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ORIGINAL RESEARCH ARTICLE

Genetic Lineage Tracing of Sca-1$^+$ Cells Reveals Endothelial but Not Myogenic Contribution to the Murine Heart

Editorial, see p 2940

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BACKGROUND: The adult mammalian heart displays a cardiomyocyte turnover rate of ≈1%/y throughout postnatal life and after injuries such as myocardial infarction (MI), but the question of which cell types drive this low level of new cardiomyocyte formation remains contentious. Cardiac-resident stem cells marked by stem cell antigen-1 (Sca-1, gene name *Ly6a*) have been proposed as an important source of cardiomyocyte renewal. However, the in vivo contribution of endogenous Sca-1$^+$ cells to the heart at baseline or after MI has not been investigated.

METHODS: Here we generated *Ly6a* gene–targeted mice containing either a constitutive or an inducible Cre recombinase to perform genetic lineage tracing of Sca-1$^+$ cells in vivo.

RESULTS: We observed that the contribution of endogenous Sca-1$^+$ cells to the cardiomyocyte population in the heart was <0.005% throughout all of cardiac development, with aging, or after MI. In contrast, Sca-1$^+$ cells abundantly contributed to the cardiac vasculature in mice during physiological growth and in the post-MI heart during cardiac remodeling. Specifically, Sca-1 lineage-traced endothelial cells expanded postnatally in the mouse heart after birth and into adulthood. Moreover, pulse labeling of Sca-1$^+$ cells with an inducible *Ly6a*-MerCreMer allele also revealed a preferential expansion of Sca-1 lineage-traced endothelial cells after MI injury in the mouse.

CONCLUSIONS: Cardiac-resident Sca-1$^+$ cells are not significant contributors to cardiomyocyte renewal in vivo. However, cardiac Sca-1$^+$ cells represent a subset of vascular endothelial cells that expand postnatally with enhanced responsiveness to pathological stress in vivo.

Key Words: cell lineage ■ endothelial cells ■ myocardial infarction ■ regeneration ■ stem cells

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Clinical Perspective

What Is New?

- This is the first study to perform genetic lineage tracing of endogenous stem cell antigen-1 (Sca-1) (gene name *Ly6a*)—positive cells throughout postnatal cardiac growth or after cardiac injury in mice.
- Cardiac Sca-1$^+$ cells contribute predominantly to the cardiac vasculature, particularly in the endothelial cell expansion and remodeling that occurs early after birth or after myocardial infarction.
- Cardiac Sca-1$^+$ lineage-traced cells can contribute to the cardiomyocyte pool of the adult mouse heart but at a level that is extremely low and physiologically insignificant (1 in 20 000) and likely the result of fusion of cardiomyocytes with Sca-1 lineage-traced leukocytes.

What Are the Clinical Implications?

- Although there is no known human homolog of Sca-1 (*Ly6a*), clinical application of patient-derived cells for heart regeneration has been proposed partly on the basis of characterization of comparable populations of Sca-1$^+$ cells in mouse models.
- Our data suggest that Sca-1 is not a marker of cells with enhanced stem- or progenitor-like activity in the heart.
- Although not cardiomyogenic, Sca-1 cells in the heart appear to represent a subset of cardiac endothelial cells that are more responsive to injury, which may affect human treatment if comparable cells can be isolated.

Studies of cardiac regeneration over the past 2 decades have offered hope in potentially restoring new cardiomyocytes to the heart after acute myocardial infarction (MI) injury or during progressive heart failure that is characterized by continuous cellular attrition.[1,2] However, the intrinsic regenerative potential of the adult mammalian heart is limited, with multiple genetic, biochemical, and radioisotope decay studies[3–5] arriving at a consensus rate of ≈1% new cardiomyocytes per year in rodents and humans.[6] Given this low rate of inherent cardiomyocyte renewal, it remains unlikely that the adult heart contains a bona fide stem cell destined to generate new cardiomyocytes.[7] Moreover, the most recent studies have suggested that this low rate of endogenous cardiomyocyte renewal is primarily the result of cell cycle activity of existing cardiomyocytes.[6]

Despite this, the concept that endogenous progenitor-like cells exist within the adult heart as a source for new cardiomyocyte formation remains firmly rooted in the literature.[8] Progenitor cells expressing the c-Kit stem cell factor surface receptor or the stem cell antigen-1 (Sca-1) marker define cells with purportedly profound cardiomyogenic potential in culture or when injected into the injured mouse or rat heart.[9–12] However, lineage tracing approaches for the c-Kit marker using *Kit* allele Cre recombinase gene–targeted mice showed that these cells do not contribute significantly to new cardiomyocyte formation during development, with aging, or after acute injury.[13–16]

Sca-1$^+$ cells were originally reported to possess myogenic differentiation capacity on isolation and reinjection into murine blastocysts or ischemic adult mouse hearts.[12,17,18] Subsequent studies have dissected the molecular signature of Sca-1$^+$ cells, which showed more cardiogenic progenitor-like features in vitro.[19] One previous study even suggested that several percent of new cardiomyocytes in the heart after injury are derived from endogenous Sca-1$^+$ cells.[20] Thus, a pressing need remains to better understand the intrinsic biological function of Sca-1$^+$ cells in the heart to address whether these cells truly have myogenic capacity in vivo.

