Cardiac Side Population Cells
Moving Toward the Center Stage in Cardiac Regeneration

Kazumasa Unno, Mohit Jain, Ronglih Liao

Abstract: Over the past decade, extensive work in animal models and humans has identified the presence of adult cardiac progenitor cells, capable of cardiomyogenic differentiation and likely contributors to cardiomyocyte turnover during normal development and disease. Among cardiac progenitor cells, there is a distinct subpopulation, termed “side population” (SP) progenitor cells, identified by their unique ability to efflux DNA binding dyes through an ATP-binding cassette transporter. This review highlights the literature on the isolation, characterization, and functional relevance of cardiac SP cells. We review the initial discovery of cardiac SP cells in adult myocardium as well as their capacity for functional cardiomyogenic differentiation and role in cardiac regeneration after myocardial injury. Finally, we discuss recent advances in understanding the molecular regulators of cardiac SP cell proliferation and differentiation, as well as likely future areas of investigation required to realize the goal of effective cardiac regeneration. (Circ Res. 2012;110:1355-1363.)

Key Words: adult stem cells ■ cardiac progenitor cells ■ cardiogenesis ■ cardiovascular disease ■ regeneration
Despite advances in medical and surgical therapies, heart disease continues to be the primary cause of death in the industrialized world. Among the sequelae of heart disease, heart failure remains the most grim, portending a prognosis comparable to the most malignant of cancers. As the median age of the population continues to advance, so does the incidence of heart failure. The fundamental premise for the pathogenesis of acquired heart failure is a reduction in the number of viable cardiomyocytes, either in a focal or sporadic distribution through the heart, and an inability of remaining cardiomyocytes to sufficiently compensate for the loss of functional cells. Cardiac and epidemiological outcomes, including the decline in cardiac function, development of heart failure, hospitalization, and even cardiovascular mortality, all strongly correlate with degree of cardiomyocyte loss. While current pharmacological therapies aim to mitigate the symptoms associated with heart failure and slow cardiac deterioration, to date the only definitive therapy for advanced heart failure remains orthotopic heart transplantation. This lifesaving procedure, however, comes with several inevitable complicating factors, including immune rejection, side effects associated with immunosuppression, and perhaps of greatest concern, the insufficient number of suitable donor hearts, limiting the overall effectiveness of this approach.

Given the relationship between loss of viable cardiomyocytes and the development of cardiac dysfunction, approaches to buttress injured myocardium with exogenous muscle cells have long been contemplated. The initial approaches, termed cardiac myoplasty and first detailed almost 30 years ago, attempted to use skeletal muscle, electrically trained for continuous rhythmic contraction, to physically encase the heart. As the regenerative potential of adult skeletal muscle has long been contemplated. The initial approaches, termed cardiac myoplasty and first detailed almost 30 years ago, attempted to use skeletal muscle, electrically trained for continuous rhythmic contraction, to physically encase the heart. As the regenerative potential of adult skeletal muscle has long been contemplated. 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progenitor cell surface markers, including c-kit and Sca1, as well as anchorage-independent growth of cardiospheres, to isolate varying populations of progenitor cells from adult myocardium. Moreover, highly sensitive tools, from immunohistochemistry to genetic fingerprinting and 14C-dating methods, have documented turnover of endogenous cardiomyocytes in vivo, including in humans, and replacement of lost cells with newly formed cardiomyocytes, indistinguishable from their surrounding counterparts. It has been proposed that these various progenitor populations serve to maintain cardiomyocyte turnover during normal development and pathophysiologic states, including after heart injury, and the cardiomyogenic potential of these cardiac progenitor cells in vitro and in vivo has been demonstrated to varying degrees. This review highlights the literature on cardiac SP cells, including their isolation, characterization, and recent study, and as examines SP cells within the context of other adult cardiac progenitor cell populations.

