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Review

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

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ABSTRACT

Stem cell mediated cardiac repair is an exciting and controversial area of cardiovascular research that holds the potential to produce novel, revolutionary therapies for the treatment of heart disease. Extensive investigation to define cell types contributing to cardiac formation, homeostasis and regeneration has produced several candidates, including adult cardiac c-Kit+ expressing stem and progenitor cells that have even been employed in a Phase I clinical trial demonstrating safety and feasibility of this therapeutic approach. However, the field of cardiac cell based therapy remains deeply divided due to strong disagreement among researchers and clinicians over which cell types, if any, are the best candidates for these applications. Research models that identify and define specific cardiac cells that effectively contribute to heart repair are urgently needed to resolve this debate. In this review, current c-Kit reporter models are discussed with respect to myocardial c-Kit cell biology and function, and future designs imagined to better represent endogenous myocardial c-Kit expression.

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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 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 α MyHCmER-Cre-mER/ZEG mouse, in which cardiomyocytes

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Table 1
Summary of c-Kit+ cardiac stem cell debate.

	Current cardiac c-Kit findings	Species	Refs.
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]
	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]

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

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