METHODS

An expanded methods section is available in the online-only Data Supplement.

All materials, data sets, and protocols used in this study will be made available to investigators on request. *Ly6a*$^{+/Cre}$ and *Ly6a*$^{+/MerCreMer}$ knock-in mice will be made available under a material transfer agreement between Cincinnati Children's Hospital and the recipient institution. Requests for resources and reagents should be directed to and will be fulfilled by the corresponding author.

Experimental Animals (Mice) and Animal Procedures

All procedures were performed according to institutional guidelines and governmental regulations (Public Health Service Animal Welfare Assurance No. D16-00068 [A3108-01]). All experimental procedures were approved by the Institutional Animal Care and Use Committee at Cincinnati Children's Hospital under protocol IACUC2015-0047.

MI was induced in mice via permanent ligation of the left coronary artery as previously described.[21] Blinding was not performed because only 1 genotype was used in each experiment (either *Ly6a*$^{+/Cre}$×*R26-TdT* or *Ly6a*$^{+/MerCreMer}$×*R26-eGFP*).

Immunohistochemistry and Confocal Microscopy

Tissues from *Ly6a*$^{+/Cre}$×*R26-TdT* or *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice were isolated and processed for immunohistochemistry as described in Methods in the online-only Data Supplement.

Flow Cytometry

For flow cytometry analysis of dissociated cardiac nonmyocytes from *Ly6a*$^{+/+}$×*R26-TdT*, *Ly6a*$^{+/Cre}$×*R26-TdT*, or *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice, single-cell suspensions were prepared via repeated rounds of enzymatic digestion and trituration with collagenase type IV (Worthington No. LS004188) and dispase II (Roche No. 10165859001) according to protocol 2 from Pinto

et al.[22] For flow cytometry analysis of whole bone marrow from *Ly6a*$^{+/+}$×*R26-TdT*, *Ly6a*$^{+/Cre}$×*R26-TdT*, Ly*6a*$^{+/+}$, or *Ly6a*$^{+/MerCreMer}$ mice, cells were isolated by cannulating dissected tibias and femurs with a 25-gauge needle and flushing with 5 to 10 mL flow cytometry staining buffer as previously described.[23]

Quantification and Statistical Analysis

For all experiments, the exact number of mice used in each experiment is reported in the figure legends for each data panel. For experiments involving MI surgery, the number of animals that received surgery was determined from prior experimentation in the laboratory, which demonstrated a perioperative surgical mortality rate of 20%. Only animals that did not survive the surgical procedure were excluded from analysis. Randomization was not necessary because the mice were genetically identical within a given group. All data subjected to statistical analysis were determined to follow a gaussian distribution via the Shapiro-Wilk normality test with α=0.05. Comparisons between exactly 2 groups were made with an unpaired 2-tailed *t* test. Comparisons between >2 groups were made with a 1-way ANOVA with the Tukey multiple-comparisons test. *P* values for each comparison are shown in each respective figure, and $P<0.05$ was considered statistically significant. All statistical tests were performed with GraphPad Prism 7.0d.

RESULTS

Cardiac Sca-1$^+$ Cells Are Predominantly Endothelial Cells in the Postnatal Heart

To assess the role that endogenous Sca-1$^+$ progenitor cells might play in the heart, here we generated gene-targeted mice with Cre recombinase inserted into the *Ly6a* gene locus. These mice were crossed with Cre-responsive *Rosa26-TdTomato* (*R26-TdT*) reporter mice to permanently label Sca-1$^+$ cells and their progeny throughout development and postnatal growth (Figure 1A). No TdTomato$^+$ cells were observed in the hearts or bone marrow of control *R26-TdT* mice lacking *Ly6a*$^{+/Cre}$, indicating the absence of reporter leak (Figure I in the online-only Data Supplement). We first performed histological assessment of the hearts from *Ly6a*$^{+/Cre}$×*R26-TdT* mice over a time course of postnatal growth. TdTomato$^+$ cells were observed as early as postnatal day 1 in the hearts of these mice, located primarily in distinct clusters scattered throughout the heart. By 1.5 months of age, these TdTomato$^+$ cells expanded evenly throughout the heart in *Ly6a*$^{+/Cre}$×*R26-TdT* mice, which was more notable by 3 months of age (Figure 1B). Further analysis of these hearts by immunohistochemistry revealed that the vast majority of TdTomato$^+$ cells were positive for the vascular endothelial cell marker CD31 at both postnatal day 1 (Figure 1C) and 1.5 months (Figure 1D). To more quantitatively assess the identity of Sca-1 lineage-traced cells, we also performed flow cytometry on dissociated heart preparations from *Ly6a*$^{+/Cre}$×*R26-TdT* mice at 3 months of age. We again observed robust labeling of the endothelium; ≈70% of Sca-1 antibody–positive cells that were CD31$^+$ also were TdTomato$^+$. In contrast, significantly fewer Sca-1$^+$ cells that were CD31$^-$ expressed TdTomato (≈8%–9%; Figure 1E and 1F). Additional flow cytometry assessment demonstrated that the majority (>90%) of these Sca-1$^+$CD31$^-$ cells were resident platelet-derived growth factor receptor alpha (PDGFRα^+) cardiac fibroblasts (Figure IIA and IIB in the online-only Data Supplement). Taken together, these data suggest a cumulative contribution of Sca-1$^+$ cells to the cardiac vasculature during physiological growth in the mouse.

