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Cardiac Stem Cell Niches

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Abstract

The critical role that stem cell niches have in cardiac homeostasis and myocardial repair following injury is the focus of this review. Cardiac niches represent specialized microdomains where the quiescent and activated state of resident stem cells is regulated. Alterations in niche function with aging and cardiac diseases result in abnormal sites of cardiomyogenesis and inadequate myocyte formation. The relevance of Notch 1 signaling, gap-junction formation, HIF-1α and metabolic state in the regulation of stem cell growth and differentiation within the cardiac niches are discussed.

Keywords

Stem cell; heart; niche; supporting cells

Introduction

Historically, the foundations of the concept that the heart is a static organ incapable of regeneration were established in the mid-1920s. A significant publication in 1925 claimed that mitotic figures are not detectable in human cardiomyocytes (Karsner et al., 1925), which were considered as cells irreversibly withdrawn from the cell cycle. This work challenged numerous studies published from 1850 to 1911 in which the general belief was that cardiac hypertrophy was the consequence of hyperplasia and hypertrophy of existing cardiomyocytes (for review see Anversa and Kajstura, 1998). The 1925 report introduced the notion that the heart is a terminally differentiated post-mitotic organ.

The conclusion that new cardiomyocytes cannot be formed in the human heart was mostly dictated by difficulties in identifying mitotic nuclei. The conviction that the pool of myocytes present at birth is irreplaceable during the lifespan of the organism gained further support from a series of autoradiographic studies conducted in the late 1960s and early

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1970s (Anversa and Kajstura, 1998; Soonpaa and Field, 1998). The degree of DNA synthesis in myocyte nuclei was negligible, and this observation, together with the inability to distinguish mitotic images in cardiomyocytes, reiterated the theory that the adult heart is composed of a homogenous population of parenchymal cells that are in a permanent state of growth arrest.

However, quantitative measurements of myocyte volume and number, performed in human hearts collected from patients who died as a result of decompensated cardiac hypertrophy and chronic heart failure, began to challenge this concept of myocardial biology. In the late 1940s and early 1950s, Linzbach documented that, in the presence of a heart weight equal to or greater than 500 g, myocyte proliferation represented the predominant mechanism of the increase in cardiac muscle mass (Linzback, 1947Linzback, 1960). These results were confirmed several years later (Adler and Friedburg, 1986; Astorri et al., 1971, 1977). In all cases, hearts weighing 500 g or more were characterized by a striking increase in myocyte number that was more prominent than cellular hypertrophy; this adaptation involved the left and right ventricular myocardium.

An inherent inconsistency became apparent. If we assume that cardiomyocytes lack the ability to reenter the cell cycle and replicate, differences in myocyte size would be expected to reflect comparable differences in the size of the organ. However, changes in heart weight and cardiomyocyte volume rarely coincide challenging the notion that the number of myocytes is an entity that remains constant throughout the organ lifespan. This discrepancy has been reported frequently with postnatal maturation, myocardial aging and cardiac diseases (Anversa and Kajstura, 1998; Anversa et al., 1998). Changes in myocyte number are the consequence of two interrelated mechanisms, myocyte death and myocyte formation. This rather simple biological principle is often ignored; the plasticity of the myocardium cannot be equated to myocyte hypertrophy only.

The critical interaction of cell death and cell renewal is not unique to the heart. Organ mass in prenatal and postnatal life is determined by the balance between cell death and cell division, which regulate the number of parenchymal cells within the tissue (Hipfner and Cohen, 2004). With various diseases, cell loss may be compensated by an increase in size of the surviving cells, although this response may become rapidly maladaptive in view of the difficulty of hypertrophied cells to perform efficiently their specialized function (Gomer, 2001).

Postnatal cardiac development, endurance exercise training, and pregnancy are typical examples of physiological cardiac hypertrophy (Dorn, 2007; Hill and Olson, 2008). The rapid expansion in myocardial mass after birth in mammals involves both an increase in size and number of cardiomyocytes, but the growth of the coronary vasculature markedly exceeds the growth of the myocyte compartment (Anversa and Olivetti, 2002; Rakusan, 1994). It is difficult to compare the dramatic increase in heart weight that occurs postnatally with the relatively modest degree of cardiac hypertrophy promoted by dynamic exercise. Additionally, there is little information concerning the cellular basis of exercise- and pregnancy-induced myocardial hypertrophy. Similarly, the mechanisms implicated in the regression of cardiac hypertrophy with loss of physical conditioning, or following delivery,

have not been determined. Whether new myocytes are formed with endurance exercise and pregnancy and whether myocyte loss, myocyte atrophy, or both, contribute to the restoration of myocardial mass with cessation of exercise and pregnancy are unknown. Thus far, the only conclusion that can be reached is that preservation of myocardial structure characterizes postnatal development, moderate endurance training, pregnancy, and the early phases of increased pressure and volume loading on the adult heart. This balanced "physiological" response, however, is temporary, and aging, strenuous exercise, and sustained workload lead to the structural and functional manifestations of "pathological" hypertrophy, pointing to "time" as the critical determinant of the transition from physiological to pathological cardiac hypertrophy (Dorn, 2007).

An adequate regenerative outcome depends on the presence of exogenous and/or endogenous stem cells, or on the existence of a pool of constantly cycling parenchymal cells. Replicating myocytes are small in size and mononucleated, suggesting that they might derive from: **a)** activation and differentiation of stem/progenitor cells; and/or **b)** proliferation of pre-existing immature myocytes. Currently, there is disagreement in the scientific community regarding the origin and magnitude of cell regeneration occurring in the mammalian heart. Labeling studies with thymidine analogs (Gonzalez et al., 2008; Hosoda et al., 2009; Kajstura et al., 2010a) and mathematical modeling of hierarchically structured cell populations (Kajstura et al., 2010b) have both documented a constant formation of new myocytes. Conversely, a minimal contribution of cardiomyogenesis has been reported during physiological aging in animals and humans (Bergmann et al., 2009; Hseih et al., 2007). The debate continues, and time will tell whether the ability of the heart to renew itself is an important variable or an inconsequential biological process of cardiac homeostasis and repair.

Tissue-specific adult stem cells

Hematopoietic stem cells (HSCs) are the first tissue-specific adult stem cells that have been described (Ema et al., 2014; Scadden, 2014), so that stem cells in other organs are typically studied based on the characteristics of these blood-forming cells. Originally, HSCs were identified as clonogenic cells that develop multilineage hematopoietic colonies in the spleen (Siminovitch et al., 1963). In addition to the fundamental attributes of self-renewal, clonogenicity and multipotentiality, HSCs have been defined as units of a hierarchically structured system, in which stem cells give rise to oligolineage progenitors that lose self-renewal properties and generate a progeny of transient amplifying cells with restricted differentiation potential.

It might be difficult today to imagine that the discovery of HSCs was received with great skepticism, comparable to that surrounding the documentation of cardiac stem cells (CSCs). In the early 1970s, the possibility that HSCs existed and regulated blood cell formation was not accepted. Bone marrow stem cells were defined elusive or enigmatic and the protocols employed for their identification were criticized (Unlisted authors, 1971). *In vivo* bioassays consisted of intravenous injection of unfractionated bone marrow cells followed by the monitoring of colonies being generated in the spleen (Chu-tse and Min-pei, 1984). By transplanting progressively smaller numbers of marrow cells, the number of colonies

detected in the spleen was proportional to the size of the inoculum, while the dimensions of the colonies was proportional to the time post-inoculation (Boggs and Boggs, 1973). These results were consistent with the hypothesis that the spleen colonies were derived from single cells. Moreover, the analysis of the cellular composition of these colonies revealed that all blood cell types were represented, supporting the multipotentiality of the founder cell (Chu-tse and Min-pei, 1984). The spleen colony forming cell, or CFU-S, was considered the definitive HSC.

Accumulating data, however, revealed that CFU-S missed one of the fundamental stem cell properties: CFU-S could not undergo long-term replication and asymmetric cell division (Schofield, 1978; Schofield and Lajtha, 1983). These processes emphasized an apparent inconsistency. On the one hand, the long-term repopulating ability of bone marrow cells did not decline significantly with age and, on the other, the bone marrow-derived spleen colonies showed an “age-structure,” which was evident in the serial transplantation assay and in mice receiving chronic irradiation (Gaidul, 1986; Schofield, 1978). Thus, Schofield concluded that the CFU-S is not the ultimate HSC.

The true HSC was described as a cell that, upon isolation and transplantation, forms spleen colonies, but resides in the bone marrow in association with one or more cells, becoming a “fixed tissue cell” (Schofield, 1978). The cellular environment inhabited by stem cells was defined as “niche” (Schofield, 1978). In the niche, stem cells remain undifferentiated and divide asymmetrically generating a daughter stem cell, which is retained within the niche, and a daughter cell, which corresponds to the first generation of colony-forming cells (CFCs) (Unlisted authors, 1971). The latter retains a certain degree of developmental plasticity and, in case of need, can occupy an empty niche, becoming a fixed stem cell. The free CFC undergoes a finite number of divisions. At each division, the replicative capacity is reduced and progressive maturation is acquired. After a given number of divisions, CFCs become differentiated, representing a cell population that has an “age structure” (Fig. 1A).

When the number of stem cells is reduced as a consequence of damaging stimuli, physiological aging or following serial transplantation in subsequent recipients, an incomplete filling of the empty niches occurs (Albright and Makinodan, 1976; Lajtha and Schofield, 1969). By introducing extra-divisions during the maturation of the CFU-S, a compensatory response for the maintenance of hematopoiesis is activated. Over time, the proportion of CFU-S with long-term repopulating ability declines and a progressively smaller number of niches are occupied. The fourth transfer is incompatible with survival because the cells formed in the last division have a very low repopulating ability (Fig. 1B). These studies opened the field of the biology of hematopoiesis (Morrison and Scadden, 2014; Scadden, 2014; Suda et al., 2011).

The niche: stem cells and supporting cells

Stem cells are stored in niches, which are located deep in the tissue for protection from damaging stimuli (Morrison and Scadden, 2014; Scadden, 2014; Solanas and Benitah, 2013). The niche constitutes a dynamic entity in which the control of stem cell function depends on the complex interaction of intrinsic and extrinsic factors. Stem cells are defined

by their behavior rather than by the specific genes that they express. The set of genes that distinguishes stem cells from the early committed cell progeny has not been determined as yet, and, given the plasticity and heterogeneity of adult stem cells, a “stemness identifier” may not exist (Fuchs and Chen, 2013).