Identification of SP Cells
As mentioned above, the SP phenotype has been used to isolate putative stem cell populations and is based on the unique ability for long-term repopulation. After identification of SP cells in BM, Hoechst efflux has been used to isolate varying populations of progenitor cells from a number of adult mammalian tissues. This method requires staining cells with DNA binding dye, Hoechst 33342, followed by FACS analysis. Stem cells highly express ABC transporters, including ABC, superfamily G, member 2 (Abcg2) (also known as Bcrp1, breast cancer resistance protein 1), or ABC, superfamily B, member 1 (Abcb1) (also known as P-glycoprotein 1, multi-drug resistance protein 1 [mdr1]), and consequently have the capacity to effectively eject toxins and dyes, with Hoechst-lowlow or negative cells typically appearing to the side of Hoechst dye-retaining cells on FACS analysis.

Hoechst dye efflux via the ABC transporters is energy dependent, and depletion of ATP limits dye transport. Moreover, the ABC transporter inhibitor fumitremorgin C or the calcium channel blocker verapamil effectively inhibits ABC transporter activity, and thereby the Hoechst efflux phenomenon (Figure 2A and 2B). One or both of these reagents are often used to set the gating of SP cells during FACS analysis. The exact mechanisms by which verapamil inhibits ABC transporter activity is not completely clear; however, it has been proposed that noncompetitive mechanisms underlie verapamil’s activity. Since verapamil is a potent calcium channel blocker, to determine whether Hoechst efflux ability is a calcium-dependent phenomenon, efflux analysis was conducted in the absence of calcium. As shown in Figure 2C, in the absence of calcium, the dye efflux capacity of the SP phenotype was maintained. Our data support the notion that in cardiac side population (CSP) cells, ABC transporter activity is independent of calcium concentrations.

The initial isolation of SP cells from murine BM (BMPSP) cell suspensions suggested that they are exceedingly rare, accounting for 0.05% to 0.1% of total BM mononuclear cells. Functionally, however, these cells were found to be very important. Competitive repopulation experiments revealed that murine BMSP were highly enriched in hematopoietic stem cells with the ability for long-term repopulation. After identification of SP cells within BM, Hoechst efflux has been used to isolate SP progenitor cells from a number of adult mammalian tissues (Table) as well as to isolate stem cells from various solid tumors. Among cancer SP cells, these cells typically are resistant to chemotherapy and demonstrate robust self-renewal as well as high tumorigenicity. In general, the SP cells found in adult tissues are quite heterogeneous in nature, with fluctuations in quantity during different stages of life.

Phenotypic Characterization of CSP Cells
Within the heart, CSP cells form a distinct cell subpopulation, ranging from 0.03% to 3.5% of total mononuclear cardiac cells, .

Figure 1. Representative FACS plot of CSP cells. Blue plots and green plots indicate CD31-negative and CD31-positive cells, respectively. CSP cells are identified as both Hoechst blue low and Hoechst red low population in the FACS plot (enclosed population).

Figure 2. Dye efflux capacity of CSP cells is calcium independent. A, Representative FACS plot of CSP isolation with regular Hoechst 33342 incubation medium (Ca^2+ concentration of 1.8 mmol/L). B, Verapamil inhibits SP phenotype completely. C, Ca^2+ is chelated from the incubation media with EGTA, CSP population is not affected by Ca^2+ deprivation, suggesting that verapamil inhibits ABC transporters through a Ca^2+-independent manner.
Table. Isolation and Identification of SP Cells in Mammalian Tissues