Sca-1$^+$ Cells Contribute Few Cardiomyocytes to the Heart Over Postnatal Growth

To assess the cardiomyogenic contribution of Sca-1$^+$ cells in vivo throughout development and postnatal growth, we performed immunohistochemistry studies in *Ly6a*$^{+/Cre}$×*R26-TdT* mice at 3 months of age using antibodies against sarcomeric α-actinin and PCM1 to label the cytoplasm and nuclei[24] of cardiomyocytes, respectively (Figure 2A and 2B). We observed rare single TdTomato$^+$ cardiomyocytes in the hearts of *Ly6a*$^{+/Cre}$×*R26-TdT* mice (Figure 2C), which, when quantified, accounted for ≈0.0035% of cardiomyocytes in these hearts (Figure 2D). Given previous observations from our laboratory and others demonstrating that leukocytes are a known source of false labeling in lineage tracing studies owing to fusion with endogenous cardiomyocytes,[13,25] we also examined the bone marrow of *Ly6a*$^{+/Cre}$×*R26-TdT* mice at 3 months of age by flow cytometry. We observed that ≈30% of total bone marrow cells were TdTomato$^+$ (Figure 2E), and these were mostly mature hematopoietic cells as indicated by surface staining for the pan-leukocyte marker CD45 (Figure 2F). This suggests an even further reduction in the potential for Sca-1$^+$ cells to form de novo cardiomyocytes because some of the TdTomato$^+$ cardiomyocytes we observed may be the result of fusion events from lineage-traced leukocytes. Taken together, our results indicate that the cardiomyocyte contribution from endogenous Sca-1$^+$ cells during development and physiological growth in mice is extremely rare and likely of no physiological significance.

Generation of an Inducible Lineage Tracing Model for Adult Sca-1$^+$ Cells

To specifically examine the contribution of endogenous Sca-1$^+$ cells to the adult heart at baseline or after injury, we generated a second mouse line in which a cDNA encoding the tamoxifen-regulated MerCreMer (modified Cre recombinase) fusion protein was targeted to the endogenous *Ly6a* locus. These mice were crossed with Cre-responsive *Rosa26-enhanced green fluorescent protein*

