects.[162] Despite such intriguing observations, the relevance of these type of molecular intervention candidates adult human myocardium remains both questionable and unknown, as studies were predominantly performed in the context of postnatal or developing myocardium that exhibits increased cardiomyocyte proliferative plasticity similar to lower vertebrates.

Multiple additional paracrine factors and associated signal transduction pathways contribute in the process of myocardial repair. For example, FGF is essential for recruitment of receptors and coordinating epicardial and myocardial activities into regenerating muscle, improving angiogenesis and myocyte survival in acutely injured mammalian hearts.[163] FGF induces downstream PI3 activity correlated with cardiac mitosis during development that is blunted by p38 MAPK (mitogen-activated protein kinase). Inhibition of p38 induces DNA synthesis and G2/M transition in cardiomyocytes, acting synergistically with FGF-1 signaling mediated by phosphoinositol 3-kinase and Akt.[163,164] Thyroid hormones regulate diverse developmental processes as both hyper- and hypothyroid conditions detrimentally impact on myocardial growth and maturation. Tri-iodo-L-thyronine (T3) serves as the primary driver of maturation and suppresses proliferation of near-term fetal ovine cardiomyocytes in vitro[165] by upregulating p21 and downregulating cyclin D1.[165,166] Preadolescent T3 surge in mice coincides with a brief cardiomyocyte proliferative burst mediated by an IGF-1/Akt pathway that may dictate binuclear cardiomyocyte number.[121]

During cardiac regeneration, fibroblasts can play an important role, via myofibroblast transdifferentiation through WNT signaling pathway. Stress fiber formation and contractile proteins expression are the hallmarks of fibroblast transdifferentiation.[167] Several attempts at genetically engineered induction of fibroblast differentiation into cardiomyocytes and CPCs using defined regulatory factors such as selected miRNAs or JAK (janus kinase) inhibitor I tout the possibility of in vivo transdifferentiation of cardiac fibroblasts into cardiomyocytes through cellular reprogramming.[168–171] However, extrapolation of any of these putative mechanisms in service of therapeutically relevant regeneration in the human (or large animal) context remains dubious because of low conversion efficiency and will require further exploration.

Fundamental biological differences between lower vertebrates and humans remain problematic for extrapolation of findings to promote myocardial repair and regeneration. For example, centrosomes of cardiomyocytes in rat hearts disassemble shortly after birth but remain intact in adult zebrafish and newts.[172] Loss of centrosome integrity in postnatal cardiomyocytes coincides with cell-cycle arrest, revealing

a potential mechanism underlying the postmitotic state of mammalian adult cardiomyocytes.[172] Mps-1 (Monopolar spindle 1) protein kinase is a mitotic checkpoint kinase in cell-cycle regulation via its function in centriole assembly and centrosome duplication in mouse and human cells.[173] In zebrafish, Mps-1 mutants fail to regenerate and restore ventricular wall, instead, the injured heart developed large connective-tissue scars 3 weeks after ventricular resection, suggesting a critical cell-cycle regulatory role of Mps-1 in proliferation.[173,174] Similarly, inhibition of the cell-cycle regulator Plk-1 (polo-like kinase 1) drastically inhibited the heart regeneration in zebrafish, resembling the regeneration perturbation seen in zebrafish Mps-1 mutants in zebrafish. This inhibition was not because of cardiomyocytes apoptosis increase, but primarily by loss of mitotic cardiomyocytes.[117,175]

Despite decades of targeted and focused efforts the adult mammalian cardiomyocyte remains remarkably refractory to molecular interventions intended to promote reentry into cell cycle and proliferation. Clearly, the neonatal and lower vertebrate myocardium is a very different molecular and cellular milieu for manipulation of cardiomyocyte proliferation relative to the adult mammalian heart.[176] Although research continues to identify novel and provocative avenues for influencing cardiomyocyte proliferation, the implementation of any such maneuvers in any therapeutic context continues to be hampered by low efficiency and debatable reproducibility. Last, almost all experimental models involve the use of lower vertebrate species and relatively young animals, both of which are problematic for translational relevance to the aged human population most likely to require regenerative therapy. Indeed, the reparative potential of myocardium inexorably declines over time and is clearly impaired in aged individuals at the time and place where it is most needed by several intractable factors as described in the following section.