Chemical and physical signals modulate the behavior of stem cells. In addition to cytokines and cell surface adhesion molecules, shear forces, oxygen tension, innervation, and ions are major determinants of stem cell function (Spiegel et al., 2008; Wang, 2011). Cell-to-cell signaling and a variety of effectors mediate the fate of stem cells within the niches, promoting self-renewal or favoring their migration and differentiation. The bidirectional communication between stem cells and supporting cells conditions the characteristics of the niche, reflecting either a quiescent or active state. A few examples of the mechanisms involved in the regulation of stem cell growth are given. Initially, the ability of the niche to preserve stemness was documented in the Drosophila ovariole that contains two germline stem cells (GSCs) surrounded by a group of differentiated somatic cells, expressing Hedgehog and Wingless. The JAK-STAT pathway ensures the renewal of GSCs by acting in concert with the bone morphogenic protein (BMP). The BMP2/4 homolog decapentaplegic constitutes a relevant factor that maintains female GSCs by inducing cell division (Xie and Spradling, 1998). Similarly, Hedgehog controls the Hippo pathway in the ovarian follicle stem cells where it preserves their stemness and proliferation (Huang and Kalderon, 2014).

The niche in the bone marrow integrates multiple participants (Zhang et al., 2003). The endosteal region is important for haematopoiesis while the mature osteolineage cells have an indirect role in modulating HSCs. The bone-forming osteolineage cells results in high local concentrations of Ca^{2+}, and the Ca^{2+}-sensing receptor promotes the engraftment of HSCs (Adams et al., 2006). In the perivascular niche, endothelial and mesenchymal stem cells, together with sympathetic nerve fibers, modulate HSC behavior (Oh and Kwon, 2010). A major regulator of stem cell function is the Notch receptor system, which ensures a proper balance between stem cells and their progeny by preventing or favoring cell commitment. This lineage switch is apparent in the skin where Notch inhibits the formation of epidermal keratinocytes and promotes the generation of hair follicles (Williams et al., 2011). Similarly, the effects of Notch on bone marrow cells vary according to the state of maturation of the stimulated cells. Synthesis of the Notch ligand Jagged1 by osteoblasts increases the number of HSCs in the bone marrow niches (Calvi et al., 2003). In contrast, Notch activation in hematopoietic progenitors induces the formation of immune cells (Radtke et al., 2013). In the brain, Notch signaling expands the pool of immature precursors and enhances glial formation (Androutsellis-Theotokis et al., 2006).

Cardiac stem cell niches: a challenging task

The importance of the niche microenvironment for understanding HSC behavior was acknowledged shortly after the discovery of this adult stem cell class. In the absence of niches, the stemness of the primitive cells cannot be preserved. Outside of their natural milieu, stem cells acquire a high probability to differentiate, a process that may lead to exhaustion of their compartment. This *in vivo* condition is consistent with the high propensity of stem cells to undergo lineage commitment in culture. A well-defined growth

medium supplemented with specific cytokines and the presence of feeder layers are often required for the maintenance of the undifferentiated state of stem cells. More recently, attempts have been made to develop artificial niches able to protect the immature stem cell phenotype (Lee-Thedieck and Spatz, 2012; Tan and Barker, 2013).

Based on the knowledge acquired in the bone marrow, CSCs were expected to reside in myocardial niches. Our current understanding of the biology of CSC niches is limited. Ten years after the discovery of resident CSCs (Beltrami et al., 2003), a PubMed search for “cardiac stem cell niche” yielded only 137 articles. These reports recognize the relevance of stem cell niches, but the large majority failed to address the issues concerning their cellular composition, distribution in the myocardium, changes in function with age and cardiac diseases, and the molecular control of CSC growth and differentiation.

For the systematic identification of stem cell niches, specific criteria have to be fulfilled: stem cells have to be recognized, the anchoring of stem cells to the supporting cells determined, and the existence of an ancestor-progeny relationship established. Because of the knowledge acquired over the years on the properties of HSCs, the approaches employed for the definition and characterization of bone marrow HSC niches (Scadden, 2014) have been considered to represent the ideal strategy for the analysis of the niche microenvironment in the myocardium. However, the variables included in the study of HSCs and their milieu cannot be translated without caveats to solid organs, including the heart and its resident CSCs.

The bone marrow structure has facilitated the identification of HSC niches and their function. The marrow is located within the bone, in which osteoblasts form a well-defined lining to which quiescent HSCs adhere (Nakamura et al., 2010; Sugimura et al., 2012). The association between these two cell types (Fig. 1C) makes the distribution of osteoblasts a valid reference point for the recognition of HSC niches (Arai et al., 2012). The technical limitations involving the assessment of the spatial and temporal relationship of stem cells and their niches in solid organs have been overcome in HSCs by dynamic confocal imaging of the bone marrow underlying the skull (Sipkins et al., 2005). The cortex of the mouse skull is relatively thin and the visualization of HSCs can be achieved with minimal manipulation (Lo Celso et al., 2011). Unique anatomical regions characterized by specialized endothelium have been identified, and the interactions between circulating and resident cells within the bone marrow have been defined (Kunisaki and Frenette, 2014; Sipkins et al., 2005).

The possibility to ablate the niches and create empty structures that can be repopulated has provided the bases for prospective studies of HSCs. The bone marrow produces blood cells that migrate continuously to the peripheral circulation, constituting an unlimited source of differentiated progeny. The availability of large numbers of committed cells and the opportunity to collect serial samples has facilitated the retrospective analysis of the cells of origin within the HSC niches and the documentation of abnormal hematopoiesis when the structure of the niche is altered.

Similarly, the rapid self-renewal kinetics and the simple and repetitive architecture of the intestinal epithelium have favored its study as a primary system for the characterization of

adult stem cells. In each crypt, stem cell differentiation proceeds orderly from the bottom to the luminal surface, suggesting that the primitive cell compartment is located at the base of the crypt. The possibility was advanced that stem cells reside within a ring of 16 cells positioned immediately above the Paneth cells (Potten, 1977; Potten and Loeffler, 1990). More recently, the columnar cells at the base of the crypt have been considered as resident stem cells (Barker, 2014). Two distinct stem cell populations expressing specific surface markers have been characterized, but whether both models are correct, or one reflects more precisely the biology of the intestine remains to be defined. Fate mapping strategies have suggested that stem cell behavior is context dependent and that the two stem cell classes may have separate functions.

The myocardium lacks the basal-apical orientation typical of epithelial organs making it difficult to delineate the precise localization of CSC niches. The epicardial lining has been employed to define anatomically several classes of niches in the adult heart (Castaldo et al., 2008; Di Meglio et al., 2010a, 2010b; Kocabas et al., 2012; Limana et al., 2007, 2010; Smart et al., 2011; Zhou et al., 2008). However, cardiac niches are not limited to the subepicardium and are dispersed throughout the myocardium (Urbanek et al., 2006). CSC niches are more numerous in the atria and apex, which represent protected anatomical areas characterized by low hemodynamic stress (Boni et al., 2008; Goichberg et al., 2011; Gonzalez et al., 2008; Hosoda et al., 2009; Sanada et al., 2014; Urbanek et al., 2006, 2010). The process of myocyte formation lacks directionality; the scattered distribution of the sites of cardiomyogenesis complicates the demonstration of the topography of cardiac niches.

The analysis of cardiac niches becomes problematic if we consider that the strategies used for the depletion of bone marrow niches cannot be implemented in the heart. The protocol of lethal and sub-lethal irradiation of the bone marrow does not result in effective ablation of CSCs in the myocardium, preventing the possibility of repopulating empty niches with fluorescently labeled stem cells. Because of its physical properties, the cardiac muscle is affected only when 30 Gy units of absorbed radiation are utilized. This dose is more than 3-fold higher than that used in the bone marrow and results in profound alterations of the myocardium, diffuse myocyte apoptosis, and high animal mortality (Leri et al., 2007).

c-kit-positive cells and cardiac niches

To establish whether CSC niches are present in the myocardium and whether CSC number is tightly regulated within the niches, the young mouse heart was studied initially by a morphometric approach (Urbanek et al., 2006). The niche was defined as a randomly oriented ellipsoid structure constituted by cellular and extracellular components (Fig. 2A). Within the niches, lineage-negative CSCs are typically clustered together with early committed cells, which continue to express the c-kit receptor but show nuclear localization of the myocyte transcription factor Nkx2.5 and cytoplasmic distribution of the contractile protein α-sarcomeric actin (Fig. 2B). The number of CSCs is higher in the atrial and apical myocardium than in the base-mid-region of the young and old heart (Fig. 2C) (Sanada et al., 2014; Urbanek et al., 2006).

The recognition of the anatomical localization of CSCs and their distribution in the cardiac chambers was complemented with functional studies, which were required for understanding the growth kinetics of CSCs in *vivo*. The transplantation assay, which involves the creation of tissue damage and the injection of exogenous stem cells, is commonly employed. CSCs engraft within the infarcted myocardium, expand their pool and differentiate in cardiomyocytes and coronary vessels (Bearzi et al., 2007; Beltrami et al., 2003; Ellison et al., 2014; Fischer et al., 2009; Konstandin et al., 2013; Mohsin et al., 2012; Smart et al., 2011; Williams et al., 2013). Additionally, a large quantity of newly-formed undifferentiated CSCs can be acquired and delivered to subsequent recipients forming both primitive and specialized muscle cells and vascular structures. This methodology, however, leaves open the question whether the properties displayed by the administered cells are intrinsic to the stem cells or are influenced by the organ injury.

Information concerning CSC function in the intact myocardium involves labeling of resident stem cells with nucleotide analogs or lentiviral fluorescent tags (Gonzalez et al., 2008; Hosoda et al., 2009; Sanada et al., 2014; Urbanek et al., 2006). The slow rate of proliferation of stem cells allows their identification within their natural milieu and the evaluation of their committed progeny (Braun et al., 2003). The administration of repeated doses of nucleotide analogs such as BrdU or 3[H]-thymidine results in labeling of cycling cells (Braun et al., 2003). These molecules are incorporated in the nuclei during S-phase. A prolonged chase period leads to the dilution of the label in cells that are rapidly replicating and its maintenance in cells that are rarely or slowly cycling. Thus, the growth and phenotypic changes occurring in CSCs during their transition from an undifferentiated to a specialized compartment can be characterized.

