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c-kit+ Cardiac stem cells: spontaneous creation or a perplexing reality

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Abstract

One of the lingering controversies in the field of cardiac regenerative medicine has been around the origin of c-kit+ cardiac stem cells in the heart and their contribution towards cardiac homeostasis. A recent study reports that a subpopulation of c-kit+ cardiac progenitors emerges from the cardiac neural crest (CNC) with the capacity to give rise to cardiomyocytes. Contribution of CNC derived c-kit+ cells to cardiac myocyte development is regulated by bone morphogenetic protein (BMP). However, ex vivo manipulation of CNC$^{c\text{-}kit}$ cells reveals efficient transformation to early cardiac progenitors.

The idea of the heart as a mitotic organ capable of cardiomyocyte renewal has been the subject of scientific polarization for over a decade. The ensuing years have witnessed a relentless quest to identify the true ‘cardiac stem cell’ capable of regulating cardiac homeostasis thereby providing evidence for any mitotic nature of cardiac tissue. Importantly, studies have effectively shown that similar to other organs, the heart contains a small population of resident stem cells responsible for cellular turnover to physiological and pathological demands[1]. Ex vivo manipulation of this cardiac stem cell population unequivocally has demonstrated the repair potential of these cells following adoptive transfer in pathological heart tissue in a variety of small[2] and large animal models[3]. That being said, the controversy has been the true identity of a putative cardiac stem cell as recent studies have shown that this cell in question does not have significant cardiomyocyte forming potential and although it may have exogenous repair processes, its endogenous repair potential is not robust[4]. The presented notion in 2003 was that the tyrosine kinase protein CD117 or c-kit was a bona fide marker to identify and study resident cardiac stem cells and these cells were an endogenous cardiac precursor cell (CPC) in the adult heart[1]. Adoptive transfer studies have extensively shown that c-kit+ CPCs possess the ability to form all three cardiac lineages (endothelial cells, vascular smooth muscle cells and cardiomyocytes) and all 3 could take part in the beneficial effects seen when injected into injured and failing hearts[1, 2]. Nevertheless, the question remains that if c-kit+ CPCs are so

Disclosures

None

primed to adopt cardiac paternity, is there any role for these cells in normal cardiac development and can they contribute to cellular turnover during physiological and pathological cardiac changes.

The primary goal of the study by Hatzistergos *et al*., was to determine the origin of c-kit+ CPCs in the heart and whether these cells contribute towards cardiomyogenesis[5]. The authors make use of the *ckit*$^{CreERT2/+}$ mouse line crossed to two reporter lines i.e. *CCAGIRG*, a novel dual reporter line expressing ds-Red ubiquitously prior to Cre recombination and enhanced green fluorescent (EGFP) following recombination, along with *R26R-LacZ* and observe appearance of EGFP+ cells indicating c-kit recombination emerge around E9.5 in the heart and contribute towards cardiomyogenesis[5]. These c-kit+ CPCs were mainly observed in embryonic melanoblasts, craniofacial cells, neural tube, dorsal root ganglion, blood, gastrointestinal cells, gonads, pulmonary cells but interestingly, appeared within the outflow tract (OFT), epicardium and myocardium. Since a majority of the EGFP cells, indicative of c-kit recombination, were found in cardiac neural crest (CNC) derived tissue, the authors set out to test whether neural crest lineage marks c-kit CPCs of the heart. Taking advantage of intersectional genetic fate-mapping approach, a novel mouse model carrying dual-recombinase system (Cre-loxP and Flp-FRT) was created to assess c-kit expression in the Wnt1-expressing CNC lineage and its derivatives[5]. The Flp-FRT technology has gained prominence recently and is a site directed recombination strategy analogous to the Cre-lox system but involves recombination of sequences flanked by FRT recognition sites by the recombinase Flp. Subsequent findings with this mouse model established a linear relationship between c-kit and the proto-oncogene protein Wnt1 that can mark a CNC lineage. Additional characterization of the developing cardiac tissue revealed expression of EGFP+ cells within the CNC derivatives including the OFT, tunica medica of the aortic arch, cardiac and aortic valves, atria, inflow tract, satellite glial progenitors and sensory cells[5]. Since ISL1 is considered to mark a number of cardiac progenitor lineages including CNC, whether CNC$^{c\text{-}kit}$ can give rise to myocyte lineage yet have a CNC origin, the *ckit*$^{CreERT2/+}$ line was crossed with mice carrying ISL1 nuclear LacZ allele. A number of EGFP+ cells co-expressing LacZ were observed in CNC derivatives, corresponding to reports showing that a part of ISL1 CPCs arise in the CNC[6]. Contrary to earlier reports on CNC contribution to cardiomyogenesis[7, 8], the authors observed a large number of EGFP+ atrial and ventricular cardiomyocytes along with CNC$^{c\text{-}kit}$ derivatives within the pericardial, endocardial and epicardial cells[5]. Remarkably, CNC$^{c\text{-}kit}$ CPCs co-expressed the endothelial cell marker PECAM1 along with smooth muscle myosin heavy chain in the OFT, yet no EGFP+ cells were found within the coronary vasculature[5]. The overall conclusion from these lineage-tracing experiments provided strong evidence towards an origin of a subpopulation of CPCs expressing c-kit in the CNC. Although CNC$^{c\text{-}kit}$ cells possess cardiomyogenic properties, the extent to which these cells contribute towards the developing cardiac tissue was found to be low.