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<th>Year</th>
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<td>1999</td>
<td>Skeletal muscle</td>
<td>Jackson et al.70 Proc Natl Acad Sci U S A. 1999</td>
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<td>2000</td>
<td>Umbilical cord blood</td>
<td>Storms et al.71 Blood. 2000</td>
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<td>2001</td>
<td>Liver (fetal)</td>
<td>Uchida et al.72 J Clin Invest. 2001</td>
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<td>2002</td>
<td>Mammary gland</td>
<td>Welm et al.75 Dev Biol. 2002</td>
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<td>2002</td>
<td>Pancreatic islets cell</td>
<td>Lechner et al.74 Biochem Biophys Res Commun. 2002</td>
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<td>2002</td>
<td>Brain</td>
<td>Murayama et al.76 J Neurosci Res. 2002</td>
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<td>2002</td>
<td>Peripheral blood</td>
<td>Preffer et al.79 Stem Cells. 2002</td>
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<td>Hierlihy et al.57 FEBS Lett. 2002</td>
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<td>2003</td>
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<td>Watanabe et al.52 FEBS Lett. 2004</td>
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<td>Chen et al.61 Endocrinology. 2005</td>
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<td>Kawanabe et al.57 Biochem Biophys Res Commun. 2006</td>
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<td>2006</td>
<td>Dental pulp</td>
<td>Iohara et al.63 Stem Cells. 2006</td>
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<td>Cregan et al.51 Cell Tissue Res. 2007</td>
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<td>Hattori et al.32 Biochem Biophys Res Commun. 2007</td>
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<td>Hoshi et al.63 Endocrinology. 2007</td>
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<td>Prostate</td>
<td>Brown et al.34 Prostate. 2007</td>
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<td>2008</td>
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<td>Teramura et al.56 BMC Musculoskel Disord. 2008</td>
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<td>2010</td>
<td>Amniotic fluid</td>
<td>Teai et al.58 Taiwan J Obstet Gynecol. 2010</td>
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<td>2010</td>
<td>Placenta</td>
<td>Ramadan et al.59 Genes Cells. 2010</td>
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isolation of other resident stem/progenitor cells from myocardium, CSP isolation is not a trivial procedure. Consequently, differences observed among laboratories in CSP quantity may very well be due to technical issues related to enzymatic digestion of cells from intact tissue, amount of Hoechst used, and FACS analysis, all of which may lead to the disparity in CSP quality and quantity.46

CSP cells generally express Sca1 but not hematopoietic markers, such as CD34 and CD45, advocating a nonhematopoietic phenotype.19,20,45 Additionally, unlike BMSP cells, CSP cells do not form colonies under conditions typical for hematopoietic stem cells.47 This particular phenotype is in contrast to SP cells isolated from other organs including skeletal muscle, lung, and liver.47–49 In addition, CSP cells phenotypically differ from their BM counterparts in the expression of ABC transporters. Although both Abcg2 and Mdr1 are expressed in BMSP cells, it is believed that Abcg2 is the sole transporter accountable for the dye efflux or SP phenotype of SP cells isolated from BM.54 Based on the data obtained in BMSP cells, the perception of Abcg2 as a molecular determinant of SP cells was established. However, such regulation, in fact, is more complicated than initially realized. Similar to BMSP cells, CSP cells also express both Abcg2 and Mdr1 transporters (Figure 3); however, using mice null for Abcg2 or Mdr1a/b, it was demonstrated that dye efflux from CSP cells is regulated by both transporters, in an age-dependent manner.50 Early postnatal (day 3), Abcg2 controls dye efflux, similar to that seen in BMSP cells, whereas Mdr1 is required for the dye efflux phenotype in CSP cells isolated from adult hearts.50 These data contest the previous perception of Abcg2 as the sole ABC transporter for the SP phenotype and suggest that the relative contributions of ABC transporters to Hoechst dye efflux may be age and tissue dependent. Consistent with this view is a recent report demonstrating the contribution of both Abcg2 and Mdr1 to the Hoechst dye efflux property in SP cells isolated from mammary glands.51

Moreover, freshly isolated CSP cells express cardiac-specific transcription factors, such as Nkx2.5 and GATA but not myofilament or structural genes,19 suggesting that CSP cells are indeed distinct from BM and other tissue SP cells and may be maintained in a primitive cardiac progenitor stage before differentiation. Although the developmental origins of
CSP cells and molecular cues that dictate CSP development remain to be determined, work from Tomita et al using mice carrying protein-0-Cre recombinase and CAG-CAT-EGFP double transgenes suggest that certain CSP cells express neutral precursor markers and may derive from neural crest cells within the heart. As mentioned, CSP cells are isolated based on a functional assay rather than specific molecular markers, which preclude tracking of the developmental origins of CSP cells in vivo using traditional techniques and markers. Certainly, future work will be required to definitely define the developmental origins of CSP cells.