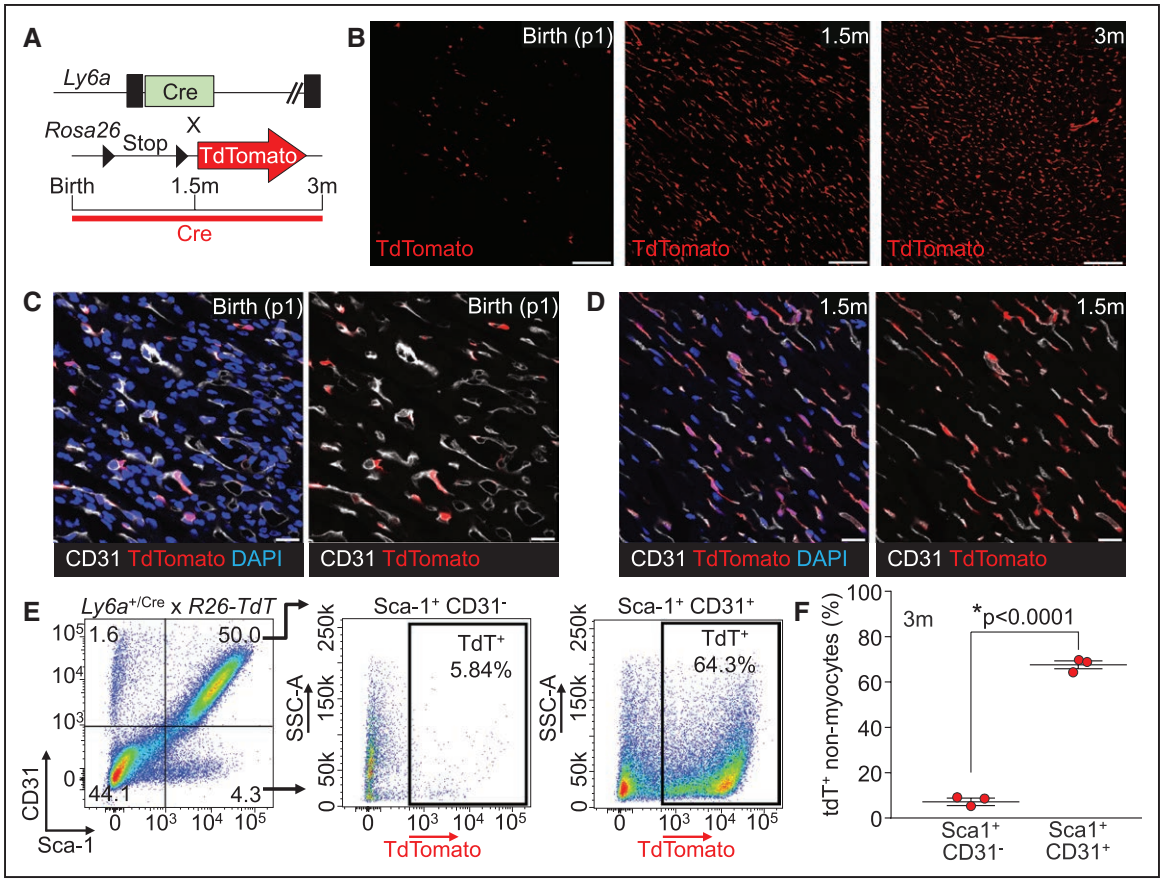

Figure 1. Cardiac stem cell antigen-1 (Sca-1)–positive cells contribute to the vasculature throughout postnatal growth.
A, Experimental scheme and timeline for genetic lineage tracing studies in this figure using constitutive Sca-1 (gene name *Ly6a*) Cre gene–targeted mice. **B**, Representative confocal micrographs of histological sections from hearts of *Ly6a*$^{+/Cre}$×*R26-TdT* mice at postnatal day 1, 1.5 months of age, or 3 months of age showing continual expansion of TdTomato$^+$ cells (red) in the heart. Scale bars=100 μm**. C** and **D**, Immunohistochemistry on cardiac histological sections was performed to detect CD31 (white) along with endogenous TdTomato fluorescence (red). DAPI (blue) was used to visualize nuclei. TdTomato$^+$ endothelial cells were seen in small clusters at postnatal day 1 (**C**) and throughout the heart at 1.5 months of age (**D**). Scale bars=10 μm. **E** and **F**, Representative flow cytometry plots (**E**) and quantification (**F**) from dissociated *Ly6a*$^{+/Cre}$×*R26-TdT* mouse hearts at 3 months of age (n=3) analyzed with antibodies against Sca-1 and CD31. The first plot shows Sca-1 positivity by fluorochrome-conjugated antibody staining (Sca-1) vs CD31 positivity also by antibody (CD31). The second plot shows TdTomato$^+$ cells (endogenous TdTomato fluorescence) vs side scatter within the Sca-1$^+$CD31$^-$ gate (lower right quadrant) shown in the first plot as indicated. The final plot shows TdTomato$^+$ cells (endogenous TdTomato fluorescence) vs side scatter within the Sca-1$^+$CD31$^+$ gate (upper right quadrant) shown in the first plot as indicated. DAPI indicates 4′,6-diamidino-2-phenylindole; SSC-A, side scatter area; and TdT, TdTomato.

(*R26-eGFP*) reporter mice to permanently label Sca-1$^+$ cells and their progeny coincident with tamoxifen administration (Figure 3A). Without tamoxifen, no eGFP–positive cells were observed in these mice, again confirming the absence of reporter leak (Figure 3B). *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice treated with tamoxifen throughout adulthood starting at 3 months of age showed endothelial cell labeling across multiple tissues examined (Figure 3C), although other cell types with known Sca-1 expression were also labeled, including liver hepatic cells,[26] parenchymal cells of the kidney,[27,28] and splenocytes.[27,29,30]

Ly6a Heterozygosity Resulting From Targeted MerCreMer Insertion Does Not Affect Endogenous Sca-1 Function

To determine whether introduction of the MerCreMer cDNA at the endogenous *Ly6a* locus resulted in heterozygosity that could affect normal Sca-1 function, we performed additional analysis of the bone marrow compartment of *Ly6a*$^{+/MerCreMer}$ or *Ly6a*$^{+/+}$ mice at 9 months of age. Although the biological roles of Sca-1 remain poorly defined, the hematopoietic system is the best-characterized tissue in terms of Sca-1 function in mice in that animals lacking all Sca-1 protein show a pronounced reduction in basal production of megakaryocytes in vivo and deficiencies in hematopoietic stem cell and T-cell responsiveness ex vivo.[31,32] However, we observed that bone marrow isolated from *Ly6a*$^{+/MerCreMer}$ mice had comparable amounts of megakaryocytes and monocytes compared with *Ly6a*$^{+/+}$ controls and only a modest decrease in granulocytes (Figure IIIA and IIIB in the online-only Data Supplement). More important, *Ly6a*$^{+/MerCreMer}$ mice showed no differences in total bone marrow Sca-1$^+$ cell content or Sca-1 surface staining compared with *Ly6a*$^{+/+}$ controls. Thus, the genetic systems used to lineage trace Sca-1$^+$ cells by the introduction of Cre recombinase driven from the endogenous

