Reassessment of Sca-1+ Progenitor Cells for Cardiomyocyte Contribution in the Adult Heart

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Introduction: The mammalian heart contains a pool of cardiac stem/progenitor cells (CSCs) initially characterized by c-kit expression. However, the lone c-kit expression does not equate to CSC identification. A variety of overlapping markers have been used to isolate CSCs. We already demonstrated that the only population of cells in the heart which possess *in vitro/in vivo* stemness property resides within the $CD45^{negative}/CD31^{negative}/c\text{-}kit^{positive/low}$ cell fraction. Half of this cell fraction express Sca-1. As a cell-fate tracking marker the Sca-1 gene has significant advantages over the complex molecular structure/regulation of the c-kit-locus. The latter has been intensively engineered generating animal models to study the contribution of c-kit+ cells to the heart with poor and misinterpreted results.

Purpose: To analyze the cardiac phenotype of transgenic Sca-1/Cre mice to further assess the evidence that c-kit expression is necessary but not sufficient to identify a population of true adult CSCs. To this aim Sca-1-expressing cells and their cell progeny were followed from embryonic life to adulthood and aging. In particular physiological CM renewal by endogenous Sca-1+ cardiac progenitors from adulthood to aging was assessed.

Material and methods: We employed $Sca\text{-}1^{Cre}$ transgenic mice, expressing a constitutive Cre recombinase driven by the entire regulatory elements of the Sca-1 locus. $Sca\text{-}1^{Cre}$ transgenic mice were crossbred to $R26^{mT/mG}$ Cre-reporter mice to permanently label all the Sca-1-expressing cells, including endogenous CSCs, and map their fate in the heart from embryo to adulthood and after injury.

Results: In double-mutant $Sca\text{-}1^{Cre}$::$R26^{mT/mG}$ mice, Sca-1 expression is activated in late fetal life when the heart is already structurally formed. From neonatal to adult life and until old age, the heart is progressively and robustly replenished of new CMs derived from Sca-1-labeled progenitors. New CM formation after injury is mainly the product of Sca-1-labeled progenitor differentiation *in vivo*. Thus, while the embryonic/fetal heart and its CM population originate from $Sca\text{-}1^{negative}$ progenitors, adult CMs are mainly formed by endogenous $Sca\text{-}1^{positive}$ CSC activation and differentiation.

Conclusions: Our results indicate that the adult heart robustly replenishes CMs lost by wear and tear and after injury. Analyzing the phenotype of c-kit+/Sca-1+ CSCs in the Tg $Sca\text{-}1^{Cre}$ mice we shown that CM formation is mainly dependent on endogenous CSCs activation and on their commitment to the myogenic lineage. Taken together our data reinforce the conclusion that CSCs are necessary and sufficient for robust cardiomyogenesis and to support myocardial regeneration/repair. These new evidences should pave the future for novel approaches to obtain functional myocardial regeneration in the everyday clinical scenario.