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Chasing c-Kit through the heart: taking a broader view

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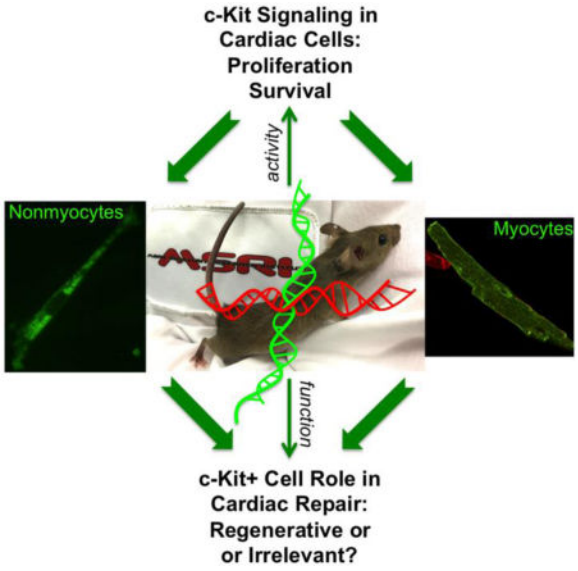
Graphical abstract



Keywords

c-Kit; cardiac; transgenic; knock-in; reporter

Limited regenerative capacity of the mammalian heart was long thought to reflect lack of a cellular reservoir for new heart muscle tissue, but over the last decade a substantial body of literature has emerged documenting the contribution of stem or progenitor cells to cardiogenesis in the postnatal heart (1–22). Numerous cell types have been identified as potential sources of *de novo* cardiomyogenesis in the adult organism, and the significance of their role in cardiac repair is the subject of ongoing intense debate. Whether cardiac regeneration occurs through proliferation of existing myocytes or differentiation of stem cells into cardiac tissue, or both, continues to be intensively studied (23–46). Identification

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of resident cardiac stem cells coupled with awareness that myocyte turnover is an ongoing process throughout life provide a rationale for new stem and regenerative therapies for diseased hearts. Clinical trials using bone marrow derived cell therapies have led the way and shown modest improvements in clinical endpoints (47–49), while further results from Phase I trials using the well characterized cardiac c-Kit+ stem cells and cardiosphere derived cells demonstrate promising improvement in cardiac function and/or structure (50, 51). Engineering c-Kit+ cardiac progenitors with Pim1 kinase to improve their reparative capacity has been validated in animal models and offers a path forward for clinical applications (52–56).

As a marker in the cardiac context, c-Kit is expressed by multiple cell types, including myocytes (22, 57–59), endothelial cells (60, 61), and cardiac stem cells such as mesenchymal and progenitor cells (1, 2, 57, 62). Debate over the contribution of c-Kit+ cells to cardiac repair and their utility in cell-based therapy applications is summarized briefly in Table 1 (1–3, 5, 8, 22, 50, 52–55, 57–61, 63–74). This overview of key publications highlights the diversity of viewpoints in the ongoing discussion among cardiac researchers regarding c-Kit+ cells. A more complex and heterogeneous expression pattern for c-Kit is emerging, as revealed by studies using various genetic animal models developed to determine which cell types participate in cardiac regeneration. Initial fate mapping models created to identify which cell types participate in cardiac repair include the αMyHCmER-Cre-mER/ZEG mouse, in which cardiomyocytes are tagged upon administration of tamoxifen, and transgenic c-KitGFP reporter mouse lines, in which GFP expression diminishes upon loss of c-Kit promoter activity (25, 31, 35, 57, 70, 72, 75). These animal models provide valuable information regarding dynamics of cardiomyocyte turnover and replacement, however they do not definitively identify the specific contribution made to these processes by the c-Kit+ cell population throughout the life of the organism. More recently, direct tagging of c-Kit expressing cells using the endogenous c-Kit promoter validated that c-Kit cells contribute to the cardiomyocyte population, albeit at a very low level, and more extensively to the endothelial and interstitial cell pools (59–61). Intriguingly, studies using a similar lineage-tracing model demonstrated cardiomyogenic capability in c-Kit+ cardiac neural crest progenitors, positing a non-permissive cardiac environment to explain low contribution of these cells to the cardiomyocyte population (22).