The cardiomyogenic potential of CSP cells has largely been documented in vitro. Initial work from Hierlhy et al suggested that CSP cells were capable of expressing cardiac muscle specific proteins when cocultured with neonatal cardiomyocytes. These observations have been extended, and CSP cells have been shown to possess the capacity for functional cardiomyogenic differentiation when cocultured with adult or neonatal cardiomyocytes, with stimulated rhythmic contraction and intracellular calcium transients indistinguishable from bona fide cardiomyocytes. Adult CSP cells when cultured on laminin-coated dishes spontaneously express muscle-specific transcription factors, such as mouse embryonic fibroblast 2 and GATA4, as well as cardiac specific proteins, including α-sarcromeric actinin and troponin I. Despite such robust cardiac specific protein expression, however, without coculture with cardiomyocytes, adult CSP cells do not exhibit proper sarcomeric organization and contractile activity. In contrast, Oyama et al showed that CSP cells isolated from neonatal rats when cultured alone are able to spontaneously differentiate into beating cardiomyocytes when stimulated with oxytoxin or trichostatin A. Furthermore, data from our laboratory has further identified specific subpopulations within CSP cells, including Sca1+/CD31-CSP cells, which were found to be enriched for cardiomyogenic potential. Whereas Sca1+/CD31-CSP cells exhibited greater differentiation into functional cardiomyocytes relative to Sca1+/CD31-CSP cells, both populations were capable of differentiation into endothelial cells when cultured in endothelial cell growth media.

Studies reporting the in vivo differentiation of CSP cells have been relatively less common than those describing their functional capacity in vitro. Oyama et al were the first to demonstrate the functional capacity of CSP cells in vivo. The authors showed that GFP+ neonatal CSP cells were able to properly migrate to areas of myocardial injury in a rat model of cryo-injury when delivered intravenously. Importantly, using immunohistochemical staining, the authors showed that implanted CSP cells were able to differentiate into cardiomyocytes, endothelial cells, smooth muscle cells, and fibroblasts. Our unpublished data support these observations and suggest that labeled adult CSP cells when injected into the remote myocardium are able to migrate into the infarct site (Liao Laboratory, unpublished data). Similar to the findings reported by Oyama et al, Liang et al recently showed that Sca1+/CD31-CSP cells are able to proliferate and differentiate into both cardiomyocytes and endothelial cells in vitro as well as migrate and vascularize in vivo after cardiac injury.

The ability of CSP cells to differentiate into all cardiac cell lineages, including cardiomyocytes, smooth muscle cells, and endothelial cells with proper stimulation, as well as migrate and home to areas of injured myocardium, further establishes CSP cells as a bona fide progenitor cell population. To date, the lack of a specific molecular marker for CSP cells has made tracking the presence and differentiation of endogenous CSP cells in vivo in various states difficult, though future approaches and studies will certainly aim to understand the differentiation process of endogenous CSP cells.

