Published in final edited form as:

Circ Res. 2017 October 27; 121(10): 1130–1132. doi:10.1161/CIRCRESAHA.117.311921.

Identity crisis for regenerative cardiac cKit⁺ cells

Onur Kanisicak, Ronald J. Vagnozzi, and Jeffery D. Molkentin

Department of Pediatrics, University of Cincinnati, Cincinnati Children's Hospital Medical Center and Howard Hughes Medical Institute, OH (O.K., R.J.V., J.D.M.)

Summary

The concept that cardiac-derived cKit⁺ cells can regenerate the injured adult heart by transdifferentiating into functioning cardiomyocytes was proposed 13 years ago, although it remains controversial because of negative data from multiple independent laboratories. Irreproducibility of cardiac cKit⁺ cell studies was attributed to the differences in cell isolation, selection and expansion before *in vivo* application. This Viewpoint will discuss recent results that again change the formula for how cardiac cKit⁺ cells must be isolated and processed in order to be cardiomyogenic, as well as discuss the uncertain *in vivo* relevance of cKit⁺ cells as putative cardiomyocyte-producing stem cells.

Keywords

Cardiac regeneration; Myocardial infarction; stem cell

Introduction to the cardiac regeneration - cKit⁺ cell controversy

The regenerative and myogenic potential of cKit⁺ cardiac-resident stem cells (CSCs) remains a dominant and contentious topic. *In vitro* expanded cKit⁺ CSCs were originally described 13 years ago as a source for robust new myocyte generation when injected directly into the adult heart post-myocardial infarction (MI)¹, and while some laboratories have confirmed the basic principle, the magnitude of the effect remains unclear². More recently, Ellison et al showed that a clonal cKit⁺ cell line derived from a population of cardiac CD45⁺ cKit⁺ cells was capable of widely regenerating isoproterenol-injured adult rodent hearts with abundant new cardiomyocytes, after simple tail vein infusion³. However, many independent laboratories reported that adult cardiac-derived cKit⁺ cells did not generate appreciable new cardiomyocytes when directly injected back into the injured rat or mouse heart^{4–7}.

In addition to controversies surrounding the regenerative potential of injected cKit⁺ cell formulations, until recently the field has not attempted to directly address if endogenous cardiac cKit⁺ cells are true cardiomyocyte producing “stem cells” of meaningful significance⁸. In an attempt to directly address this shortcoming, 4 independent laboratories

Correspondence to Jeffery D. Molkentin, PhD, Howard Hughes Medical Institute, Cincinnati Children's Hospital Medical Center, 240 Albert Sabin Way, Cincinnati, OH 45229. jeff.molkentin@cchmc.org.

Disclosures

None.

separately generated and characterized mice with Cre recombinase (or H2B-tdTomato) targeted to the *Kit* gene (encodes cKit protein), to permit lineage tracing in vivo^{9–12}. These studies uniformly showed that endogenous cardiac cKit⁺ cells did not generate appreciable new cardiomyocytes in the mouse heart. In response to these data and the ongoing controversy in the field, a collection of established cardiac-based researchers released a 2017 consensus statement in *Circulation* that provides guidance on cardiac regeneration. The general conclusion is that the adult mammalian heart only generates ~1% new cardiomyocytes each year, which fate-mapping studies suggest is mediated primarily by cardiomyocyte proliferation¹³. However, this consensus statement did not dispute the likelihood that exogenously delivered cardiac cKit⁺ cells could have a beneficial effect when given to the injured heart, such as by enhancing vascular regrowth, extracellular matrix conditioning and by paracrine-mediated induction of endogenous repair pathways^{14, 15}.

New study from Vicinanza et al changes formula for cardiogenic cKit⁺ cells

A new study from Vicinanza et al has reignited the debate over cardiomyogenesis from cKit⁺ CSCs, as they now report that roughly 99% of primary cKit⁺ cells isolated from the heart lack cardiomyogenic potential and have no rejuvenating benefit when injected into the injured hearts of mice or rats¹⁶. Instead, only a rare sub-fraction of cardiac cKit⁺ cells defined by: 1) expression of the marker cKit, 2) absence of the hematopoietic marker CD45 and 3) having been clonally derived and expanded in vitro, are able to regenerate the heart with new cardiomyocytes. It is this fraction, representing only 1% of total cardiac cKit⁺ cells, that Vicinanza et al¹⁶ re-define as the “true cKit⁺ cardiac stem cell.” Such a model, if correct, warrants re-examination of an entire body of past literature, as it calls into question the identity and phenotype of isolated cKit⁺ CSCs that have already been used in numerous animal studies and in ongoing human clinical trials^{2, 14}.