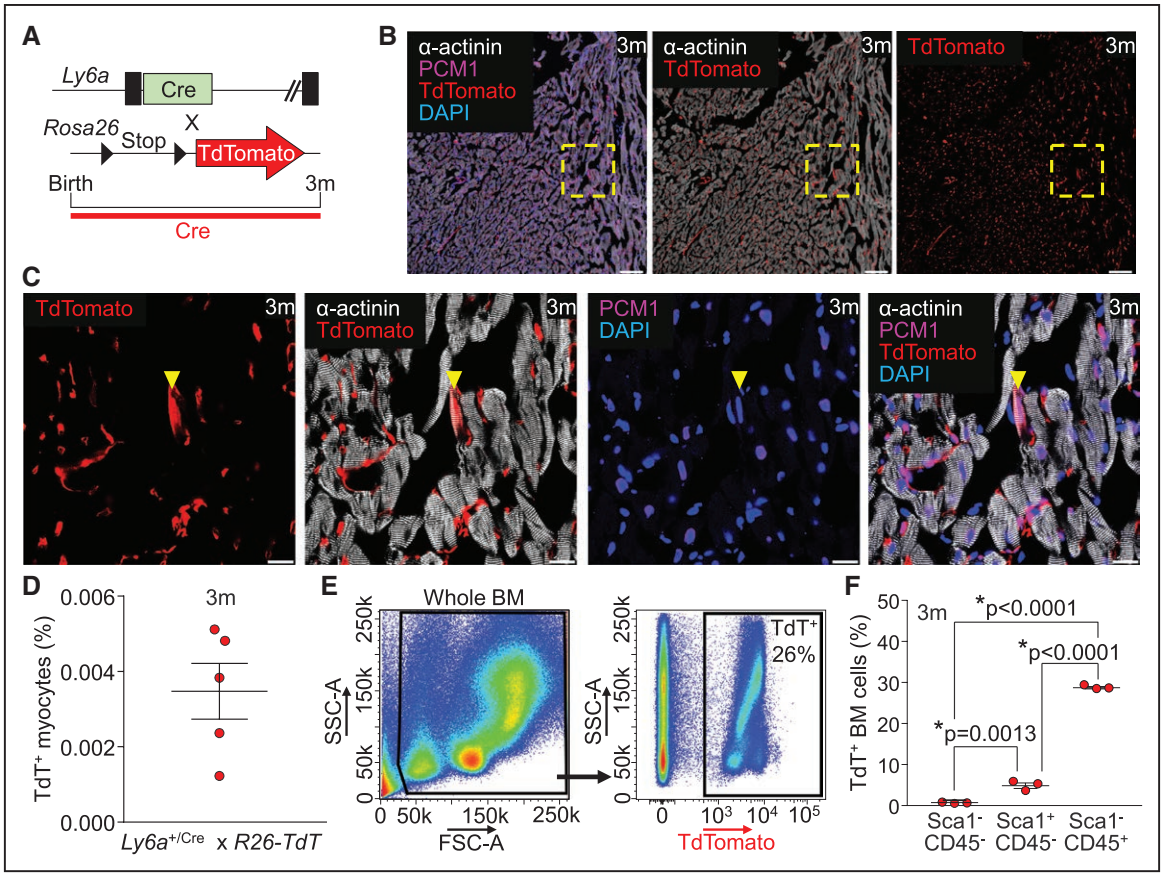

Figure 2. Cardiac stem cell antigen-1 (Sca-1)–positive cells contribute few cardiomyocytes throughout postnatal growth.
A, Experimental scheme and timeline for genetic lineage tracing studies in this figure using *Ly6a*-Cre gene-targeted mice. **B**, Immunohistochemistry was performed on cardiac histological sections from *Ly6a*$^{+/Cre}$×*R26-TdT* mice at 3 months of age with antibodies against sarcomeric α-actinin (white) and PCM1 (purple). DAPI (blue) was used to visualize nuclei. Representative confocal micrograph shows a rare TdTomato$^+$ cardiomyocyte (yellow box). Scale bar=100 μm. **C**, High-magnification confocal micrographs of the yellow boxed area denoted in **B**. Yellow arrowhead indicates a TdTomato$^+$ cardiomyocyte (α-actinin$^+$ PCM1$^+$). Scale bars=10 μm. **D**, Quantification of TdTomato$^+$ cardiomyocytes from histological sections of hearts from *Ly6a*$^{+/Cre}$×*R26-TdT* mice at 3 months of age. Quantification is shown from hearts of n=5 mice over 144 histological sections and 303 260 total cardiomyocytes counted. **E** and **F**, Representative flow cytometry plots (**E**) and quantification (**F**) from the bone marrow (BM) of *Ly6a*$^{+/Cre}$×*R26-TdT* mice at 3 months of age (n=3). The first plot shows forward (FSC-A) vs side (SSC-A) scatter to determine size distribution of the bone marrow. The second plot shows TdTomato$^+$ cells (endogenous TdTomato fluorescence) vs side scatter from the gate shown in the first plot as indicated (**E**). Cells within this second gate were stained with antibodies against Sca-1 and CD45, which showed primarily mature CD45$^+$ leukocytes that were TdTomato$^+$ (**F**). DAPI indicates 4′,6-diamidino-2-phenylindole; and TdT, TdTomato.

Ly6a locus do not alter Sca-1 surface expression or cell composition in the bone marrow, which is the major site of Sca-1 functionality in vivo.