**Functional Significance of CSP Cells**

The presence of endogenous CSP cells with capacity for cardiomyogenic differentiation raises the critical question as to the functional significance of these cells during normal growth and in pathological states after cardiac injury. It is apparent that despite the presence of cardiac progenitor cells, they are inadequate for regenerating a substantial loss of cardiomyocytes, as occurs with a myocardial infarction. Indeed, the function of CSP cells may be largely to maintain the slow turnover of cardiomyocytes during normal growth rather than to compensate for a sudden large loss of cardiomyocytes. That said, after focal cardiac injury in mice, CSP cells are dynamically regulated, with CSP pools decreasing rapidly, as early as 1 day after MI, and returning to basal levels within 7 days. The restoration of endogenous CSP cells is accomplished in part by increased proliferation of endogenous CSP cells, as determined by expression of the proliferation marker ki67, as well as by replenishment by exogenous cells. Reconstitution of animals with GFP-labeled BM has revealed that BMSP cells home to the heart after MI and contribute to the restoration of resident CSP cells in the heart. Similarly, BM-derived stem cells have been shown to contribute to skeletal muscle stem cell populations. Interestingly, BMSP cells only contribute to the maintenance of resident CSP pool after injury and do not contribute to the physiological turnover of CSP cells during normal development. Once BMSP home to the heart after injury, they undergo phenotypic conversion, with loss of the hematopoietic marker, CD45, to attain a CSP phenotype. Approaches that limit the replenishment of CSP cells after MI also result in worsening of cardiac function, suggesting a potential role for CSP cells in post-MI regeneration. Using a similar approach of BM transplantation of CD34+ckit+Sca1+ lineage- SP cells, after granulocyte colony-stimulating factor stimulation, Fujita et al found that BM-derived SP cells home to injured myocardium and contribute to cardiac repair, in part, by increasing BM-derived myofibroblast differentiation in the infarct area. Collectively, these studies highlight the intricate relationship between BM, notably BMSP cells, and myocardium after cardiac injury and the critical role of BM cells in contributing to cardiac repair, whether through...
Maintenance of the CSP cell pool or active differentiation into myofibroblasts or other cell types.

**Molecular Regulation of CSP Cells**

It is clear that CSP cells are dynamically regulated, sensing both local loss of cardiac cells as well as depletion of their own pools, and respond to these varied signals. The molecular cues and mechanisms that dictate CSP differentiation and proliferation, however, are an area of ongoing, active investigation. Gene expression profiling of CSP cells after MI has demonstrated a downregulation of Wnt-related signals in conjunction with increased CSP cell proliferation. Consequently, treatment of isolated CSP cells in vitro with canonical Wnt agonists or recombinant Wnt inhibited the proliferation of CSP cells with partial G1 cell cycle phase arrest. Regulation of CSP proliferation by Wnt signals was further found to be dependent on the IGF-binding protein IGF-binding protein 3. Consistent with these observations, in vivo administration of Wnt ligand or IGF-binding protein 3 to the heart after MI depleted CSP cells, through a decrease in CSP proliferation capacity, and worsened post-MI cardiac remodeling. Consistent with this observation, antagonizing Wnt activity by delivery of sFRP improves post-MI remodeling. It is likely that many signals serve to regulate CSP fate and function, and although Wnt is certainly one such molecular regulator of CSP activity, future work will continue to elucidate the signaling pathways active in CSP cells and their role in dictating CSP function.

In addition to endogenous signaling proteins, CSP cell function is also critically dependent on expression of the ABC transporter that gives SP cells their distinct functional characteristic. In adult CSP cells, although Abcg2 does not contribute to the Hoechst efflux ability, it is important in the maintenance of CSP cells by supporting their proliferation and differentiation capacity as well as survival against oxidative stress. In non-SP cells, including primitive cells, loss of Abcg2 results in an accumulation of porphyrins, heme degradation products and poor survival in low oxygen conditions. Abcg2 is also important in protecting trophoblasts and hematopoietic stem cells against oxidative stress. Consistent with its role during hypoxia, Abcg2 is regulated transcriptionally by hypoxia activated transcriptional factors, including hypoxia-inducible transcription factor. The cytoprotective effects of Abcg2 have also been demonstrated in C2C12 skeletal myoblast and mouse embryonic fibroblasts. Similar to its role in primitive cells, recent data has suggested that the expression of Abcg2 in CSP cells is critical in rendering protection against apoptotic and necrotic cell death under the condition of elevated oxidative stress. In addition to its role in cytoprotection, Abcg2 directly regulates cell cycle progression in CSP cells. Overexpression of Abcg2 augmented CSP proliferation capacity, whereas deficiency of Abcg2 hindered CSP cell proliferation. These results, suggesting a role for Abcg2 in dictating cell function, are consistent with reports that Abcg2 is greatly expressed in several highly proliferative stem/progenitor cells and tumor cell lines. Conversely, forced expression of Abcg2 in CSP cells inhibits differentiation. These data are in line with the data obtained from retinal and hematopoietic stem cells showing that Abcg2 maintains stem cells in a proliferative stage and is downregulated during lineage commitment.