Perhaps most striking, the new claims by Vicinanza et al contradict the original characterization of cKit⁺ CSCs reported in *Cell* in 2003 (Beltrami et al) by some of the same senior authors (Drs. Nadal-Ginard and Torella)¹. The 2003 study which began much of this field used total cKit⁺ cells to create 70% new functioning myocardium when directly injected into the infarcted rodent heart¹. Cardiac cKit⁺ cells without additional isolation criteria were reported to be self-renewing, clonogenic, and multipotent, as well as widely expressing the cardiogenic transcription factor Nkx2.5¹. Vicinanza et al now contend that CD45 negative selection is required to designate a cardiac cKit⁺ cell as a CSC, and that among all CD45[−] cKit⁺ cells, only a small portion (10%) have the potential to generate clones in culture with cardiomyogenic potential¹⁶. However, the histologic endpoints for cardiac renewal and the physiologic endpoints for functional improvement in Vicinanza et al¹⁶ versus Beltrami et al¹ appear virtually identical, despite the fact that these new data contend that the population used in the original Beltrami et al study was probably not cardiomyogenic. In addition, the new results by Vicinanza et al are also at odds with their Ellison et al study in which they stated that “many of the c-kit^{POS} eCSCs expressed GATA4 and Nkx2.5, two early transcription factors of the cardiac lineage”³.

More perplexing, Vicinanza et al reported that injection of broadly isolated cKit⁺ cells not negatively sorted for CD45 produced essentially no new cardiomyocytes (0.0001%) and no

functional benefits in injured rodent hearts¹⁶. Again, this result stands in contrast to their original observations from 13 years ago¹, and it calls into question the newly emerging paracrine hypothesis¹⁴ and the potential validity of ongoing clinical trials that also use broadly isolated cardiac cKit⁺ cells (NCT02501811). Recent studies have shown that the vast majority of cKit⁺ cells directly injected in the rodent heart simply die in a few days, while the cells that persist lack appreciable cardiomyogenic potential⁷. The results of Vicinanza et al are at odds with these newer concepts for how cardiac cKit⁺ cells might truly perform when injected back into the injured myocardium.

Understanding the teleology of endogenous cKit⁺ cells

The potentially larger issue to consider is that Vicinanza et al only showed that *in vitro* clonally-derived cKit⁺ CD45⁻ cells could generate new cardiomyocytes when injected into the rat or mouse heart¹⁶. This same basic experimental approach with clonally redrived cKit⁺ cells (in this case injected into the tail vein) was also used in Ellison et al as the means of achieving meaningful regeneration of the mouse and rat heart³. We make no scientific argument against the likelihood that selected cardiac-resident mesenchymal cell types can be reprogrammed to adopt a more cardiomyogenic fate, whether by addition of cardiac-lineage specifying transcription factors, or perhaps by rigorous *in vitro* selection to identify a rare clonal cKit⁺ cell with myogenic characteristics. Our contention is related to interpretation, as *in vitro* clonally selected cKit⁺ cells do not directly inform as to the true endogenous function of these cells within the heart.

To directly address the cardiomyogenic potential of endogenous cKit⁺ cells *in vivo*, we and others have taken a genetic approach by inserting Cre recombinase (or H2B-tdTomato) into the *Kit* gene. Our collective results showed that cardiac resident cKit⁺ cells have extremely limited cardiomyogenic potential *in vivo*, either at baseline, with aging or following injury^{9, 10, 12}. However, Vicinanza et al claim that cKit⁺ cells are true cardiac stem cells, albeit a very rare population of them, by again attempting to discredit *Kit* allele knock-in lineage tracing approaches as flawed. As this argument was previously addressed⁸ but still invoked by these authors, here we provide a simple figure of data definitively showing that lineage tracing with Kit-Cre knock-in mice identifies a majority of CD45⁻ cKit⁺ cells from the adult heart (Figure 1). This is the same starting population of cells that Vicinanza et al claims have enhanced cardiomyogenic potential if clonally selected. Hence, disrupting one of the 2 *Kit* alleles in performing lineage tracing does not compromise identification of the newly proposed “true” cardiogenic cKit expressing subpopulation described by Vicinanza et al¹⁶.