Cardiac Aging-Associated With Impairment of Reparative and Regenerative Potential

Cardiac aging is a heterogeneous process characterized by increased levels of reactive oxygen species, genomic DNA damage, epigenetic modifications, and telomere shortening. Consequences of aging pursuant to these deleterious changes include defective protein homeostasis, progressive loss of quality control processes, and accumulation of dysfunctional organelles that directly impact cardiomyocyte, fibroblast, and stem cell populations. Such stochastic insults initiated by both extrinsic and intrinsic stimuli eventually lead to impaired contractile function, lower hemodynamic performance as well as defective regenerative responses to injury and stress stimuli.

Need for ongoing myocardial reparative and regenerative maintenance is due in part to deterioration of cardiomyocyte biological and functional competency as a consequence of aging. A hallmark of cardiac aging is decreased a total number of cardiomyocytes by apoptotic or necrotic cell death. Multiple growth and antiapoptotic molecules including survivin, PKC (protein kinase C), protein kinase B (PKB/Akt), STAT-3 (signal transducer and activator of transcription 3), and metallothioneins are downregulated with age.[177–179] Although levels of some apoptotic markers decrease with age, there is a clear shift toward prodeath signals in aging cardiomyocytes, hence, a consensus that apoptosis is a major contributor to deteriorating function in aging hearts.[180] Cell death-induced inflammation[181] leads to oxidative stress and damage through the generation of reactive oxygen species that interact with and alter the properties of biological molecules including nucleic acids, proteins, lipids, and sugars.[182] In turn, oxidative damage induces inflammation creating a vicious cycle in the aging myocyte. Oxidative stress-induced damage and gradual deterioration of cardiomyocyte function also can be mediated by extrinsic environmental factors such as exposure to ionizing radiation or chemicals through ingestion, inhalation, or skin contact.[183,184] Cardiomyocyte mechanics, calcium dynamics, and contractile potential are also significantly altered during the aging process.[185] Upregulation of cytoskeletal proteins and a transcriptional switch of contractile protein from fast to slow myosin are evident during aging.[186] Simultaneous deposition of ECM proteins, mainly collagen, upregulation of MMPs (matrix metalloproteinases) and downregulation of TIMP result in increased stiffness.[187–189] This is associated with defective myocyte activation, contraction, and relaxation. Age-related dysregulation of contractile machinery has primarily attributed to decreased responsiveness of β-adrenergic signaling, the most important regulatory mechanism of cardiovascular performance, possibly because of reduced β-adrenergic receptor density and some defects involving the adenylyl cyclase.[190] Additionally, defective myocyte activation results from downregulation of SERCA2 (sarcoplasmic reticulum Ca^{2+}-ATPase) and Na^{+}/Ca^{2+} exchanger.[191] Altered calcium transients during aging also arise from deposition of cellular garbage such as lipofuscin because of impaired autophagy.[192] Autophagy is a major quality control degradation pathway primarily regulated by mTOR. Interestingly, activation of autophagy through the administration of rapamycin (mTOR inhibitor) or caloric restriction preserves cardiac function with age in rodent animal models.[193,194] Indeed, electric coupling and cell–cell communication are also substantially impaired as a consequence of aging in large animal models where downregulation and misdistribution of connexin 43, a critical gap junction protein, occurs in guinea pig sinoatrial node and rabbit hearts, respectively.[195,196] Overall, defective cardiomyocyte contractile function in addition to global cardiomyocyte loss lead to enlargement of the remaining cardiomyocytes leading to left ventricular hypertrophy to preserve cardiac function.