**Comparison of CSP Cells to Other Cardiac Stem/Progenitor Cells**

As mentioned above, the last decade of myocardial biology has led to the identification of a number of cardiac progenitor pools in the adult heart, isolated based on the expression of cell surface markers or functional characteristics. In examining CSP cells within the broader context of other identified cardiac progenitor cells, it is apparent that these progenitor populations are all probably nonhematopoietic, based on the lack of CD45 expression, as well as have the capacity for some degree of self-renewal and differentiation into cardiomyocytes and endothelial and smooth muscle cells. Therefore, it is attractive to speculate that various progenitor cell populations represent a continuum of cells during different stages of cardiomyogenesis rather than developmentally and functionally distinct entities. Important similarities and distinctions among various progenitor cell populations do warrant mentioning, though. The most well-characterized and extensively studied progenitor cell population is c-kit-positive stem cells (CSC), and to date, only these cells have been shown to meet the stringent criteria for true "stem cells." Although CSC do not exhibit the Hoechst dye efflux property, subpopulations of CSC are found to express Mdr-1 on their cell surface. It is important to highlight that although c-kit is present in CSP cells at the gene level, we were not able to detect the c-kit antigen using FACS analysis. The c-kit receptor is known to be rather sensitive to enzymatic cleavage, and the lack of detectable expression of c-kit may be due to enzymatic cleavage during sample preparation. 80% to 90% of adult CSP cells do express Sca1; however, only a very small percentage (<1%) of Sca1+ cardiac cells exhibits the SP phenotype. Similar to c-kit–positive CSC and Sca1-positive cells, CSP cells do not express Islt1 at either the gene or protein levels. Functionally, both CSP and CSC augment their proliferation capacity after injury. Using unbiased global gene expression profiling, Martin et al demonstrated that CSP cells shared some similarity in gene expression patterns, particularly within the Notch and TGFβ signaling networks and cell cycle regulation, with embryonic stem cells. Genes implicated in detoxification and stress pathways, however, were selectively upregulated in CSP as compared with embryonic stem cells and hematopoietic stem cells. These early studies point to commonalities among various progenitor cell populations, particularly in their potential for cardiomyogenic differentiation, and future investigation remains to systematically compare and contrast these progenitor cell populations.

**Future Outlook**

During the past decade, myocardial biology has undergone an intense redefining. The notion of the adult heart as a regenerative organ, complete with endogenous progenitor cells, is now a generally accepted concept. Promoting physiologically relevant cardiac regeneration will require understanding the molecular mechanisms that regulate progenitor cell biology to stimulate endogenous regenerative capacity or directly...
supplement with “reprogrammed” exogenous progenitor cells. Indeed, recent animal data have suggested that cell implantation may synergistically stimulate resident stem cells and thus activate the intrinsic regeneration capacity of the heart.68 To date, human trials of resident cardiac stem/progenitor cell implantation remains early in their design and implementation, though initial results have been promising.69

More and more, the concept of cell therapy for heart disease is moving from fantasy to practice, and with both vigorous basic science studies in animals and cautiously organized human trials, true cardiac regeneration will hopefully be achieved in the not-to-distant-future. For CSP cells, certainly work has pointed to these cells as dynamic members of the cardiac community, responsive to endogenous stimuli. Identification of specific molecular markers for CSP cells is still outstanding and will eventually allow for labeling and tracking the fate of these cells endogenously in vivo during normal development and after cardiac injury. Moreover, such markers will allow for specific genetic ablation or augmentation of CSP pools to more clearly define CSP’s role in cardiac physiology and pathophysiology. Future work will also strive to understand the complex regulation of CSP function, particularly in identifying the molecular cues that dictate the survival, proliferation, and differentiation of CSP, as well as other cardiac progenitor cells, and harnessed these signals to pharmacologically or genetically boost cardiac regeneration.

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Disclosures

None.

References


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