As the lineage tracing experiments revealed an unusual pattern of CNC$^{c\text{-}kit}$ contribution towards cardiomyogensis, the next set of experiments were aimed at characterizing whether CNC$^{c\text{-}kit}$ cells exhibit similar or better cardiac differentiation potential ex vivo and providing a mechanistic insight for the observed low myogenic ability in vivo[5]. The authors answer

this important question by generating iPSCs from *ckit*$^{CreERT2/+}$ mice and determine their cardiac differentiation potential. Induction of differentiation was based on either ascorbic acid that produces an intermediate stage of cardiac differentiation or BMP antagonism, known to regulate development of both mesodermal and neural crest lineages. Of interest, both treatments led to enrichment of EGFP+ cells in beating embryoid bodies (EBs), however there was significantly more EGFP+ EBs in the dorsomorphin (BMP antagonist) treatment group[5]. Increased cardiac transcription markers such as ISL1/NKX-2.5 were also found to be upregulated in the dorsomorphin treatment group and although c-kit expression increased over time, there was no difference in basal c-kit expression after both ascorbic acid and dorsomorphin treatments[5]. Therefore, these authors conclude that CNC$^{c\text{-}kit}$ cells possess the ability to form cardiomyocytes ex vivo however, this cardiomyogenic potential is retarded in vivo due to a BMP gradient in the cardiac tissue[5].

Overall, this study provides significant new information potentially important for cardiac regeneration and provides new evidence to support the establishment of a relationship between origin of c-kit+ CPCs and CNC. Of particular interest is the finding that the CNC harbors any population of cardiac progenitors contradicting earlier reports associating CNC lineage mainly to outflow tract and its derivatives[8, 9], although a pluripotent nature of the neural crest has been previously reported[8, 10]. Moreover, disruption of the CNC can lead to abnormalities in myocardial contractility, myocyte calcium handling and a thin ventricular myocardium[11–13], raising the possibility for CNC contribution towards development of myocardium.

On the surface, the study described above appears to be in contrast to two recent studies using different strategies to attempt to lineage trace the origin and cardiomyocyte potential of c-kit CPCs[13–14]. The overall conclusions of all three studies are quite clear: that is these cells do not contribute significantly to adult cardiomyocytes and are likely inefficient to mount robust endogenous myocyte replenishment without exogenous administration. First, a study by Van Berlo *et al*. utilized a novel c-kit lineage tracing mouse model to determine the relative contribution of the c-kit+ CPCs towards cardiomyogenesis[14]. In their assessment, the authors concluded that c-kit expressing cells contribute rarely to cardiomyocyte formation during heart development; rather, they primarily differentiate into coronary endothelial cells[13]. Although this study was meticulously conducted, the mouse model as all models could have limitations and other studies are warranted to address the true origin and fate of these cells. Accordingly, a recent study using another mouse model aimed to address the limitations of the Van Berlo *et al*. data and actually provides a more definitive answer to contribution of the endogenous c-kit+ CPCs to cardiomyocyte formation in the heart[15]. This study utilized a number of reporter mice targeting the c-kit locus and found that c-kit expression rarely co-localizes with cardiac markers such as NKX-2.5 or cardiac troponin T (cTnT); rather, c-kit expression was predominantly observed in cardiac endothelial cells[14]. The latter findings are consistent with Van Berlo *et al*. [13] and again support the conclusion that these CPCs have only rare potential for cardiomyogenesis, which is also a conclusion from the study by Hatzistergos *et al*.[5]. However, further studies are needed to determine if altering the BMP gradient may increase the potential for these cells to provide significant cardiomyogenesis as suggested above.

