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A Re-assessment of c-Kit in Cardiac Cells: A Complex Interplay Between Expression, Fate, and Function

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Introduction

c-Kit (CD117) is a type III receptor tyrosine kinase that activates a downstream signaling cascade upon binding to stem cell factor (SCF). Studies from the past three decades have demonstrated the expression of c-Kit in various cell types including embryonic, spermatagonial, and hematopoietic stem cells as well as differentiated cells such as melanocytes, neurons, Leydig cells in the testis, and mast cells.[1] The expression of c-Kit to mark a stem cell population in the adult heart was initially reported by Anversa and colleagues[2] and subsequent studies to validate or extend this work has led to tremendous conflicts and controversies. Much of the disagreements involve inconsistent results and discrepant conclusions that stem from the use of distinct tools and models that each carries its own advantages and disadvantages. In general, studies that employed single allele knock-in replacement of the endogenous *C-KIT* locus have demonstrated fewer labeled cells than studies that utilized transgenic reporters (discussed below). Furthermore, a functional role for c-Kit signaling in cardiac cells has been raised but not convincingly demonstrated.

c-Kit$^+$ Cells in Cardiac Development and Regeneration

Following the initial report of c-Kit as a marker for adult cardiac stem cell (CSC),[2] numerous studies describe the isolation, expansion, and *in vitro* differentiation of adult c-Kit$^+$ cardiac cell into multiple cardiovascular cell lineages (for a review see Hesse et al[1]). The

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Disclosures

None.

expression of c-Kit in cardiac cells declines from embryonic stages and is nearly completely absent in the adult heart. In developing embryos, a population of cardiomyogenic c-Kit$^+$ cardiac progenitors is present in the neural crest.[3] The ability of a rare number of neonatal c-Kit$^+$ non-myocytes to differentiate *in vitro* into beating cardiomyocytes were shown by two independent groups[4, 5] Interestingly neither group was able to demonstrate cardiomyogenic differentiation from adult c-Kit$^+$ non-myocytes either *in vitro* or *in vivo*. These studies raised an interesting possibility that both the down regulation of c-Kit expression in cardiac cells and the loss of cardiomyogenic potential in resident non-myocytes (of which some may be c-Kit$^+$ CSCs) may account for the decline in cardiomyogenesis in adult hearts compared with neonatal hearts. While the discrepancy in the cardiomyogenic potential of adult c-Kit$^+$ cells between various studies remains to be clarified, human c-Kit$^+$ cardiac cells were isolated, *ex vivo* expanded, and transplanted into myocardial infarction patients in Phase I/IIa studies (SCIPIO[6] and CADEUCES[7]). Although the patient numbers were relatively small, both studies demonstrated safety and hints of cardiac functional improvement in patients injected with either *ex vivo* expanded c-Kit$^+$ cells (SCIPIO)[6] or cardiosphere-derived cells that contains c-Kit$^+$ cells (CADEUCES)[7]. Since c-Kit$^+$ cells were delivered by intracoronary infusion in both studies, it is likely that the reported benefit is due to paracrine mechanisms given the difficulty in demonstrating long-term cell engraftment following transplantation in human.[8] The interest in further validating the benefit of adult c-Kit$^+$ cells in a larger patient population from the clinical community contrasts sharply with the ongoing debate surrounding the cardiomyogenic capacity of adult c-Kit$^+$ cardiac cell in the basic research community. While clinical studies in human may be argued as the most relevant model for investigation, the contribution from mouse models that offers precise targeting of specific gene for improved mechanistic understanding of the biology at work is critical and necessary to guide future clinical studies. A series of recent studies from multiple labs to lineage trace c-Kit expressing cells in mice using direct or inducible Cre/LoxP-based strategy have attempted to address the ability of c-Kit$^+$ cells to give rise to cardiomyocytes in the heart *in vivo* throughout the entire lifespan from fetal to adult stages.

Lineage Tracing Studies to Address the Contribution of cKit$^+$ Cells to Cardiomyocytes

The effort to lineage trace c-Kit$^+$ CSCs *in vivo* was first performed by injection of lentivirus expressing Cre recombinase under c-Kit promoter.[9] Ellison et al. showed the Cre-labeled c-Kit$^+$ cells contributed to a substantial number of cardiomyocytes after isoproterenol-induced heart injury.[9] Since the transgenic c-Kit promoter used to drive Cre expression may exhibit off-target labeling, given the short promoter used and the unexpectedly large number of cardiomyocytes labeled, the strategy to target Cre recombinase into endogenous gene locus (knock-in) was recently employed for tracing c-Kit$^+$ cells. Using similar approaches, three independent groups found that c-*Kit-Cre* labeled very few, if any, cardiomyocytes in the adult heart,[10–12] raising concerns over the previously reported claim that c-Kit$^+$ CSCs give rise to new cardiomyocytes. Interestingly, the c-Kit-Cre and inducible Cre generated by independent labs all resulted in the loss of c-Kit expression in one allele,[10–12] raising the possibility of under-reporting of cells that express low level of c-Kit and a defect in the differentiation of endogenous c-Kit$^+$ CSCs into cardiomyocytes. The haploinsufficiency of

c-Kit in all published knock-in studies begs the question of whether there is a role for c-Kit protein to regulate cardiac cell function (e.g. proliferation, survival and differentiation). In this regard, a new transgenic mouse line that employs a longer regulatory element from the *C-KIT* locus and leaves the endogenous c-Kit gene unperturbed would be very helpful to reassess the labeling of c-Kit$^+$ cells in the adult heart and examine the functional requirement of c-Kit.