To identify the label-retaining cells within the myocardium, BrdU was administrated and the intensity of the BrdU signal was examined after a prolonged chasing period (Urbanek et al., 2006). As expected, the number of bright BrdU-CSCs in the atria decreased rapidly after chasing while the number of dim BrdU-CSCs increased over time. The bright BrdU-CSCs detected after a chase period of 2–5 months (Fig. 2C) constitutes the subset of slow-cycling stem cell pool in the atria. Similar changes were seen in the base-mid-region and apex. The aggregate number of CSCs remained constant throughout the period of observation indicating that the growth kinetics of CSCs tends to preserve the pool of primitive cells in the young healthy heart (Urbanek et al., 2006). Thus, cardiac niches harbor a subset of BrdU-retaining cells; these CSCs may correspond to the category of undifferentiated cells that are self-renewing, clonogenic and multipotent *in vitro* (Beltrami et al., 2003; Bearzi et al., 2007, 2009).

The BrdU pulse-chase protocol provides information on the growth kinetics of the CSC population but does not allow the recognition of the mechanism of CSC division at the single cell level. Stem cells can divide symmetrically and asymmetrically. When stem cells engage themselves in asymmetric division, one daughter-stem cell and one daughter-amplifying cell are formed. When stem cells divide symmetrically, two self-renewing daughter cells or two committed amplifying cells are generated. In the young mouse heart, the homeostasis of the cardiac niches is mediated by asymmetric and symmetric division of CSCs (Urbanek et al., 2006). Asymmetric division, however, is the predominant form of

CSC replication; it accounts for ~65% of proliferating CSCs. This mechanism of cell renewal is termed “invariant” and typically occurs in organs in a steady state (Watt and Hogan, 2000).

Recently, a lineage tracing study in the mouse has challenged the implications that c-kit-positive cardiac progenitors have in the modulation of myocyte turnover and regeneration in the mammalian heart (van Berlo et al., 2014), questioning the results obtained by fate mapping in rodents (Ellison et al., 2014). There are several variables that have to be considered in an effort to reconcile these contrasting findings. There is no perfect experimental strategy that can provide an undisputable answer to any scientific question. Genetic manipulations have limitations as any other methodology. The knock-in strategy employed leads inevitably to the loss of one allele of the c-kit gene which may affect significantly the physiological role of the c-kit receptor and the ability of c-kit-positive CSCs to proliferate and form a myocyte progeny (Nadal-Ginard et al., 2014). This defect in c-kit receptor function may be organ specific and the myocardium might have been more severely affected than other organs, i.e., the bone marrow and the lung. A good example consistent with this possibility can be found in the discrepancy between the number of cardiomyocytes derived from c-kit-positive CSCs in this model that is in sharp contrast with the large formation of lung cells (van Berlo et al., 2014), an organ in which c-kit-positive cells have been shown to regulate the growth and differentiation of epithelial cells (Kajstura et al., 2011). Sca1-positive cardiac progenitors are implicated in cardiomyocyte formation in the mouse (Bailey et al., 2011; Uchida et al., 2013), in which an almost 100% co-expression of c-kit and Sca1 in CSCs was detected (Urbanek et al., 2005). In contrast, Sca1-positive progenitors are not present in large mammals and humans. Thus, data in mice cannot be translated to human beings without caveats.

Cardiac niches contain lineage negative cells nested together with cardiac progenitors and precursors. In close proximity to lineage negative cells, myocytes and fibroblasts are commonly visible. Within the niches, stem cells are connected to the supporting cells by gap and adherens junctions composed of connexins and cadherins, respectively. The recognition that junctional complexes are present between CSCs and myocytes or fibroblasts suggests that these cell types act as supporting cells within the cardiac niches. Conversely, endothelial cells and smooth muscle cells do not exert this role. On this basis, the cross-talk between CSCs and cardiomyocytes and fibroblasts has been carefully documented by a series of *in vitro* assays, which have provided strong evidence in favor of the functional coupling of these three cell populations; the transfer of dyes via gap junction channels between CSCs and cardiomyocytes or fibroblasts was shown by multiple protocols (Bearzi et al., 2009; Urbanek et al., 2006). Additionally, the translocation of microRNA-499 from cardiomyocytes to CSCs may occur physiologically resulting in lineage specification of CSCs and myocyte formation (Hosoda et al., 2011).

Cardiac niches and the Notch receptor system

The Notch pathway is an evolutionary conserved intercellular and intracellular mechanism that controls stem cell fate (Bray, 2006). The Notch gene family encodes transmembrane receptors that are activated by ligand binding and proteolytic cleavage, resulting in the

release of the Notch intracytoplasmic domain (NICD). To date, four Notch isoforms have been described (Tanigaki and Honjo, 2007). Upon translocation to the nucleus, NICD develops a complex with the DNA recombinant binding protein RBP-Jk, which then loses its repressor function and initiates transcription (Borggrefe and Oswald, 2009). Direct targets of NICD/RBP-Jk include the Hes and Hey family of proteins that are transcriptional repressors of Notch-dependent genes (Iso et al., 2003).

Notch ligands are membrane-bound proteins and Notch activation links the fate decision of one cell to that of its neighboring cell through lateral inhibition or inductive interaction (Bray, 2006). The effects of Notch on the maintenance of stemness or initiation of differentiation are context-dependent and time-dependent (Androutsellis-Theotokis et al., 2006). The Notch1 receptor and its ligand Jagged1 are critical components of the cardiac niche (Boni et al., 2008; Urbanek et al., 2010). In the neonatal and adult mouse heart, most cardiac niches contain Notch1-positive CSCs, which are nested in the myocardial interstitium (Fig. 3A and 3B). The Notch ligand Jagged1 is expressed in cardiomyocytes and is distributed at the interface between CSCs and myocytes (Fig. 3C and 3D). The extracellular domain of Notch1 is restricted to lineage-negative CSCs, which do not express transcription factors and markers of cell commitment. Importantly, the Notch receptor isoforms Notch2-4 are present only in ~10% of CSCs (Fig. 3E), pointing to Notch1 as the major constituent of the Notch pathway in CSCs within the niches. The expression of the Notch1 receptor on the plasma membrane in the absence of the nuclear translocation of its intracellular domain may be linked to a permissive state in which the multipotentiality of CSCs is preserved. In contrast, activation of the Notch1 receptor in CSCs results in upregulation of Nkx2.5, *in vitro* and *in vivo* (Fig. 4A). Activation of the lineage commitment marker Nkx2.5 failed to inhibit cell proliferation. Nuclear co-localization of Nkx2.5 and Notch1 intra-cellular domain (N1ICD) (Fig. 4B) is coupled with entry in the cell cycle and enhanced myocyte formation (Boni et al., 2008; Urbanek et al., 2010). These observations support the notion that Notch1 regulates the transition of CSCs from the primitive immature phenotype to the compartment of amplifying myocytes. This cell category has the ability to divide and simultaneously differentiate.

In stem cell-regulated organs, the pool size of transient amplifying cells defines the growth reserve of the organ and its ability to regulate tissue homeostasis and promote the structural recover following injury (Doupé and Jones, 2012; Ihrie and Alvarez-Buylla, 2011) Activation of the Notch1 pathway favors the commitment of CSCs to the myocyte lineage and controls the size of the compartment of replicating myocytes *in vitro* and *in vivo*. This behavior may correspond to a model of differentiation delay in which sustained upregulation of Notch1 signaling prolongs the amplifying state of CSC-derived myocytes and prevents terminal differentiation and growth arrest. This function of Notch1 involves the expression of the transcription factor Nkx2.5, which represents a novel target gene of Notch1, and drives the acquisition of the myocyte lineage in resident CSCs.

Notch1 appears to control in part the activation or repression of the complex transcriptional network that modulates myocyte formation in the adult heart (Boni et al., 2008; Gude et al., 2008). Interference with cardiomyogenesis by inhibition of Notch1 has a powerful negative effect on the anatomy and hemodynamics of the growing heart, resulting in a dilated

cardiomyopathy with high mortality (Fig. 5A–5F). The number of Nkx2.5-positive cells is severely affected, resulting in attenuation in the generation of cardiomyocytes and reduction of heart weight. The decrease in left ventricular mass was dictated by a 54% smaller number of cardiomyocytes which were 55% larger. Following Notch1 inhibition, the number of capillary profiles per mm^2 of myocardium did not decrease as a result of myocyte hypertrophy, suggesting that the coronary vasculature grew in proportion to the increase in myocyte size. Although ~10% of endothelial cells in the coronary vasculature express the Notch1 receptor, inhibition of this pathway did not affect their proliferation rate. Importantly, restoration of cardiomyogenesis by reestablishing Notch1 signaling reverses the dilated myopathy and promotes the recovery of the function and structure of the myocardium (Urbanek et al., 2010).

Sustained, constitutive activation of Notch1 signaling or stimulation with the Jagged1 soluble ligand maintains long-term proliferation of cultured cardiomyocytes, delaying their differentiation into mature beating cells (Boni et al., 2008). This phenomenon should not be interpreted as cell dedifferentiation. Activation of the Notch1 receptor does not reprogram newly formed cardiomyocytes into precursor cells, but prolongs the proliferative phase of cardiomyocytes, favors their survival and extends their lifespan. Additionally, N1ICD dictates the acquisition of the cardiomyocyte phenotype in endothelial progenitor cells and this response is enhanced by co-culture with neonatal myocytes secreting Jagged1 (Koyanagi et al., 2007). These findings strengthen the relevance of N1ICD for myocyte renewal postnatally. A similar mechanism is operative in the adult heart after infarction. Activation of the Notch1 receptor is a critical determinant of the transition of adult CSCs to the compartment of amplifying myocytes, and inhibition of this pathway has dramatic negative consequences on the replacement of myocytes and the adaptation of the myocardium to ischemic injury (Boni et al., 2008).