Our previous lineage tracing results in the adult mouse heart showed detectable levels of new cardiomyocytes from cKit⁺ lineages⁹ and while this rate was extremely low (1:20,000, when fusion was subtracted), it is consistent with the recent results of Vicinanza et al whereby only 1% of the total cKit⁺ cells isolated from the heart can become cardiomyogenic when clonally selected *in vitro*. For example, when Vicinanza et al inject total primary isolated cKit⁺ cells into the MI-injured heart, which would contain these putative rare cardiomyogenic cKit⁺ cells, they report a rate of new cardiomyocyte production of 0.0001%. Hence, the results of Vicinanza et al support the emerging consensus in the field that

endogenous cardiac cKit⁺ cells are not true cardiomyocyte producing stem cells of physiologic significance. However, this does not discredit the observation that *in vitro* clonal selection of a subfraction of cardiac cKit⁺ cells can produce a cell line with enhanced cardiomyogenic potential, as appears to have been the case behind the Ellison³ and Vicinanza¹⁶ et al manuscripts. Such highly selected cKit⁺ clonal cells could also be therapeutically relevant in treating patients, especially if they were autologously generated.

Implications of Vicinanza et al in looking forward

In moving forward, it will be important to better define the mechanisms of action underlying cardiac cell therapy while not forgetting the overarching concept that the adult mammalian heart is not an inherently regenerative organ and as such, it is unlikely to contain a physiologically relevant cardiomyocyte producing “stem cell”, cKit expressing or otherwise. However, despite the ongoing controversy in the field we should not be dissuaded from continuing our efforts to enhance the effectiveness of cell therapeutic approaches, as the majority of animal studies continue to show a benefit of cKit⁺ cell administration when directly injected into the injured heart.

Acknowledgments

Sources of Funding

J.D.M. and R.J.V. were supported by grants from the National Institutes of Health (F32 HL128083 to R.J.V). J.D.M. was also supported by the Howard Hughes Medical Institute.

References

1. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003; 114:763–776. [PubMed: 14505575]
2. Nigro P, Perrucci GL, Gowran A, Zanolini M, Capogrossi MC, Pompilio G. C-kit(+) cells: The tell-tale heart of cardiac regeneration? *Cell Mol Life Sci*. 2015; 72:1725–1740. [PubMed: 25575564]
3. Ellison GM, Vicinanza C, Smith AJ, Aquila I, Leone A, Waring CD, Henning BJ, Stirparo GG, Papait R, Scarfò M, Agosti V, Viglietto G, Condorelli G, Indolfi C, Ottolenghi S, Torella D, Nadal-Ginard B. Adult c-kit(pos) cardiac stem cells are necessary and sufficient for functional cardiac regeneration and repair. *Cell*. 2013; 154:827–842. [PubMed: 23953114]
4. Zaruba MM, Soonpaa M, Reuter S, Field LJ. Cardiomyogenic potential of c-kit(+)-expressing cells derived from neonatal and adult mouse hearts. *Circulation*. 2010; 121:1992–2000. [PubMed: 20421520]
5. Jesty SA, Steffey MA, Lee FK, Breitbach M, Hesse M, Reining S, Lee JC, Doran RM, Nikitin AY, Fleischmann BK, Kotlikoff MI. C-kit+ precursors support postinfarction myogenesis in the neonatal, but not adult, heart. *Proc Natl Acad Sci U S A*. 2012; 109:13380–13385. [PubMed: 22847442]
6. Cheng K, Ibrahim A, Hensley MT, Shen D, Sun B, Middleton R, Liu W, Smith RR, Marbán E. Relative roles of cd90 and c-kit to the regenerative efficacy of cardiosphere-derived cells in humans and in a mouse model of myocardial infarction. *J Am Heart Assoc*. 2014; 3:e001260. [PubMed: 25300435]
7. Tang XL, Li Q, Rokosh G, Sanganalmath SK, Chen N, Ou Q, Stowers H, Hunt G, Bolli R. Long-term outcome of administration of c-kit(pos) cardiac progenitor cells after acute myocardial infarction: Transplanted cells do not become cardiomyocytes, but structural and functional improvement and proliferation of endogenous cells persist for at least one year. *Circ Res*. 2016; 118:1091–1105. [PubMed: 26838790]