Genetic reporter models are imperfect reproductions of endogenous gene expression, whether employing an exogenous promoter segment or exploiting the endogenous gene via knock-in recombination. Transgenic promoter segments may lack important regulatory elements, while knock-in reporters often disable one allele of the gene-of- interest. Specifically, applying knock-in technology for c-Kit lineage tracing silences at least one allele of the c-Kit gene and has been reported to disrupt known regulatory elements in exon 1, thereby perturbing endogenous c-Kit biology with potentially significant consequences for stem cell function (76). c-Kit signaling has been shown to promote growth, survival and proliferation in human CPCs *in vitro* (77), while W locus mouse mutants (W/Wv) exhibit c-Kit cell dysfunction (78, 79). W/Wv mice display impaired cardiac recovery after infarction (80), diminished cardiac function with advanced age (81), and compromised c-Kit cell differentiation into cardiomyocytes (58, 82). Bone marrow c-Kit+ cells from W locus mutants or cells in which c-Kit has been molecularly silenced *in vitro* exhibit blunted

reparative responses to myocardial injury (80, 82–84). Given the importance of functional c-Kit in cardiac maintenance and repair, current c-Kit knock-in mice may harbor similar c-Kit cell related defects. Additionally, reporter expression constrained to one allele of the endogenous promoter, coupled with decreased c-Kit function, could manifest as decreased reporter sensitivity and consequent underrepresentation of the tagged c-Kit cell population (85, 86). Recently, levels of c-Kit expression were shown to influence hematopoietic stem cell (HSC) function and regenerative capacity such that HSCs with relatively low c-Kit surface expression exhibited more stem-like properties of self-renewal and multipotency, whereas high c-Kit surface expression corresponded to compromised self-renewal and a propensity toward megakaryocyte differentiation (87). Low expressing c-Kit cells that constitute a more stem-like population would likely be under-represented in genetic tagging systems with an inherent bias toward high expressing cells. Finally, given the potentially compromised function of the c-Kit population in hemizygous reporter models, they cannot be used to assess the contribution of c-Kit+ cells that have been isolated according to c-Kit expression, then expanded and modified by passaging *in vitro*, as the selection pressures of tissue culture likely favor a subpopulation with enhanced survival and proliferative potential relative to the initial isolates. As such, knock-in studies do not inform upon the role of c-Kit + cells in adoptive transfer therapeutic applications in the clinical context, where cardiomyogenic and regenerative potential are undoubtedly much different from endogenous repair alone.

C-Kit as a cardiac cell marker. The hematopoetic stem cell marker c-Kit has been used to isolate and characterize adult cardiac stem cells in numerous studies. c-Kit expressing cells derived from adult cardiac tissue exhibit stem cell properties of self renewal, clonogenicity, and ability to differentiate into adult cardiac lineages (1, 2, 26, 88). The SCIPIO trial provides compelling clinical evidence that autologous c-Kit expressing adult cardiac stem cells function in a cell therapy application to improve cardiac performance in patients suffering from severe heart disease (50, 63). Direct comparison between human c-Kit+ cardiac progenitors (hCPCs) and bone marrow derived mesenchymal stem cells (hMSCs) reveals a 30 fold greater potency in cardiac repair of hCPCs over hMSCs after adoptive transfer into infarcted hearts of SCID mice, further illustrating the efficacy of c-Kit+ cells in therapeutic applications (89). Distribution of c-Kit expressing cells in the developing heart and their response to pathologic cardiac injury in the adult heart have been monitored using transgenic reporter lines expressing enhanced green fluorescent protein (EGFP) under control of the c-Kit promoter (25, 26, 57, 90). In addition to purported contributions of c-Kit + stem cells to myocardial adaptation and repair, cardiomyocyte de-differentiation and proliferation may also represent important mechanisms of cardiac regeneration. However, interpretations of the role for myocytes as contributors to cardiomyogenesis in the adult vary from little or none to substantial (30, 70, 91–93). For example, two separate studies from the same laboratory assert that multi-isotope mass spectrometry performed with mice demonstrates pre-existing cardiomyocytes are the dominant source of cardiomyocyte replacement in the adult mammalian heart following injury (93), while a prior report supports the possibility that nonmyocyte cells contribute to cardiac repair (70). Similarly, in lower vertebrate species such as zebrafish that exhibit remarkable myocardial regenerative potential throughout life, the robust reparative response after injury occurs via de-