Following myocardial infarction, cardiomyocytes in the border zone are characterized by marked upregulation of activated N1ICD, which co-localizes with the nuclear c-Met receptor (Gude et al., 2008). The expression of the Notch ligand Jagged1 is significantly increased 4 days after infarction. Similarly, Delta4 is enhanced in interstitial areas. Injection of HGF and forced overexpression of activated N1ICD lead to nuclear accumulation of phosphorylated Akt an improved ventricular hemodynamics. The integrated and coordinated activity of the HGF/Notch/c-Met/Akt signaling axis prevents cell death after ischemia, exerting a cardioprotective effect on the myocardium.

Myocyte formation (Boni et al., 2008), together with the increased survival of pre-existing myocytes (Gude et al., 2008), decreases infarct size and improves ventricular performance. Thus, manipulation of Notch activation may have a beneficial therapeutic impact on the failing post-infarcted heart.

Epicardial progenitor cell niches

An alternative source of progenitor cells has been identified in the epicardium, which represents an epithelial sheet on the cardiac surface. The contribution of epicardium-derived cells (EPDCs) to cardiac lineages is a matter of debate. EPDCs express the transcription

factors Wt1, Tbx18, Tcf21, and the retinoic acid (RA) synthesizing enzyme, Raldh2. The contribution of EPDCs to coronary vascular smooth muscle cells and fibroblasts has been carefully documented. In contrast, the role of EPDCs in the formation of the coronary endothelium and cardiomyocytes is unclear. Initially, the controversy surrounding the fate of EPDCs was dictated by divergent findings in chick and mouse studies. More recently, the disagreement has involved the interpretation of lineage tracing in cre-loxP-based reporter mouse lines.

Fate mapping studies using Wt1 and Tbx18 Cre lines have suggested that epicardial progenitors contribute significantly to the developing myocardium in the mouse (Cai et al., 2008; Zhou et al., 2008). However, these findings were challenged based on the observation that Tbx18 is expressed in the inter-ventricular sulcus and left ventricular myocardium, even in epicardium-deficient embryos (Christoffels et al., 2009). Moreover, the leaky and inefficient recombination detected in the Wt1 reporter lines and the endogenous endocardial Wt1 expression has questioned the participation of EPDCs in cardiomyogenesis (Rudat and Kispert, 2012). These data emphasize the need to ensure the specificity of Cre-lox based fate mapping and the rigorous characterization of the native expression pattern of the gene driving the Cre recombinase. Clonal assays should be introduced to validate the findings.

In the adult mouse heart, the embryonic epicardial gene program is downregulated. Following injury, the epicardium acts as a multipotent cell source and releases trophic factors that modulate tissue healing, a response characterized by the reactivation of the embryonic gene program. However, Wt1 positive sub-epicardial cells form primarily fibroblasts and a modest number of myocytes that is insufficient for effective myocardial regeneration (van Wijk et al., 2012).

Recently, a population of epicardially-derived CPCs with mesenchymal-like properties has been characterized in the developing and adult mouse heart (Chong et al., 2011). The documentation of these cells was obtained by introducing the in *vitro* colony-forming unit (CFU) assay, which is commonly used for the analysis of mesenchymal stem cells (MSCs) in the bone marrow. Enzymatically dissociated cardiac mononuclear cells formed colonies in culture composed of fibroid cells named cardiac CFU-fibroblasts (cCFU-Fs). These cells were defined as MSCs because of their capacity for clonogenic propagation, long-term *in vitro* growth, and multilineage differentiation. The cCFU-Fs isolated from embryonic and adult hearts express the MSC markers CD44, CD90, CD29 and CD105 and the embryonic stem cell markers Oct4 and cMyc. Additionally, cCFU-Fs are positive for Sca1 and PDGFRα, but are negative for hematopoietic and endothelial antigens. By employing fate-mapping strategies, the origin of cCFU-Fs was meticulously investigated. Mesp1 (mesodermal) and Wnt1 (neural crest) Cre-recombinase lineage tracing assays revealed that cCFU-Fs originate from the mesoderm but not from the neural crest. Additional lineage tracing studies using Wt1-Cre and Gata5-Cre epicardial lineage tags found that cCFU-Fs arise from the proepicardium and epicardium. Interestingly, the ability of this population to produce cCFU-Fs appeared to decline in the adult heart. Fate mapping with Myl2-Cre and Nkx2.5-Cre did not label substantial numbers of cCFU-Fs, which suggests that they do not arise from Nkx2.5 cardiac progenitors. This putative stem cell population occupies a

perivascular, adventitial niche adjacent to coronary arterioles. The relevance of these cells for tissue homeostasis and repair in vivo remains to be documented.

Mesenchymal stem cells

The concept of cooperation between stem cells and cardiomyocytes, functioning as supporting cells within the myocardial niches, has been documented carefully. The interaction between exogenously delivered bone marrow MSCs and endogenous c-kit-positive CSCs following myocardial infarction has been reported recently (Hatzistergos et al., 2010). This process combines the regenerative potential of CSCs with the powerful secretory phenotype of MSCs. The relatively low efficiency of transdifferentiation of MSCs has been integrated with their ability to recruit and expand the local CSC pool and create a large myocyte progeny and abundant coronary microcirculation. After MSC injection, chimeric clusters containing MSCs of exogenous origin and endogenous CSCs develop in the infarcted myocardium and in the border zone (Fig. 6). CSCs form connexin-43 mediated gap junctions and N-cadherin mechanical connections with cardiomyocytes and MSCs (Hatzistergos et al., 2010).

Additionally, the remarkable recovery in the anatomical and functional integrity of the infarcted myocardium following combined administration of human MSCs and human c-kit-positive CSCs (Williams et al., 2013) raises important clinical questions. Whether the administration of MSCs and CSCs leads to the formation of temporary and/or permanent niches within the host myocardium has not been resolved and constitutes a major challenge for future research (Leri and Anversa, 2013). The actual transfer of molecules between MSCs and CSCs remains to be shown to uncover the fundamental cellular processes involved in the extensive repair of the damaged heart. However, the “cooperative” interaction regulating stem cell function may be one of the most logical outcomes of this complex therapeutic strategy.

The survival of exogenously administered stem cells requires their integration with the recipient myocardium. The region bordering the infarct is exposed to increased wall stress and functional demand, which promotes myocyte hypertrophy, activation of interstitial fibroblasts and collagen accumulation. The secretome of the delivered MSCs may provide an environment favoring the engraftment of CSCs and the acquisition of the cardiogenic fate.

Hypoxic niches

Stem cell niches in the bone marrow are characterized by low oxygen tension, a condition that offers a selective advantage to stem cells favoring their quiescent primitive state (Simsek et al., 2010; Suda et al., 2011). A similar mechanism may be operative in the myocardium, and CSC behavior may be regulated by the O_2 gradient within the tissue. The long-term preservation of the CSC compartment may require a hypoxic milieu, although physiological normoxia may be necessary for active cardiomyogenesis. In fact, niches composed of hypoxic c-kit-positive CSCs have been found throughout the myocardium, although they accumulate preferentially in the atria and apex of the left ventricle (Sanada et al., 2014). At all ages, clusters of CSCs with low content of O_2 coexist with niches

comprising normoxic CSCs. Hypoxic CSCs are unable to reenter the cell cycle and divide. By necessity, normoxic CSCs are forced to undergo intense proliferation and differentiation with progressive telomere erosion, and formation of senescent dysfunctional cardiomyocytes (Rota et al., 2007). Hypoxic CSCs, particularly in the atria, correspond to quiescent long-term label retaining cells that rarely divide, while normoxic CSCs reflect cells that proliferate frequently and generate a specialized progeny. Additionally, the expression of the myocyte markers Nkx2.5 and GATA4 is restricted to the normoxic CSC compartment (Fig. 7A–7D). The balance between these two stem cell classes is critical for the regulation of myocardial homeostasis and the preservation of the CSC compartment during the organ lifespan. Aging, however, expands dramatically the pool of hypoxic quiescent CSCs so that the smaller pool of cycling CSCs sustains cell turnover.

Hypoxic cardiac niches are numerous in the epicardium and subepicardium of the adult mouse heart (Kimura and Sadek, 2012; Kobacas et al., 2012), which houses a metabolically distinct population of glycolytic progenitor cells (GPCs). In vivo perfusion quantification with Hoechst dye has been combined with metabolic profiling to recognize poorly oxygenated myocardial areas and cells with low mitochondrial content. GPCs possess some of the properties of progenitor cells; they are self-renewing, clonogenic and multipotent. GPCs do not express c-kit, Sca-1, endothelial and hematopoietic markers but are positive for the transcription factors Nkx2.5, GATA4, Wt1, and Tbx18. The metabolic phenotype of GPCs is regulated by HIF-1α. Downregulation of HIF-1α promotes in GPCs a metabolic shift from glycolysis to mitochondrial oxidative phosphorylation resulting in a decrease in proliferation with loss of the primitive state (Kobacas et al., 2012). Similarly, hypoxic c-kit-positive CSCs showed a lower mitochondrial activity with respect to normoxic CSCs (Sanada et al., 2014). Thus, intact functional niches ensure the adequate response of stem cells to the needs of the organ. Disruption of regenerating niches may be a critical component of tissue injury, and the reconstitution of the microenvironment in which stem cells operate may represent an essential target for the treatment of myocardial aging and cardiac diseases.

Conclusive remarks

Although the utilization of a single marker for the identification of stem cells may be risky, the stem cell antigen c-kit recognizes a population of CSCs that are self-renewing, clonogenic and multipotent *in vitro* and replace necrotic myocardium with functioning tissue *in vivo*. It is becoming apparent that the pool of CSCs is heterogeneous, including quiescent and actively proliferating cells, migratory and adherent cells, young and senescent cells, and uncommitted and early committed cells. A major effort is being made currently to search for additional surface epitopes able to identify pools of CSCs with specific properties. The surface expression of Notch1 recognizes multipotent CSCs, which are poised for lineage commitment, while the presence of c-Met and the EphA2 receptor reflect cells with a particular propensity to migrate out of the niche area. The IGF-1 receptor is expressed in a compartment of CSCs that can be coaxed to regenerate large portions of damaged myocardium, while the IGF-2 receptor is present in CSCs with high susceptibility to senesce and undergo apoptosis. With the exception of c-kit-positive CSCs, GPCs and epicardially-derived MSCs, niches hosting other classes of myocardial progenitors have not been shown.