8. Cai CL, Molkentin JD. The elusive progenitor cell in cardiac regeneration: Slip slidin' away. *Circ Res.* 2017; 120:400–406. [PubMed: 28104772]
9. van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin SC, Middleton RC, Marbán E, Molkentin JD. C-kit+ cells minimally contribute cardiomyocytes to the heart. *Nature.* 2014; 509:337–341. [PubMed: 24805242]
10. Sultana N, Zhang L, Yan J, Chen J, Cai W, Razzaque S, Jeong D, Sheng W, Bu L, Xu M, Huang GY, Hajar RJ, Zhou B, Moon A, Cai CL. Resident c-kit(+) cells in the heart are not cardiac stem cells. *Nat Commun.* 2015; 6:8701. [PubMed: 26515110]
11. Hatzistergos KE, Takeuchi LM, Saur D, Seidler B, Dymecki SM, Mai JJ, White IA, Balkan W, Kanashiro-Takeuchi RM, Schally AV, Hare JM. Ckit+ cardiac progenitors of neural crest origin. *Proc Natl Acad Sci U S A.* 2015; 112:13051–13056. [PubMed: 26438843]
12. Liu Q, Yang R, Huang X, Zhang H, He L, Zhang L, Tian X, Nie Y, Hu S, Yan Y, Qiao Z, Wang QD, Lui KO, Zhou B. Genetic lineage tracing identifies in situ kit-expressing cardiomyocytes. *Cell Res.* 2016; 26:119–130. [PubMed: 26634606]
13. Eschenhagen T, Bolli R, Braun T, Field LJ, Fleischmann BK, Frisén J, Giacca M, Hare JM, Houser S, Lee RT, Marbán E, Martin JF, Molkentin JD, Murry CE, Riley PR, Ruiz-Lozano P, Sadek HA, Sussman MA, Hill JA. Cardiomyocyte regeneration: A consensus statement. *Circulation.* 2017; 136:680–686. [PubMed: 28684531]
14. Sanganalath SK, Bolli R. Cell therapy for heart failure: A comprehensive overview of experimental and clinical studies, current challenges, and future directions. *Circ Res.* 2013; 113:810–834. [PubMed: 23989721]
15. van Berlo JH, Molkentin JD. An emerging consensus on cardiac regeneration. *Nat Med.* 2014; 20:1386–1393. [PubMed: 25473919]
16. Vicinanza C, Aquila I, Scalise M, Cristiano F, Marino F, Cianflone E, Mancuso T, Marotta P, Sacco W, Lewis FC, Couch L, Shone V, Gritti G, Torella A, Smith AJ, Terracciano CM, Britti D, Veltri P, Indolfi C, Nadal-Ginard B, Ellison-Hughes GM, Torella D. Adult cardiac stem cells are multipotent and robustly myogenic: C-kit expression is necessary but not sufficient for their identification. *Cell Death Differ.* 2017 Epub ahead of print.

Figure 1. Kit-Cre lineage tracing of CSCs

Flow cytometry plots of isolated cardiac interstitial cells pre-gated for the $CD45^-/cKit^+$ population from either *Rosa26-loxP-eGFP* (Cre-dependent reporter mouse line, R26-eGFP) or *Kit^{+/Cre} × R26-eGFP* mice. Approximately 66% of $CD45^-/cKit^+$ cells are eGFP-positive and 34% did not show recombination of the R26-eGFP locus (Representative of cells analyzed from n=4 mice at 12 weeks of age). These data indicate that the Kit-Cre allele effectively lineage traces cardiac $cKit^+$ cells with the same antibody marker profile used in enriching for cardiomyogenic cells by Vicinanza et al¹⁶.