differentiation and proliferation of existing cardiomyocytes (34, 94). Myocardial regeneration of this magnitude can also occur in mice, but only during fetal and very early neonatal development where c-Kit+ cells predominate (37, 95). Treatments that induce cardiomyocyte de-differentiation with c-Kit+ re-expression (72) and proliferation (32, 96, 97) in adult mice have been described, and expression of c-Kit has been reported in neonatal mouse cardiomyocytes undergoing terminal differentiation a few days after birth (58, 66). These “noncanonical” expression patterns of c-Kit have profound implications for lineage tracing studies employing c-Kit promoters. As traditional lines continue to blur between differentiated cardiomyocytes and the resident cardiac stem cell pool, with both populations potentially contributing to expansion and deployment of c-Kit+ cells engaging in heart growth and repair, more nuanced interpretation of results utilizing genetic models for c-Kit cell labeling becomes essential.

Beyond c-Kit+ stem cells and myocytes, cardiac interstitial cells, which comprise vascular and perivascular cells of the coronary circulation, and stromal and immune cells, represent the majority cell types of the heart within a dynamic and interconnected environment supporting cardiomyocyte function, homeostasis and repair. In addition to structural, sensing and adaptive functions, these cells are governed by their own stem cell hierarchies, and help to configure the niche for all stem cell populations of the heart. The adult epicardium, where c-Kit+ cells are known to reside (98, 99), is also emerging as a potentially significant player in heart repair. In the injured adult heart the epicardium reactivates its developmental transcriptional program (100, 101) and contributes new fibroblasts, perivascular cells and potentially cardiomyocytes to the injury site, stimulating angiogenesis and repair processes (102–104).

New approaches are needed to identify the distribution and proportion of all adult cardiac cell types expressing c-Kit or derived from c-Kit progenitors, establishing a direct readout of c-Kit+ cell participation in cardiac homeostasis and repair. An undeniable need exists for a better understanding of c-Kit+ as a marker of the regenerative cell population in the adult mouse. Although the list of candidate cardiac stem cells continues to grow, there is no clear understanding of whether these populations are inter-related functionally, or if a cellular stem cell hierarchy exists within the adult mammalian heart. As regenerative medicine further expands to embrace novel approaches for treatment of cardiovascular disease, robust, reliable, and consensual experimental models to study the cellular basis of tissue repair are needed now more than ever. As one of the very first endogenous cardiogenic cell populations identified in the adult mammalian heart, c-Kit+ stem cells have been advanced to clinical implementation for treatment of heart failure even as their role in myocardial repair continues to be contested.

Possession of fate mapping information for c-Kit+ cells in the adult mammalian heart represents powerful methodology as well as an important conceptual advance for revealing the true basis of endogenous c-Kit+ cell biology in the myocardium. Tagging of c-Kit+ can be performed during development, after injury, or in the aged heart to assess the incorporation of c-Kit+ cells, and to resolve longstanding debates resulting from indirect assessment of c-Kit+-based myocardial biology, or studies performed in models with potentially compromised endogenous c-Kit function. Recently, an inducible transgenic