At present, bioartificial niches are being developed by introducing nanomaterial, scaffolds and cardiac patches in an attempt to improve the myocardial recovery after infarction.

The limitations in the healing response of the adult heart, however, should not be inappropriately interpreted as evidence against CSCs and their role in tissue homeostasis and regeneration following injury. The recurrent statement made against the presence of resident CSCs and the significant growth reserve of the human heart is that spontaneous cardiac repair does not occur after infarction, and the necrotic tissue is not restored by intact myocardium (Steinhauser and Lee, 2011); the healing process evolves and a thick scar is formed. It might come as a surprise, but the lack of endogenous regeneration after infarction is present in solid and non-solid organs including the skin, liver, intestine, kidney, and bone marrow. In all cases, occlusion of a supplying artery leads to scar formation mimicking cardiac pathology (Anversa et al., 2013). With polyarteritis nodosa and vasculitis, microinfarcts develop in the intestine and skin, and resident stem cells do not restore the damaged tissue. Infarcts of the bone and bone marrow occur with sickle cell anemia, and HSCs and MSCs do not reconstitute the structural integrity of the organ. The simplest example is provided by the occlusion of a branch of a mesenteric artery; a segmental infarct of the intestine develops rapidly, and the viable stem cells fail to regenerate the necrotic tissue, although the stem cells of the crypts renew the mucosa every ~4 days (Anversa et al., 2013). The stem cell compartment appears to be properly equipped to regulate tissue homeostasis but does not respond effectively to ischemic injury, or late in life to aging and senescence of the organ and organism.

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Abbreviations

BMP	bone morphogenic protein
CFC	colony-forming cells
CFU-S	spleen colony forming unit-spleen
cCFU-Fs	CFU-fibroblasts
CSCs	cardiac stem cells
EPDCs	epicardium-derived cells
GPC	glycolytic progenitor cells
GSCs	germline stem cells
HSCs	hematopoietic stem cells
MSCs	mesenchymal stem cells
NICD	Notch intracytoplasmic domain
RA	retinoic acid

References

Adler CP, Friedburg H. Myocardial DNA content, ploidy level and cell number in geriatric hearts: post-mortem examinations of human myocardium in old age. J Mol Cell Cardiol. 1986; 18:39–53. [PubMed: 3950970]

Albright JW, Makinodan T. Decline in the growth potential of spleen-colonizing bone marrow stem cells of long-lived aging mice. J Exp Med. 1976; 144:1204–1213. [PubMed: 993725]

Adams GB, Chabner KT, Alley IR, et al. Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. Nature. 2006; 439:599–603. [PubMed: 16382241]

Androutsellis-Theotokis A, Leker RR, et al. Notch signalling regulates stem cell numbers in vitro and in vivo. Nature. 2006; 442:823–826. [PubMed: 16799564]

Anversa P, Kajstura J. Ventricular myocytes are not terminally differentiated in the adult mammalian heart. Circ Res. 1998; 83:1–14. [PubMed: 9670913]

Anversa P, Kajstura J, Rota M, et al. Regenerating new heart with stem cells. J Clin Invest. 2013; 123:62–70. [PubMed: 23281411]

Anversa P, Leri A, Beltrami CA, et al. Myocyte death and growth in the failing heart. Lab Invest. 1998; 78:767–786. [PubMed: 9690556]

Anversa, P.; Olivetti, G. Cellular basis of physiological and pathological myocardial growth. In: Page, E.; Fozzard, HA.; Solaro, RJ., editors. Handbook of Physiology. New York, NY: Oxford University Press; 2002. p. 75-144.Section 2, The cardiovascular System, vol. I, The Heart

Arai F, Hosokawa K, Toyama H, et al. Role of N-cadherin in the regulation of hematopoietic stem cells in the bone marrow niche. Ann NY Acad Sci. 2012; 1266:72–77. [PubMed: 22901259]

Astorri E, Chizzola A, Visioli O, et al. Right ventricular hypertrophy--a cytometric study on 55 human hearts. J Mol Cell Cardiol. 1971; 2:99–110. [PubMed: 4255912]

Astorri E, Bolognesi R, Colla B, et al. Left ventricular hypertrophy: a cytometric study on 42 human hearts. J Mol Cell Cardiol. 1977; 9:763–775. [PubMed: 143539]

Bailey B, Fransioli J, Gude NA, et al. Sca-1 knockout impairs myocardial and cardiac progenitor cell function. Circ Res. 2012; 111:750–760. [PubMed: 22800687]

Barker N. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat Rev Mol Cell Biol. 2014; 15:19–33. [PubMed: 24326621]

Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. Proc Natl Acad Sci USA. 2007; 104:14068–14073. [PubMed: 17709737]

Bearzi C, Leri A, Lo Monaco F, et al. Identification of a coronary vascular progenitor cell in the human heart. Proc Natl Acad Sci USA. 2009; 106:15885–15890. [PubMed: 19717420]

Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003; 114:763–776. [PubMed: 14505575]

Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. Science. 2009; 324:98–102. [PubMed: 19342590]

Boni A, Urbanek K, Nascimbene A, et al. Notch1 regulates the fate of cardiac progenitor cells. Proc Natl Acad Sci USA. 2008; 105:15529–15534. [PubMed: 18832173]

Boggs SS, Boggs DR. Cell-cycling characteristics of exogenous spleen colony-forming units. J Lab Clin Med. 1973; 82:740–753. [PubMed: 4583582]

Borggrefe T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. Cell Mol Life Sci. 2009; 66:1631–1646. [PubMed: 19165418]

Braun KM, Niemann C, Jensen UB, et al. Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis. Development. 2003; 130:5241–5255. [PubMed: 12954714]

Bray SJ. Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol. 2006; 7:678–689. [PubMed: 16921404]

Cai CL, Martin JC, Sun Y, et al. A myocardial lineage derives from Tbx18 epicardial cells. Nature. 2008; 454:104–108. [PubMed: 18480752]

Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003; 425:841–846. [PubMed: 14574413]

Castaldo C, Di Meglio F, Nurzynska D, et al. CD117-positive cells in adult human heart are localized in the subepicardium, and their activation is associated with laminin-1 and alpha6 integrin expression. Stem Cells. 2008; 26:1723–1731. [PubMed: 18436868]

Chu-tse W, Min-pei L. Characteristics of proliferation and differentiation of spleen colony-forming cells from bone marrow. Int J Cell Cloning. 1984; 2:69–80. [PubMed: 6707493]

Chong JJ, Chandrakanthan V, Xaymardan M, et al. Adult cardiac-resident MSC-like stem cells with a proepicardial origin. Cell Stem Cell. 2011; 9:527–540. [PubMed: 22136928]

Christoffels VM, Grieskamp T, Norden J, et al. Tbx18 and the fate of epicardial progenitors. Nature. 2009; 458:E8–9. [PubMed: 19369973]

Di Meglio F, Castaldo C, Nurzynska D, et al. Localization and origin of cardiac CD117-positive cells: identification of a population of epicardially-derived cells in adult human heart. Ital J Anat Embryol. 2010a; 115:71–78. [PubMed: 21072993]

Di Meglio F, Castaldo C, Nurzynska D, et al. Epithelial-mesenchymal transition of epicardial mesothelium is a source of cardiac CD117-positive stem cells in adult human heart. J Mol Cell Cardiol. 2010b; 49:719–727. [PubMed: 20566360]

Dorn GW. The fuzzy logic of physiological cardiac hypertrophy. Hypertension. 2007; 4:962–970. [PubMed: 17389260]

Doupé DP, Jones PH. Interfollicular epidermal homeostasis: dicing with differentiation. Exp Dermatol. 2012; 21:249–253. [PubMed: 22417300]

Ellison GM, Vicinanza C, Smith AJ, et al. Adult c-kit(pos) cardiac stem cells are necessary and sufficient for functional cardiac regeneration and repair. Cell. 2014; 154:827–842. [PubMed: 23953114]

Ema H, Morita Y, Suda T. Heterogeneity and hierarchy of hematopoietic stem cells. Exp Hematol. 2014; 42:74–82. [PubMed: 24269919]

Fischer KM, Cottage CT, Wu W, et al. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. Circulation. 2009; 120:2077–2087. [PubMed: 19901187]

Fuchs E, Chen T. A matter of life and death: self-renewal in stem cells. EMBO Rep. 2013; 14:39–48. [PubMed: 23229591]

Gaidul KV, Tsyrlova IG, Kozlov VA. Hemopoietic stem cells “age” structure revealed by the exogenous colony forming method. Biomed Pharmacother. 1986; 40:114–115. [PubMed: 3527289]

Goichberg P, Bai Y, D’Amario D, et al. The ephrin A1-EphA2 system promotes cardiac stem cell migration after infarction. Circ Res. 2011; 108:1071–1083. [PubMed: 21415392]

Gomer RH. Not being the wrong size. Nat Rev Mol Cell Biol. 2001; 2:48–54. [PubMed: 11413465]

Gonzalez A, Rota M, Nurzynska D, et al. Activation of cardiac progenitor cells reverses the failing heart senescent phenotype and prolongs lifespan. Circ Res. 2008; 102:597–606. [PubMed: 18202313]

Gude NA, Emmanuel G, Wu W, et al. Activation of Notch-mediated protective signaling in the myocardium. Circ Res. 2008; 102:1025–1035. [PubMed: 18369158]

Hatzistergos KE, Quevedo H, Oskouei BN, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circ Res. 2010; 107:913–922. [PubMed: 20671238]

Hill JA, Olson EN. Cardiac plasticity. N Engl J Med. 2008; 358:1370–1380. [PubMed: 18367740]

Hipfner DR, Cohen SM. Connecting proliferation and apoptosis in development and disease. Nat Rev Mol Cell Biol. 2004; 5:805–815. [PubMed: 15459661]

Hosoda T, D’Amario D, Cabral-Da-Silva MC, et al. Clonality of mouse and human cardiomyogenesis in vivo. Proc Natl Acad Sci USA. 2009; 106:17169–17174. [PubMed: 19805158]

Hosoda T, Zheng H, Cabral-da-Silva M, et al. Human cardiac stem cell differentiation is regulated by a mircrine mechanism. Circulation. 2011; 123:1287–1296. [PubMed: 21403094]

Hsieh PC, Segers VF, Davis ME, et al. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. Nat Med. 2007; 13:970–974. [PubMed: 17660827]

Huang J, Kalderon D. Coupling of Hedgehog and Hippo pathways promotes stem cell maintenance by stimulating proliferation. J Cell Biol. 2014; 205:325–338. [PubMed: 24798736]

Ihrie RA, Alvarez-Buylla A. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. Neuron. 2011; 70:674–686. [PubMed: 21609824]

Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. J Cell Physiol. 2003; 194:237–255. [PubMed: 12548545]

Kajstura J, Gurusamy N, Ogórek B, et al. Myocyte turnover in the aging human heart. Circ Res. 2010b; 107:1374–1386. [PubMed: 21088285]

Kajstura J, Urbanek K, Perl S, et al. Cardiomyogenesis in the adult human heart. Circ Res. 2010a; 107:305–315. [PubMed: 20522802]

Kajstura J, Rota M, Hall SR, et al. Evidence for human lung stem cells. N Engl J Med 2011. 2011; 364:1795–1806.