Adult Sca-1$^+$ Endothelial Cells Expand During Aging and Post-MI Remodeling

In the heart, *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice again showed predominantly endothelial cell labeling by immunohistochemistry; thus, we went on to further characterize the endothelial contribution of Sca-1$^+$ cells to the heart specifically after the completion of physiological growth or after MI (Figure 4A). *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice treated with tamoxifen starting at 3 months of age showed predominant labeling of the cardiac endothelium as indicated by CD31 immunohistochemistry (Figure 4B), although examples of larger vessels lacking eGFP positivity were observed (Figure 4C), suggesting that Sca-1$^+$ cells contribute mostly to the microvascular capillary network of the heart. By flow cytometry, >80% of Sca-1 antibody–positive CD31$^+$ cells but significantly fewer (≈10%) Sca-1 antibody–positive CD31$^-$ cells were labeled with eGFP (Figure 4D). We again observed very rare eGFP$^+$ cardiomyocytes in these mice, which by histological quantification accounted for <0.002% of all myocytes analyzed (Figure 4E).

Finally, to examine the role of Sca-1$^+$ endothelial cells in the cardiac injury response, we subjected *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice to permanent occlusion MI injury after a 4-week regimen of tamoxifen to pulse-label a subset of Sca-1$^+$ cells. Before MI, isolated eGFP$^+$CD31$^+$ endothelial cells were observed throughout the heart with tamoxifen administration (Figure 4F). After MI, we observed a gradual increase in the proportion of CD31$^+$ endothelial cells labeled with eGFP (Figure 4G). By 8 weeks after MI, branching eGFP$^+$ vascular clusters also were observed (Figure 4H). Quantification of total endothelial cell con-