It is critical to point out that all of these studies do not take away from studies explicitly showing that adoptive transfer of exogenously expanded and maintained c-kit+ CPCs can restore cardiac function in a damaged heart as a consequence of forming cardiomyocytes, endothelial and smooth muscle cells along with the ability to release paracrine mediators at the site of injury[4, 16]. The well-documented efficacy of exogenous CPCs and the recent availability of these novel lineage tracing mice models provided the perfect opportunity to develop a system assessing exogenous CPCs ability to form cardiac lineages to endogenous c-kit precursors. Of note, none of the studies to date have isolated and characterized c-kit+ CPCs from the heart of these novel mouse models and conducted a head to head comparison of their cardiac repair ability including cardiomyogenic potential to the endogenous c-kit population labeled after recombination strategies in these mice. The conclusion from c-kit lineage tracing studies was that the resident cardiac c-kit cells have minimal contribution towards cardiomyogenesis so whether isolated c-kit CPCs behave similarly in vitro and after transplantation in the heart remains to be tested. Additionally, the conclusion that c-kit+ CPCs have minimal role in cardiomyogenesis has been prematurely interpreted as c-kit+ CPCs lacking the ability to form cardiomyocytes. The reality may well be that the heart simply doesn't have enough c-kit+ CPCs to mount an efficient regenerative response or the c-kit+ CPCs find cardiac transformation hard due to a different developmental origin as reported in the study by Hatzistergos *et al*.,[5]. On the other hand, c-kit expression seems to be synonymous with the ability of pluripotent cells including embryonic stem cells or iPSCs to transgress towards the cardiomyocyte lineage characterized by an intermediate stage of cardiac progenitors expressing c-kit in corroboration with early cardiac transcription factor such as NKX-2.5, ISL1[17]. Similarly, cardiomyocyte dedifferentiation induced by different treatments leads to the acquisition or reappearance of c-kit[18]. One take home message from the Hatzistergos *et al*. study is certainly the testable hypothesis that blocking BMP gradient may increase cardiac myocyte generation from c-kit CPCs but BMP importance for normal vascular development may potentially be a concern.

In summary, a number of important considerations discussed above suggest a diverse nature of c-kit+ CPCs in the heart and their associated effects towards cardiac regeneration. However, most importantly, since the first identification of this adult c-kit+ CPC population over a decade ago, a plethora of studies have either showed direct cardiac regeneration by CPC transplantation or enhancement of cardiac function due to activation of resident c-kit+ cells via paracrine/autocrine mechanisms[4, 16]. Exciting pre-clinical results formed the basis of a recently conducted phase 1 clinical trial designed to examine the safety and feasibility of c-kit+ CSCs for treatment of patients with heart failure[19], and a new study has been initiated studying a combination of c-kit CPCs and bone marrow-derived stem cells[20]. Thus, regardless of their origin and potential to participate in the turnover of cardiomyocytes during aging, stress or disease, their therapeutic potential either directly or indirectly through secreted entities is what should be the focus going forward. Indeed, although, lineage-tracing stands out as one of the most efficient ways to determine cellular ancestry, the methodology utilized to date, like any other technology, has certain limitations described above that must not obscure the quest to find the true regenerative cardiac cell. The goal of all who study cardiac regeneration should be to improve the outcomes of heart failure for which we are in need of new therapies.

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