Karsner HT, Saphir O, Todd TW. The state of the cardiac muscle in hypertrophy and atrophy. Am J Pathol. 1925; 1:351–372. [PubMed: 19969656]

Kimura W, Sadek HA. The cardiac hypoxic niche: emerging role of hypoxic microenvironment in cardiac progenitors. Cardiovasc Diagn Ther. 2012; 2:278–289. [PubMed: 24282728]

Kocabas F, Mahmoud AI, Sosic D, et al. The hypoxic epicardial and subepicardial microenvironment. J Cardiovasc Transl Res. 2012; 5:654–665. [PubMed: 22566269]

Konstandin MH, Toko H, Gastelum GM, et al. Fibronectin is essential for reparative cardiac progenitor cell response after myocardial infarction. Circ Res. 2013; 113:115–125. [PubMed: 23652800]

Koyanagi M, Bushoven P, Iwasaki M, et al. Notch signaling contributes to the expression of cardiac markers in human circulating progenitor cells. Circ Res. 2007; 101:1139–1145. [PubMed: 17967789]

Kunisaki Y, Frenette PS. Influences of vascular niches on hematopoietic stem cell fate. Int J Hematol. 2014; 99:699–705. [PubMed: 24756874]

Lajtha LG, Schofield R. Kinetics of the stem cell compartment. Haematol Lat. 1969; 12:457–465. [PubMed: 5396819]

Lee-Thedieck C, Spatz JP. Artificial niches: biomimetic materials for hematopoietic stem cell culture. Macromol Rapid Commun. 2012; 33:1432–1438. [PubMed: 22815039]

Leri, A.; Boni, A.; Siggins, R., et al. Cardiac stem cells and their niches. In: Leri, A.; Anversa, P.; Frishman, WH., editors. Cardiovascular Regeneration and Stem Cell Therapy. Blackwell Futura; Malden, MA: 2007. p. 87-94.

Leri A, Kajstura J, Anversa P. Role of cardiac stem cells in cardiac pathophysiology: a paradigm shift in human myocardial biology. Circ Res. 2011; 109:941–961. [PubMed: 21960726]

Leri A, Anversa P. Stem cells and myocardial regeneration: cooperation wins over competition. Circulation. 2013; 127:165–168. [PubMed: 23224060]

Limana F, Zacheo A, Mocini D, et al. Identification of myocardial and vascular precursor cells in human and mouse epicardium. Circ Res. 2007; 101:1255–1265. [PubMed: 17947800]

Limana F, Bertolami C, Mangoni A, et al. Myocardial infarction induces embryonic reprogramming of epicardial c-kit(+) cells: role of the pericardial fluid. J Mol Cell Cardiol. 2010; 48:609–618. [PubMed: 19968998]

Linzbach AJ. Mikrometrische und histogische analyse hypertropher menschlicher herzen. Virchow Arch Pathol Anat Physiol. 1947; 314:534–594.

Linzbach AJ. Heart failure from the point of view of quantitative anatomy. Am J Cardiol. 1960; 5:370–382. [PubMed: 14417346]

Lo Celso C, Lin CP, Scadden DT. In vivo imaging of transplanted hematopoietic stem and progenitor cells in mouse calvarium bone marrow. Nat Protoc. 2011; 6:1–14. [PubMed: 21212779]

Mohsin S, Khan M, Toko H, et al. Human cardiac progenitor cells engineered with Pim-I kinase enhance myocardial repair. J Am Coll Cardiol. 2012; 60:1278–1287. [PubMed: 22841153]

Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature. 2014; 505:327–334. [PubMed: 24429631]

Nadal-Ginard B, Ellison GM, Torella D. The absence of evidence is not evidence of absence: the pitfalls of Cre Knock-Ins in the c-kit locus. Circ Res. 2014; 115:415–418. [PubMed: 24965482]

Nakamura Y, Arai F, Iwasaki H, et al. Isolation and characterization of endosteal niche cell populations that regulate hematopoietic stem cells. Blood. 2010; 116:1422–1432. [PubMed: 20472830]

Oh IH, Kwon KR. Concise review: multiple niches for hematopoietic stem cell regulations. Stem Cells. 2010; 28:1243–1249. [PubMed: 20517982]

Potten CS. Extreme sensitivity of some intestinal crypt cells to X and gamma irradiation. Nature. 1977; 269:518–521. [PubMed: 909602]

Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt Development. 1990; 110:1001–1020.

Radtke F, MacDonald HR, Tacchini-Cottier F. Regulation of innate and adaptive immunity by Notch. Nat Rev Immunol. 2013; 13:427–437. [PubMed: 23665520]

Rakusan K, Cicutti N, Kazda S, et al. Effect of nifedipine on coronary capillary geometry in normotensive and hypertensive rats. Hypertension. 1994; 24:205–211. [PubMed: 8039845]

Rota M, Hosoda T, De Angelis A, et al. The young mouse heart is composed of myocytes heterogeneous in age and function. Circ Res. 2007; 101:387–399. [PubMed: 17601802]

Rudat C, Kispert A. Wt1 and epicardial fate mapping. Circ Res. 2012; 111:165–169. [PubMed: 22693350]

Sanada F, Kim J, Czarna A, et al. c-kit-positive cardiac stem cells nested in hypoxic niches are activated by stem cell factor reversing the aging myopathy. Circ Res. 2014; 114:41–55. [PubMed: 24170267]

Scadden DT. Nice neighborhood: emerging concepts of the stem cell niche. Cell. 2014; 157:41–50. [PubMed: 24679525]

Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978; 4:7–25. [PubMed: 747780]

Schofield R, Lajtha LG. Determination of the probability of self-renewal in haemopoietic stem cells: a puzzle. Blood Cells. 1983; 9:467–483. [PubMed: 6372899]

Siminovitch L, McCulloch EA, Till JE. The distribution of colony-forming cells among spleen colonies. J Cell Physiol. 1963; 62:327–336. [PubMed: 14086156]

Simsek T, Kocabas F, Zheng J, et al. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. Cell Stem Cell. 2010; 7:380–390. [PubMed: 20804973]

Sipkins DA, Wei X, Wu JW, et al. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature. 2005; 435:969–973. [PubMed: 15959517]

Smart N, Bollini S, Dubé KN, et al. De novo cardiomyocytes from within the activated adult heart after injury. Nature. 2011; 474:640–644. [PubMed: 21654746] Cell Stem Cell. 2008; 3:484–492. [PubMed: 18983964]

Spiegel, A.; Kalinkovich, A.; Shivtiel, S., et al. Stem cell regulation via dynamic interactions of the nervous and immune systems with the microenvironment. 2008.

Solanas G, Benitah SA. Regenerating the skin: a task for the heterogeneous stem cell pool and surrounding niche. Nat Rev Mol Cell Biol. 2013; 14:737–748. [PubMed: 24064540]

Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. Circ Res. 1998; 83:15–26. [PubMed: 9670914]

Steinhauser ML, Lee RT. Regeneration of the heart. EMBO Mol Med. 2011; 3:701–712. [PubMed: 22095736]

Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell. 2011; 9:298–310. [PubMed: 21982230]

Sugimura R, He XC, Venkatraman A, et al. Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche. Cell. 2012; 150:351–365. [PubMed: 22817897]

Tan S, Barker N. Engineering the niche for stem cells. Growth Factors. 2013; 31:175–184. [PubMed: 24274105]

Tanigaki K, Honjo T. Regulation of lymphocyte development by Notch signaling. Nat Immunol. 2007; 8:451–456. [PubMed: 17440450]

Uchida S, De Gaspari P, Kostin S, et al. Sca1-derived cells are a source of myocardial renewal in the murine adult heart. Stem Cell Reports. 2013; 1:397–410. [PubMed: 24286028]

The enigmatic bone-marrow stem cell. Lancet. 1971; 2:304–305. Unlisted authors. [PubMed: 4104983]

Urbanek K, Cesselli D, Rota M, et al. Stem cell niches in the adult mouse heart. Proc Natl Acad Sci USA. 2005; 103:9226–9231. [PubMed: 16754876]

Urbanek K, Cabral-da-Silva MC, Ide-Iwata N, et al. Inhibition of notch1-dependent cardiomyogenesis leads to a dilated myopathy in the neonatal heart. Circ Res. 2010; 107:429–441. [PubMed: 20558824]

Urbanek K, Rota M, Cascapera S, et al. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. Circ Res. 97:663–673. [PubMed: 16141414]

van Berlo JH, Kanisicak O, Maillet M, et al. c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature. 2014; 509:337–341. [PubMed: 24805242]

van Wijk B, Gunst QD, Moorman AF, et al. Cardiac regeneration from activated epicardium. PLoS One. 2012; 7:e44692. [PubMed: 23028582]

Wang LD, Wagers AJ. Dynamic niches in the origination and differentiation of haematopoietic stem cells. Nat Rev Mol Cell Biol. 2011; 12:643–655. [PubMed: 21886187]

Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science. 2000; 287:1427–1430. [PubMed: 10688781]

Williams SE, Beronja S, Pasolli HA, et al. Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature. 2011; 470:353–358. [PubMed: 21331036]

Williams AR, Hatzistergos KE, Addicott B, et al. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation. 2013; 127:213–223. [PubMed: 23224061]