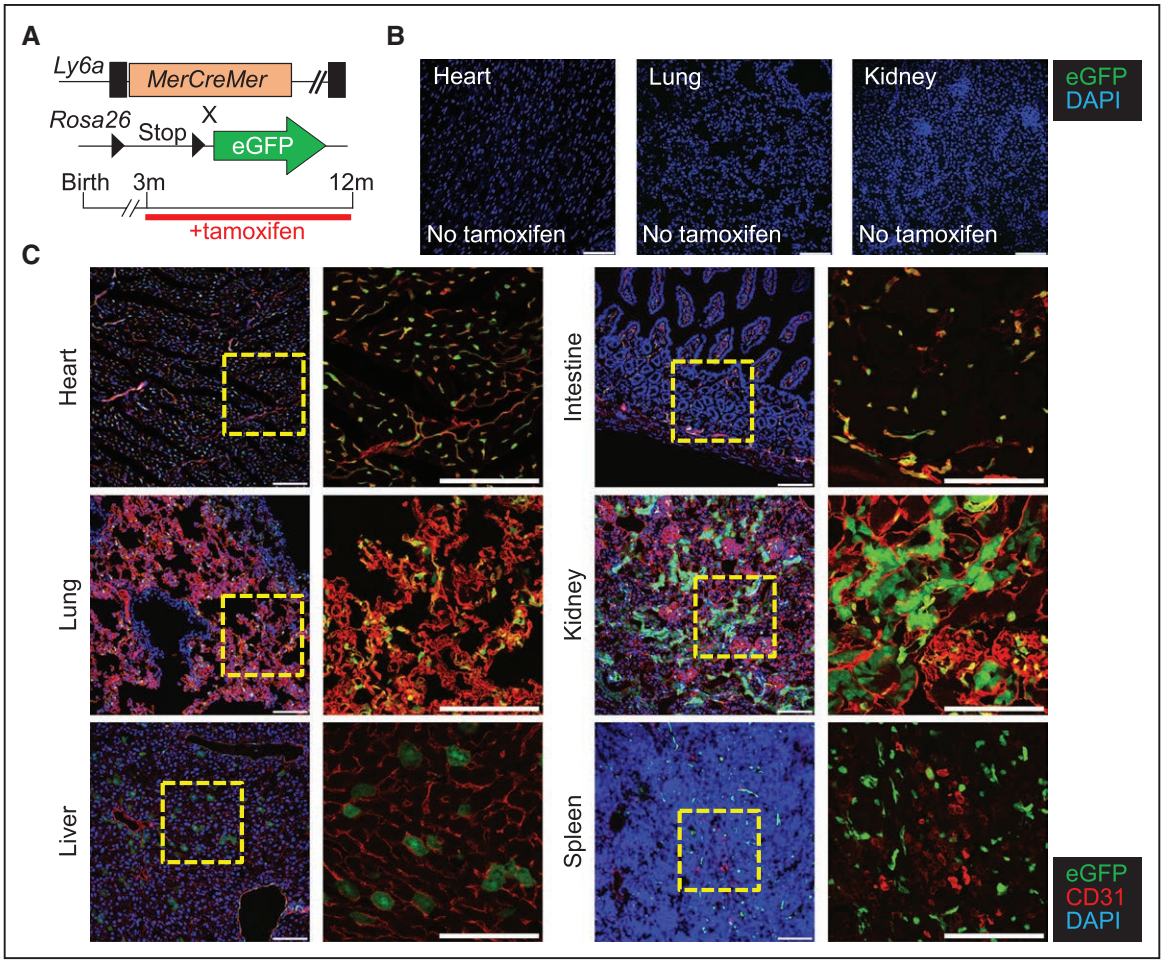

Figure 3. Inducible genetic lineage tracing in the adult mouse tracks stem cell antigen-1 (Sca-1)–positive cells during aging.
A, Experimental scheme for genetic lineage tracing studies in this figure using a tamoxifen-inducible *Ly6a*-MerCreMer gene–targeted mouse line and the R26–enhanced green fluorescent protein (eGFP) reporter line. **B**, eGFP reporter expression is dependent on tamoxifen induction because untreated *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice aged 1 year do not show eGFP^{+} cells across multiple tissues surveyed. **C**, *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice treated with tamoxifen starting at adulthood (3 months) out to 1 year of age showed endothelial cell labeling (CD31, red) of eGFP^{+} cells (green) throughout the heart, lung, liver, intestine, and kidney. Yellow boxes indicate areas shown to the right of each image in an enlarged view. Other cell types previously reported to express Sca-1 are also labeled with eGFP. DAPI (blue) was used to visualize nuclei. Scale bars=100 μm. DAPI indicates 4′,6-diamidino-2-phenylindole.

tent revealed an increase in cardiac capillary density after MI, consistent with compensatory vascular remodeling[33,34] (Figure 4I). Moreover, the percentage of CD31^{+} endothelial cells that were eGFP^{+} significantly increased, roughly doubling during this time despite the prior withdrawal of tamoxifen. These results suggest an angiogenic expansion of previously pulse-labeled Sca-1^{+} vessels during vascular remodeling of the post-MI heart. Of note, we were unable to detect any eGFP^{+} cardiomyocytes using this tamoxifen pulse-labeling protocol at any of the time points examined after MI, indicating that MI did not increase the cardiomyogenic potential of Sca-1^{+} cells in vivo.

DISCUSSION

In this study, we generated 2 genetic lineage tracing mouse models to examine the contribution of endogenous Sca-1^{+} cells to the heart under physiological and pathological conditions. We observed that cardiac Sca-1^{+} cells contribute robustly to the vasculature but to <0.005% of the total cardiomyocyte pool in mice throughout development and aging. This percentage is likely even much lower given 80% fusion rates observed in the heart from labeled leukocytes using a similar lineage tracing approach.[13] After MI injury, this apparent rate of new cardiomyocyte generation from Sca-1^{+} cells did not change and remained exceedingly low; however, expansion and vascular branching of Sca-1^{+} endothelial cells were observed, collectively indicating that the Sca-1^{+} marker reflects endothelial cell identity in the murine heart in vivo and not a subset of endogenous stem cells.

One caveat of our present study is that only 8% to 10% of Sca-1^{+}CD31^{-} cells were recombined with either Cre line. Thus, our observed values of Sca-1^{+} cell–derived myocytes may be underestimated because not all presumed "progenitor-like" Sca-1^{+} cells would be labeled. However, not all nonmyocytes and non-CD31

