SCIENCE CHINA
Life Sciences

• COMMENTARY •

September 2014 Vol.57 No.9: 949–950
doi: 10.1007/s11427-014-4690-6

Has the cardiac stem cell controversy settled down?

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Received April 28, 2014; accepted May 5, 2014; published online June 19, 2014

Citation: Wu MF, Meng FX. Has the cardiac stem cell controversy settled down? Sci China Life Sci, 2014, 57: 949−950, doi: 10.1007/s11427-014-4690-6

Although the human adult heart was considered a terminally differentiated organ and incapable of renewal after injury or with aging for almost a century, recent studies have shown that the heart is capable of new cardiomyocyte formation and possesses varying degrees of regenerative potential throughout mammalian life. However, whether the cardiac stem cells (CSC) or the pre-existing cardiomyocytes contribute to the new cardiomyocyte formation remains controversial.

This controversy started when Orlic and others reported in 2001 that bone marrow derived c-Kit positive (c-Kit) cells could regenerate infarcted myocardium, implying that these c-Kit cells are the CSC, while Murry and others demonstrated that c-Kit cells do not possess the ability to differentiate to cardiomyocytes three years later. This controversy was temporarily settled by the narrower definition of CSC, i.e., only c-Kit cells that reside in the heart, instead of all the circulating c-Kit cells in the heart, are considered CSC. The controversy seems to reappear because Jesty and others observed in 2012 that adult c-Kit CSCs do not contribute to cardiomyocytes and Senyo et al. [1] reported that newly formed cardiomyocytes originate from pre-existing cardiomyocytes, however, Ellison et al. [2] and some other studies demonstrated that these CSCs have the potential to differentiate into a variety of cardiac cell types and are the primary source for myocardium regeneration in both mouse and human hearts. Whether c-Kit CSCs contribute to the new cardiomyocyte formation during regeneration and/or with aging was not determined until a recent paper was published [3] in which genetic lineage tracing was employed to show that c-Kit cells minimally contributed to cardiomyocytes under both injury and normal conditions.

In the study by van Berlo et al. [3], a cDNA encoding recombinase (Cre) fused to an internal ribosome entry sequence (IRES) and enhanced green fluorescent protein (eGFP) (Cre-IRES-eGFP) was knocked-in at the locus of *c-kit* to generate the cKit-Cre-IRES-eGFP mouse line. They found that the expression pattern of GFP and Cre recapitulated the expression pattern of c-Kit protein in this knock-in mouse. When all c-Kit expressing cells were labeled permanently by crossing the cKit-Cre-IRES-GFP mouse to a reporter mouse Rosa26-CAG-loxP-STOP-loxP-eGFP, it is found that the c-Kit labeled cells only contributed to 0.027% or less of total cardiomyocytes during development or in adult heart. If the cardiomyocytes that were fused with the c-Kit cells are excluded, the percentage of c-Kit derived cardiomyocytes is about five-times lower than the initial prediction. The low percentage of c-Kit derived cardiomyocytes is further confirmed by the inducible c-Kit-Cre labeling. These c-Kit cells can differentiate to cardiomyocyte-like cells *in vitro*, as they express cardiac markers GATA4, α-actinin and troponin T, which is consistent with the previous *in vitro* experiment. However, these differentiated cells do not form a sarcomere. The authors concluded that the percentage of cardiomyocytes derived from the c-Kit lineage was astonishingly low and hence highly unlikely to considerably affect cardiac function [3]. The improved heart function mediated by the exogenous c-Kit cells [2] is unlikely attributable to c-Kit derived new cardiomyocyte formation, and the mechanisms underlying the im-

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proved functions need further study [3]. This study echoes Porrello and Olson's opinion that "It is possible that the proliferating c-kit cells, myocyte precursors, and mature cardiomyocytes represent distinct cell populations that do not share a direct lineage relationship and are not hierarchically connected. Genetic lineage-tracing techniques in mice may help resolve these outstanding questions, but current models suggest that stem cells do not contribute to myocyte turnover during normal aging in mice" [4].

The conclusion that c-Kit labeled cells minimally contribute to cardiomyocytes based on the genetic fate mapping [3] is inconsistent with work from Hsieh et al. [5]. Using double-transgenic MerCreMer-ZEG reporter mice by cross-breeding transgenic Myh6-MerCreMer mice with ACTB-ZEG mice, Hsieh et al. found that after injury, about 15% of newly formed cardiomyocytes in areas bordering a myocardial infarction were not derived from the pre-labeled existing cardiomyocytes (about 83% of the cardiomyocytes could be labeled). Their results indicate that CSCs and/or the non-labeled cardiomyocytes contribute to the new cardiomyocyte formation. The discrepancy between the above two studies regarding the contribution of c-Kit cells to cardiomyocyte renewal can be explained by several possibilities. First, the lineage-tracing system may slightly under-represent all the c-Kit cells or cardiomyocytes. The genetic labeling systems in van Berlo et al.'s study represent about 80% of the c-Kit cells in the heart and the systems in Hsieh et al.'s study represent about 80% of all the cardiomyocytes. Second, except for the c-Kit cells and pre-existing cardiomyocytes, there might be other sources of cardiac progenitor cells such as SCA-1 cells, sidepopulation or cardiosphere-derived cells that contribute to cardiomyocyte renewal during aging or regeneration. Studies based on the genetic-lineage-tracing experiments should be able to help clarify.

We apologize that we did not cite all the references, as there is a number limit.

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