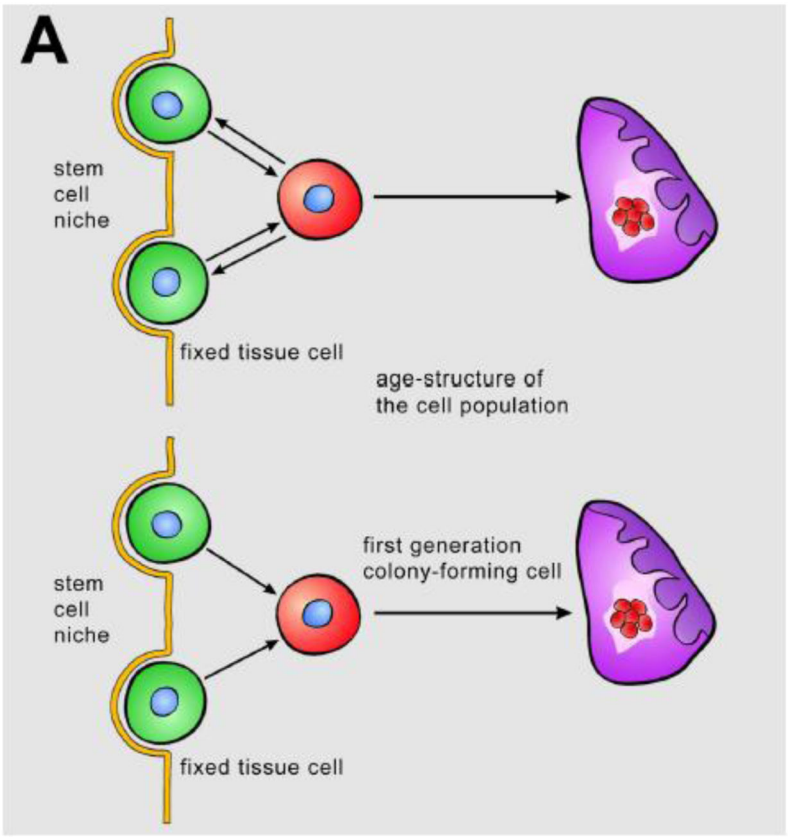
Xie T, Spradling AC. decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell. 1998; 942:251–260. [PubMed: 9695953]

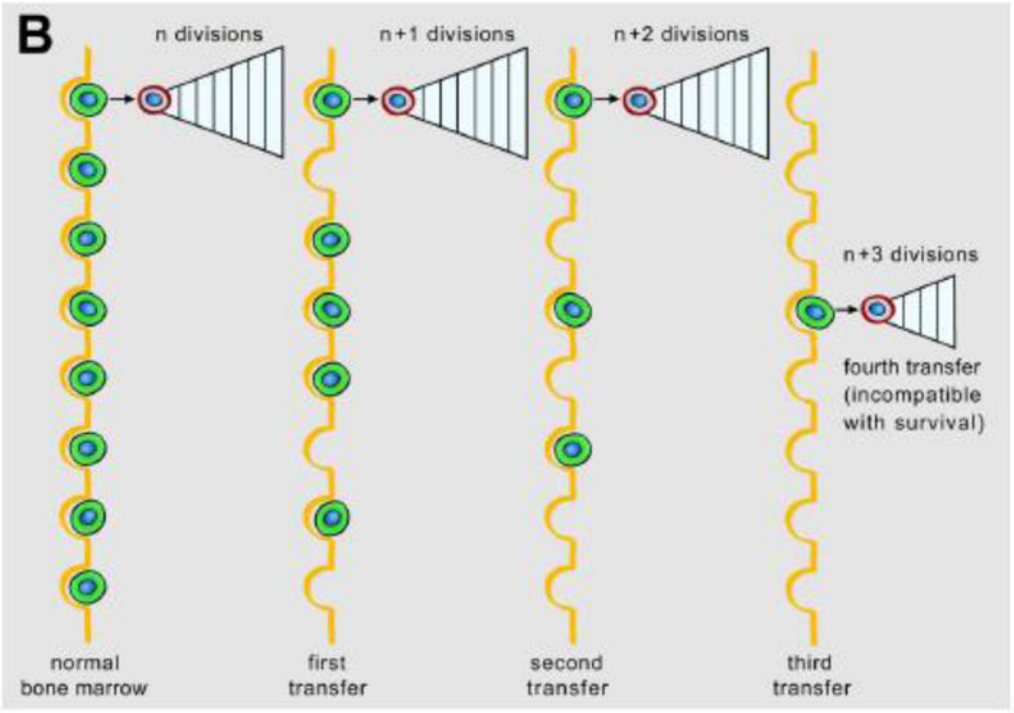
Zhang J, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003; 425:836–841. [PubMed: 14574412]

Zhou B, Ma Q, Rajagopal S, et al. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature. 2008; 454:109–113. [PubMed: 18568026]

Highlights

- The adult heart contains stem cell niches.
- The c-kit receptor identifies a pool of resident cardiac stem cells.
- Hypoxic and normoxic niches coexist in the myocardium.
- Cardiac mesenchymal stem cells are nested in perivascular niches.





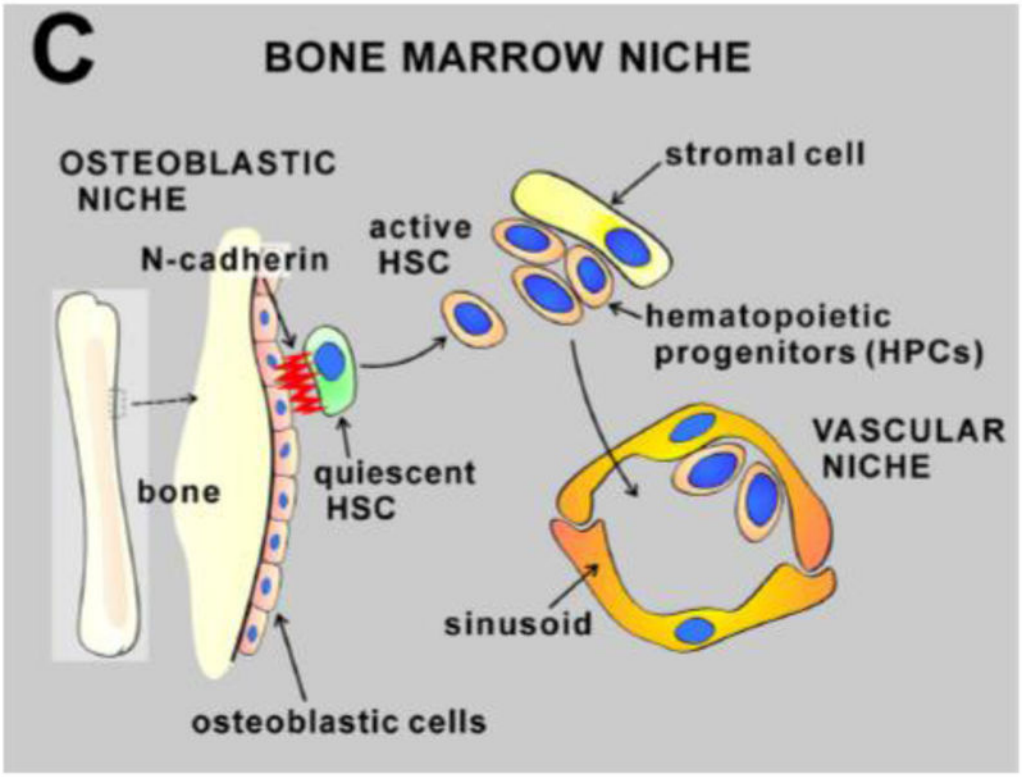
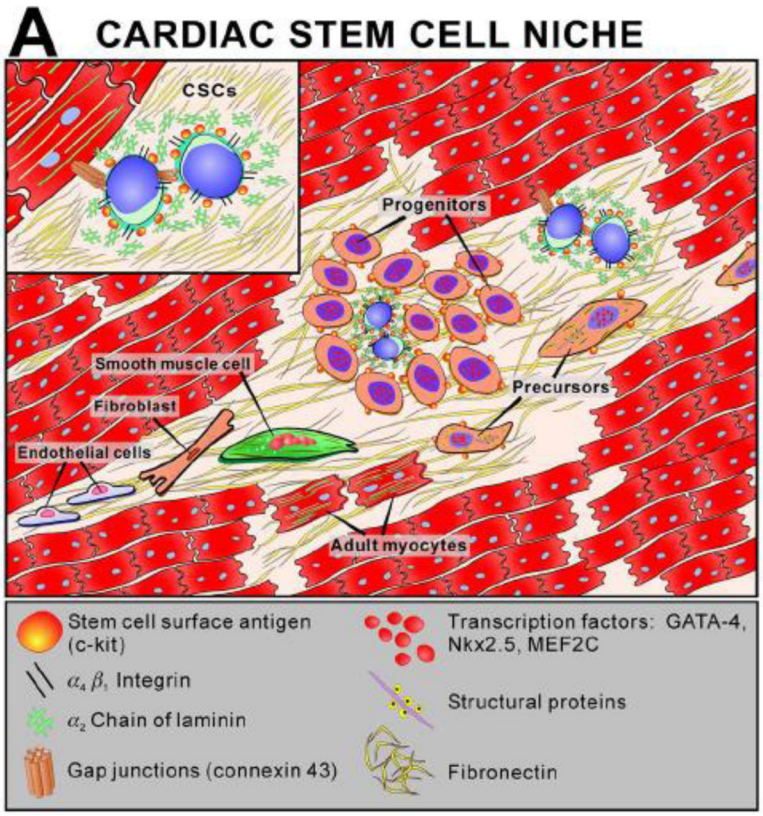
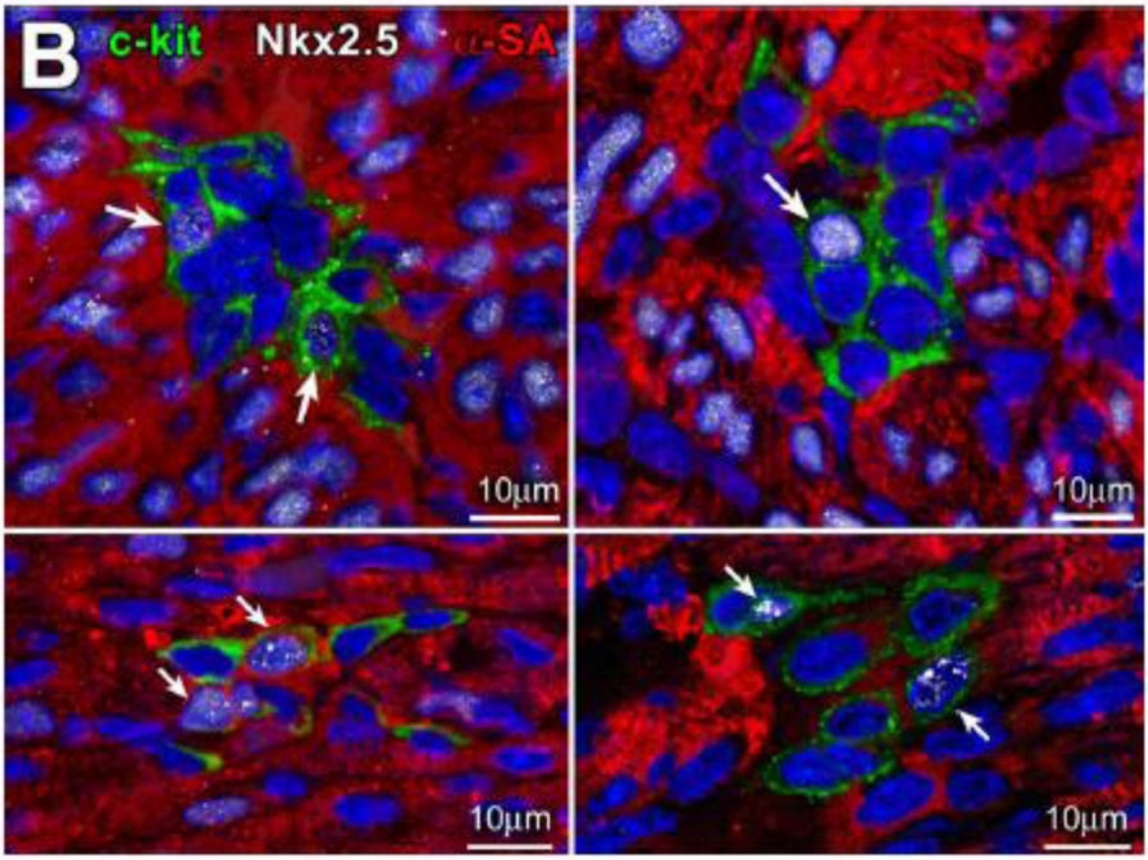