overexpression model has been developed to tag all various c-Kit+ cells including cardiomyocytes and stem cells either reversibly or permanently, thereby circumventing disruption of the endogenous c-Kit gene and potentially identifying cells within a broader range of c-Kit expression. Findings using this model are expected to reveal previously unrecognized aspects of c-Kit expression and biology that will contribute to the overall understanding of cardiac c-Kit cell function and therefore improve the potential for use of these cells in the treatment of heart disease (unpublished observations). Likewise, it is possible to envision reporter models targeting the endogenous c-Kit locus without disrupting native expression using new gene editing technologies such CRISPR/Cas9 to insert an IRES-EGFP in the 3'-UTR, or to generate a reporter fused to the endogenous c-Kit protein. Studies based on these mouse models will add missing information to the cardiac c-Kit cell literature and address important questions regarding the relevance of the c-Kit+ stem cell and c-Kit expression in the adult heart.

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Table 1

Summary of c-Kit+ cardiac stem cell debate.

	CURRENT CARDIAC c-KIT FINDINGS	SPECIES	REFERENCES
c-Kit **YES**	Mammalian hearts possess c-Kit+ adult stem cells that contribute to cardiac formation, homeostasis and repair.	mouse	Beltrami et al, Cell, 2003
			Ellison et al, Cell, 2013
			Nadal-Ginard et al, Stem Cell Res, 2014
			Anversa et al, JCI, 2013
			Hatzistergos et al, PNAS 2015
			Tallini et al, 2009
			(1–3,5,22,57)
	Adoptive transfer of autologous cardiac c-Kit+ cells improves cardiac function in heart failure patients.	human, pig	Bolli et al, Lancet, 2011
			Chugh et al, Circulation, 2012
			Quevedo et al, PNAS, 2009
			Schuleri et al, Eur Heart J, 2009
			McCall et al, Nature protocols, 2012
			(50,63,64,8,65)
	Cardiac c-Kit+ progenitor cells engineered to overexpress Pim1 engraft, differentiate and improve cardiac function better than non-engineered cells upon adoptive transfer into infarcted myocardium.	mouse, pig	Fisher et al, Circulation, 2009
			Mohsin et al, Circ Res, 2011
			Mohsin et al, JACC, 2012
			Mohsin et al, Circ Res, 2013
			(52–55)
	c-Kit+ cell fate mapping models show that c-Kit+ cells contribute to cardiogenesis during development and repair.	mouse	Hatzistergos et al, PNAS, 2015
			van Berlo et al, Nature, 2014
			(22,60)
	c-Kit is expressed in neonatal myocytes during terminal differentiation	mouse	Li et al, Circ Res, 2008
			Naqvi et al, Ped cardiol, 2009
			(58,66)
c-Kit **No**	c-Kit+ cells are not adult cardiac stem cells and do not contribute to cardiac formation, homeostasis or repair.	mouse,	Balsam et al, Nature, 2004
			Sultana et al, Nat Comm, 2015
			Zaruba et al, Circulation, 2010
			(74,61,67)
	Exogenous c-Kit+ cells do not repair injured myocardium through *de novo* formation of cardiac tissue.	mouse	Murry et al, Nature, 2004
			(68)
	c-Kit+ cells are irrelevant in human cardiosphere cell therapy applications.	human	Cheng et al, JAHA, 2014
			(69)
c-Kit **MAYBE**	Cardiomyocyte fate mapping models suggest that c-Kit+ cells could contribute to cardiogenesis following injury.	mouse	Hsieh et al, Nat Med, 2007
			(70)
	c-Kit+ cells contribute to neonatal but not adult cardiac repair in mouse.	mouse	Jesty et al, PNAS, 2012
			(71)

	CURRENT CARDIAC c-KIT FINDINGS	SPECIES	REFERENCES
	Normal, injured or dedifferentiated cardiomyocytes may express c-Kit.	mouse	Liu et al, Cell Res, 2016
			Tallini et al, PNAS, 2009
			Zhang et al, PloS One, 2010
			Kubin et al, Cell Stem Cell, 2011
			(59,57,72,73)