Endogenous cardiac regeneration: identification of the true cardiac stem cells.

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Background: Multipotent adult resident cardiac stem cells (CSCs) were first identified by the expression of c-kit, the stem cell factor receptor. However, in the adult myocardium c-kit alone cannot distinguish CSCs from other c-kit-expressing (c-kitpos) cells. The adult heart contains a heterogeneous mixture of c-kitpos cells, mainly composed of mast and endothelial/progenitor cells. This heterogeneity of cardiac c-kitpos cells has been overlooked in recent c-kitpos cell-fate mapping publications which have equated the in vitro and in vivo cardiomyogenic properties of the whole myocardial c-kitpos population, which is minimal, to that of the CSCs, a result at odds with previous publications. To shed light on the causes of this discrepancy, we have assessed the identity, abundancy, self-renewal and myogenic potential of the true-multipotent CSCs within the total c-kitpos cardiac cell cohort.

Methods: c-kitpos cardiac cells were separated through CD45 positive or negative sorting followed by c-kitpos sorting. The blood/endothelial lineage-committed (Lineagepos) CD45posc-kitpos cardiac cells were compared to CD45neg(Lineageneg/Linneg) c-kitpos cardiac cells for stemness and myogenic properties in vitro and in vivo.

Results: The majority (~90%) of the resident c-kitpos cardiac cells are blood/endothelial lineage-committed CD45posc-kitpos cells. In contrast, the Linneg CD45negc-kitpos cardiac cell cohort, which represents ≤10% of the total c-kitpos cells, are enriched for cells that express c-kit at low level and possess all the properties of multipotent stem/progenitor cells for cardiomyocytes, smooth- and endothelial-vascular and connective tissue cells. These characteristics are absent from the c-kitneg and the lineage-committed, CD45posc-kitpos, cardiac cells. Single Linneg(CD45neg)c-kitpos cell-derived clones, which represent only 1-2% of total c-kitpos myocardial cells, when stimulated with TGF-□/Wnt molecules, acquire full transcriptome and protein expression, sarcomere organization, spontaneous contraction and electrophysiological properties of differentiated cardiomyocytes.

Genetically-tagged cloned progeny of one Linneg(CD45neg)-ckitpos cell when injected into the myocardial infarcted myocardium, results in significant regeneration of new myocytes, arterioles and capillaries, derived from the injected cells. The CSC's myogenic regenerative capacity is dependent on commitment to the cardiomyocyte lineage through activation of the SMAD2 pathway. Such regeneration was not apparent when total lineage-committed c-kitpos cardiac cells were injected.

Conclusions: The cardiac c-kitpos cells with properties of true multipotent cardiac tissue-specific stem cells represent a very small fraction (~1-2%) of the total myocardial c-kitpos population. Linneg(CD45neg)-c-kitpos cardiac cells at single cell level have all the characteristics of true CSCs and exhibit robust self-renewing and myogenic properties in vitro and in vivo.