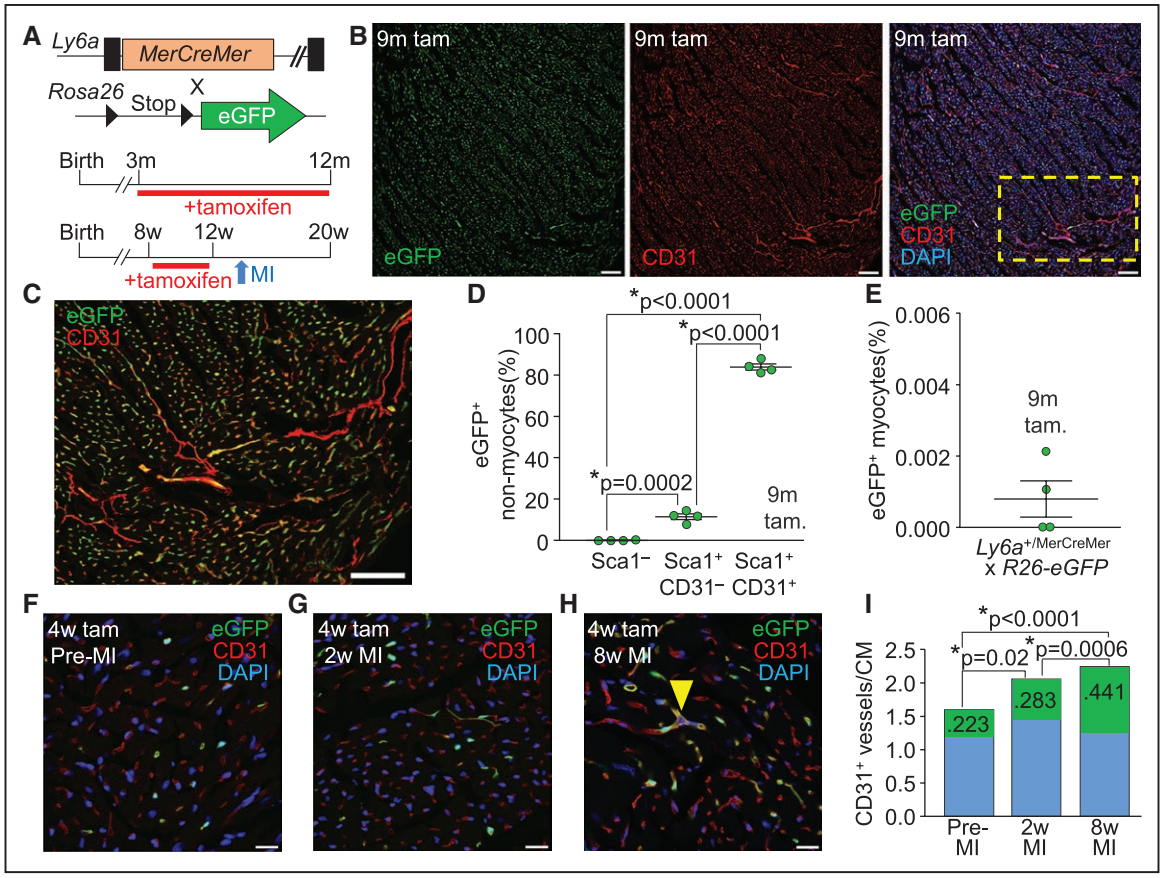

Figure 4. Stem cell antigen-1 (Sca-1)–positive cells contribute to the vasculature in the murine heart during adulthood and after injury. **A**, Experimental scheme for genetic lineage tracing studies in this figure using *Ly6a*-MerCreMer mice along with the R26–enhanced green fluorescent protein (eGFP) reporter line during aging (timeline 1) or after myocardial infarction (MI; timeline 2). **B** and **C**, Representative confocal micrographs (**B**) showing colocalization of CD31 (red) and eGFP (green) in cardiac histological sections in *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice continually treated with tamoxifen from 3 months to 1 year of age (9 months of labeling). Scale bars=100 μm. Yellow box denotes area shown in enlarged view in **C**, demonstrating eGFP^{+} capillaries and small vessels adjacent to eGFP^{-} large vessels. Scale bar=100 μm. **D**, Quantification of eGFP^{+} cells from dissociated *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* hearts (n=4) by flow cytometry with antibodies against Sca-1 and CD31. **E**, Quantification of eGFP^{+} cardiomyocytes (CMs) from hearts of *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice over 9 months of tamoxifen labeling in adulthood. Quantification was from n=4 mice and 374 832 total cardiomyocytes counted from histological sections. **F** through **I**, *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice were given tamoxifen for 4 weeks, followed by MI injury. Representative high-magnification confocal micrographs of heart histological sections labeled with CD31 (red) and DAPI (blue) are shown before MI (**F**) or at 2 weeks after MI (**G**) and at 8 weeks after MI (**H**). Yellow arrowhead in **H** indicates a vascular branching event by previously pulse-labeled eGFP^{+} endothelial cells. Scale bars=10 μm. **I**, Quantification of overall cardiac capillary density and the percentage of CD31^{+} endothelial cells labeled with eGFP in *Ly6a*$^{+/MerCreMer}$×*R26-eGFP* mice after MI. Green bars represent the fraction of CD31^{+} endothelial cells that were eGFP^{+} relative to total endothelial cells (green plus blue bars) (n=4 to 5 mice per time point). DAPI indicates 4′,6-diamidino-2-phenylindole; and tam., tamoxifen.

cells in the heart that express Sca-1 are progenitor in nature, such as the known expression of this marker on a select pool of fibroblasts (Figure II in the online-only Data Supplement and Pinto et al[22]). Moreover, there is a large family of closely related *Ly6* gene members in the mouse[35,36] that might be obscuring the difference between lineage tracing from the *Ly6a* locus and antibody-based detection of cells by flow cytometry if the antibodies used are not exquisitely specific for just Sca-1 (*Ly6a* gene) protein. Another consideration is that the apparent cardiomyogenic frequency of Sca-1^{+} lineage-traced cells was 10 times lower than what we previously observed with c-Kit^{+} cell–dependent lineage tracing.[13] That finding notwithstanding, the rates of Sca-1^{+} or c-Kit^{+} cells contributing to apparent new cardiomyocyte formation remain several orders of magnitude below accepted rates of new cardiomyocyte formation during aging or after injury, rates that are thought to arise primarily through cardiomyocyte cell cycle activity.[6] Finally, a very recent study that used a dual recombination genetic labeling system with Cre and Dre recombinases in the mouse, which labels all nonmyocytes independently of the *Kit* or *Ly6a* allele, showed no contribution of nonmyocytes, progenitor cell or otherwise, in generating myocytes within the adult heart, further proving that the adult heart lacks a myocyte-producing stem cell of any sort.[37] However, we showed that Sca-1 cells in the heart have the capacity to produce additional endothelial cells, suggesting a potential application for therapeutic revascularization strategies.

ARTICLE INFORMATION

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Disclosures

None.

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