CPCs are severely impacted by aging and undergo drastic phenotypic and functional alterations. Stem cell niches are the microenvironment where CPCs reside in a quiescent metabolically dormant state of reversible cell-cycle arrest awaiting injury stimulus to be activated, undergo proliferation, and lineage commitment in addition to self-renewal to maintain a functionally competent pool of stem cells.[139,197] In aging hearts, niches secrete signaling factors that prematurely activate CPCs in the absence of injury stimuli, which may result in exhaustion of the stem cell pool.[198] Moreover, the pool of hypoxic niches that favor a more quiescent state of CPCs increases with age while normoxic niches that favor CPC activation, proliferation, and commitment decrease with age contributing to a decline in intrinsic regenerative capacity.[198] Changes in CPC microenvironment are indeed accompanied by changes at the cardiac progenitor cellular

level in terms of morphology, expression levels of markers of stemness, and multipotency as well as multinucleation status with an expected outcome of impaired proliferative, migratory, and commitment capabilities.[199] Enhanced senescence in adult human CPCs from old heart failure patients is associated with impaired proliferative capacity compared with human fetal CPCs.[200] Thus, cardiac aging is associated with a drastic decrease in the number of functionally competent stem cells along with the accumulation of senescent stem cells, which at least partly account for the overall impairment of regenerative response to injury in the aged heart.

Collectively, aging prompts impairment of myocardial biology for both myocytes as well as interstitial cell populations displaying signs of senescence and compromised functional capacity. Consequently, there is an increasing need for homeostatic maintenance with advancing age that unfortunately coincides with progressive deterioration of reparative potential. Thus, aging is typified by the accumulation of poorly functioning senescent cells further exacerbated by the loss of regenerative replacement leading to functional decompensation. The issue of aging is particularly relevant from a translational perspective insofar as any therapeutic approach that depends on endogenous reparative potential will need to overcome the intrinsic, inherent limitations of aged cells and tissues.

Summary

Remarkable progress has been made in a little over a decade since the revolutionary concept was advanced that the human heart is not a postmitotic organ incapable of regeneration and repair. Tissue regeneration and organ repair documented and accepted for several lower vertebrate species in the laboratory setting prompted researchers to ponder whether such processes could be recapitulated in the mammalian setting. The once universally held dogma of the mammalian heart as a postmitotic organ without regenerative potential was crushed by an avalanche of new research. Although none could argue that the process in adult mammals was as efficient as in several lower vertebrates, some provocative parallels were advanced between early pre- and postnatal development and retention of regenerative capacity. Thus, a primary focus of research continues to concentrate on what is present in the nascent developing myocardium that is lost on maturation into early adulthood. Concurrently, devotees of regenerative species that retain the capability for myocardial repair throughout life are keen to identify those molecular and cellular mechanisms preserved in lower vertebrates that disappear (or become latent) in mammals. Molecular control of cellular behavior seems to be the key, but regulation of cellular regenerative potential in vivo has proven to be far more nuanced and challenging. Progress has been frustratingly slow to deliver efficacious translational solutions to impact human health, but it is important to recognize that the concept of mammalian myocardial regeneration debuted shortly after the turn of the century and has provided a wealth of return on investment with expanding knowledge and innovative approaches. The pressure for deliverables has been intense, leading to clinical testing that some have dubbed as premature and of questionable value. However, conclusions drawn from experience in the clinical arena are quite contrary to the view of skeptics and demonstrate not only safety but also modest (albeit variable) efficacy. These initial forays into treatment also have highlighted theoretical and practical challenges that need to be conquered to improve on pioneering cell therapy trials. The future belongs to optimists who recognize and acknowledge limitations of current approaches while promoting their view that if we can all agree that regeneration occurs, no matter how inefficiently, then we should stop debating relevance for cardiac biology and concentrate instead upon improving the process and outcome.

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Drs Sussman and Broughton have a significant interest in CardioCreate, Inc. The other authors report no conflicts.

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