Comparison of A New c-Kit Transgenic Reporter with the Previous Knock-in Model

In this Issue, Gude *et al.* generated a new transgenic c-Kit reporter system to study Kit$^+$ cell function and biology *in vivo* and *in vitro*.[13] In this new system, the cDNA expressing reverse tetracycline transactivator (rtTA) was driven by a longer 14kb fragment of mouse c-Kit promoter and activates *H2BEGFP* reporter driven by a tetracycline response element (TRE). This transgenic reporter system, named as CKH2B, labeled ~80% of c-Kit$^+$ non-cardiomyocytes,[13] indicating a high efficiency of c-Kit$^+$ cell labeling by the transgene. Gude *et al.* then compared the labeling of c-Kit$^+$ cells between c-*Kit-MerCreMer* (CKmCm) lineage tracing model and the CKH2B transgene model. While there was no significant difference in the expression level of the reporter between these two strategies, the frequency of c-Kit$^+$ cell detected on heart sections and by flow cytometry analysis in CKH2B model was over two folds higher than that in CKmCm model.[13] The substantial increase in labeled cell population among nonmyocytes in the CKH2B model over CKmCm model could be explained by the differences in the reporter strategy (e.g. direct reporter versus lineage tracing) and expression level of c-Kit (and Cre), given that the efficiency of Cre-mediated excision of the LoxP-flanked sequence vary significantly between cell types. In this regard, the haploinsufficiency of c-Kit in the knock-in models may result in incomplete labeling of cells that express c-Kit at a lower level.

To explore whether c-Kit signaling has any biological function in cardiac cells, the authors isolated the presumed CSCs population from the adult heart and examined the dynamics of c-Kit expression under normal and stress conditions. Both c-Kit mRNA and protein were upregulated in isolated cells under stress such as serum starvation.[13] Treatment of SCF on these cells significantly enhanced their proliferation and survival with reduced apoptosis and necrosis,[13] supporting the pro-proliferative and anti-apoptotic activities of c-Kit signaling in CSCs and consistent with results from an independent study showing that c-Kit$^+$ CSCs isolated from *c-Kit-Cre* knock-in mice showed defect in differentiation into cardiomyocytes.[14] Taken together, these studies provided new functional evidence, albeit *in vitro*, that c-Kit may serve not only as a marker but also an important receptor in the biology of cardiac cells.

Interestingly, Gude *et al.* also reported that CKH2B transgene tagged a subpopulation of adult cardiomyocytes,[13] a finding consistent with previous studies.[4, 12] Similar to CSCs, adult cardiomyocytes also express increased c-Kit mRNA and protein after isoproterenol treatment,[13] suggesting a potential role for c-Kit signaling in the cardiomyocyte injury response. The finding of c-Kit expression in adult cardiomyocytes requires a re-interpretation of the previous *c-Kit-Cre* lineage tracing data regardless of whether there was

minimal or significant labeling of cardiomyocytes.[10, 11] This finding suggests that the direct but rare expression of c-Kit in cardiomyocytes might account for the labeled cardiomyocytes observed in the adult heart, adding another layer of complexity to the ongoing discussion regarding the contribution of a stem/progenitor-like cell population in the adult heart. By employing dual recombinases (Cre and Dre) that only labels c-Kit$^+$ non-myocytes, He *et al.* demonstrate the lack of contribution by c-Kit$^+$ non-myocytes to new cardiomyocytes in both adult cardiac homeostasis and after injury.[15] Future studies that compare c-Kit$^+$ from c-Kit$^-$ cardiomyocytes *in vivo* may provide new knowledge regarding whether there are myocyte subpopulations that exhibit greater propensity for cardiac repair and regeneration.

Conclusion

Both transgene and knock-in alleles have inherent strengths and weaknesses in fate mapping of endogenous CSCs. Interpretation of the fate mapping data from different genetic tools needs to consider its caveats and limitations. While knock-in strategy keeps all regulatory elements intact, current c-*Kit-Cre* knock-in alleles all have reduced c-Kit expression. Transgenic approach avoids disruption of the endogenous allele but whether the selected fragment of promoter faithfully recapitulates the *in vivo* c-Kit gene expression can be difficult to ascertain. The creation of a new knock-in mouse line that introduces Cre into the *C-KIT* locus without disrupting c-Kit gene expression (e.g. by P2A self-cleaving peptide after coding element of endogenous c-Kit gene) may help to resolve this issue. Aside from the strength of labeling problem, the expression of c-Kit in both cardiomyocytes and non-myocytes clearly confound any interpretation of fate mapping using c-Kit as a marker. The use of a more precise lineage tracing tool such as the dual recombinases system may alleviate this issue.[19] Questions such as what are the *in vivo* functions of c-Kit gene in CSCs and cardiomyocytes during cardiac homeostasis and regeneration and what is responsible for the down-regulation of the expression of c-Kit in the heart and the decline in cardiomyogenic capacity of c-Kit$^+$ cells with age will require further investigation. With the advent of more powerful tools, we believe the confusion surrounding c-Kit cardiac biology can be clarified in the near future.

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