Figure 1. Bone marrow niches

A–C, Schematic representation of bone marrow niches. See text for detail.





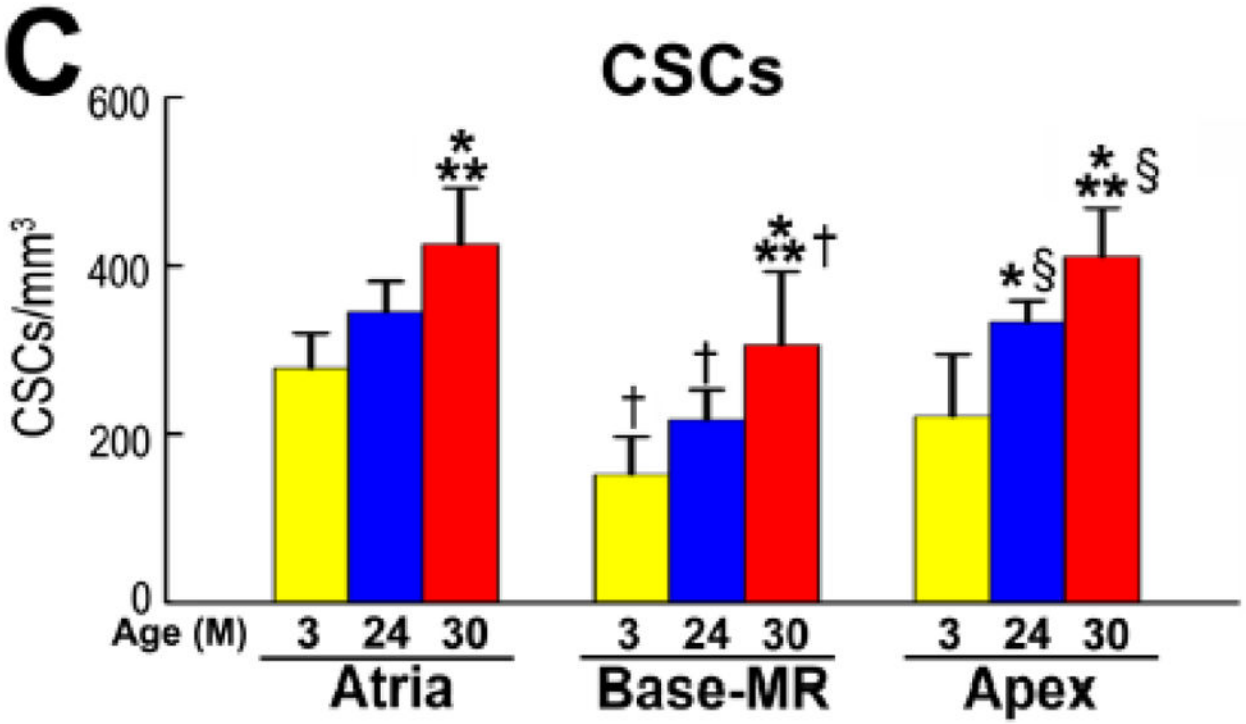
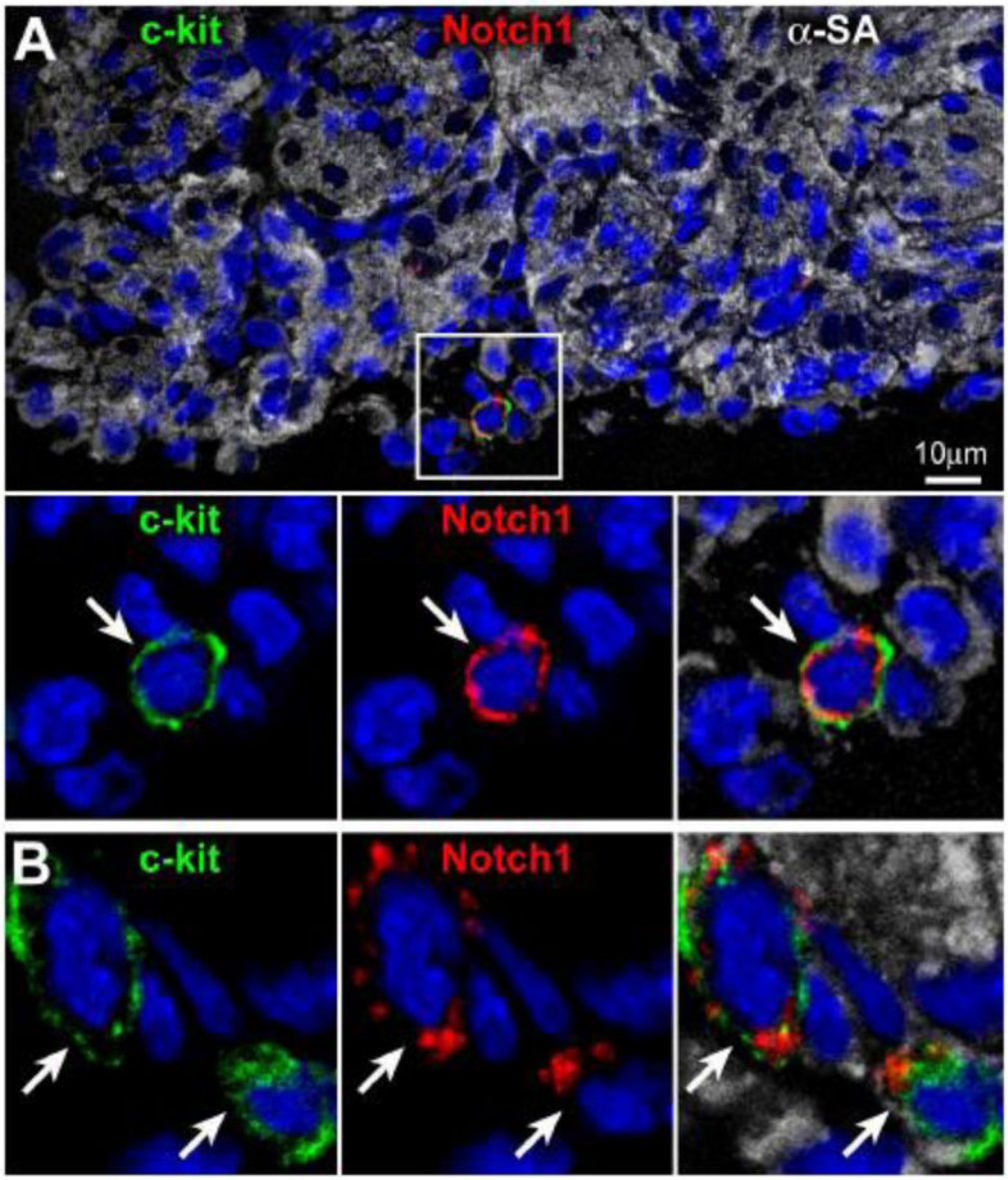


Figure 2. CSC niches

A, Schematic representation of the cellular and extracellular components of a CSC niche. **B,** Atrial (upper panels) and ventricular (lower panels) niches at 7 days after birth composed of clusters of undifferentiated c-kit-positive CSCs (green). Some of these cells express Nkx2.5 alone (white; arrows) or Nkx2.5 together with a thin layer of α-SA (red; arrows). **C,** The number of CSCs per mm^3 of myocardium increases with age in the atria, Base-MR and apex. M, months. *,**,†,§P<0.05 vs. 3M, 24M, Atria, Base-MR, respectively. (Panel A from Leri A, Boni A, Siggins R, Nascimbene A, Hosoda T. Cardiac stem cell niches. In: *Cardiovascular Regeneration and Stem Cell Therapy*, Leri A, Anversa P, Frishman WH, editors. Malden, Massachusetts: Blackwell Publishing; 2007. p. 87–94. Panel B from Urbanek et al., 2010. Panel C from Sanada et al., 2014).



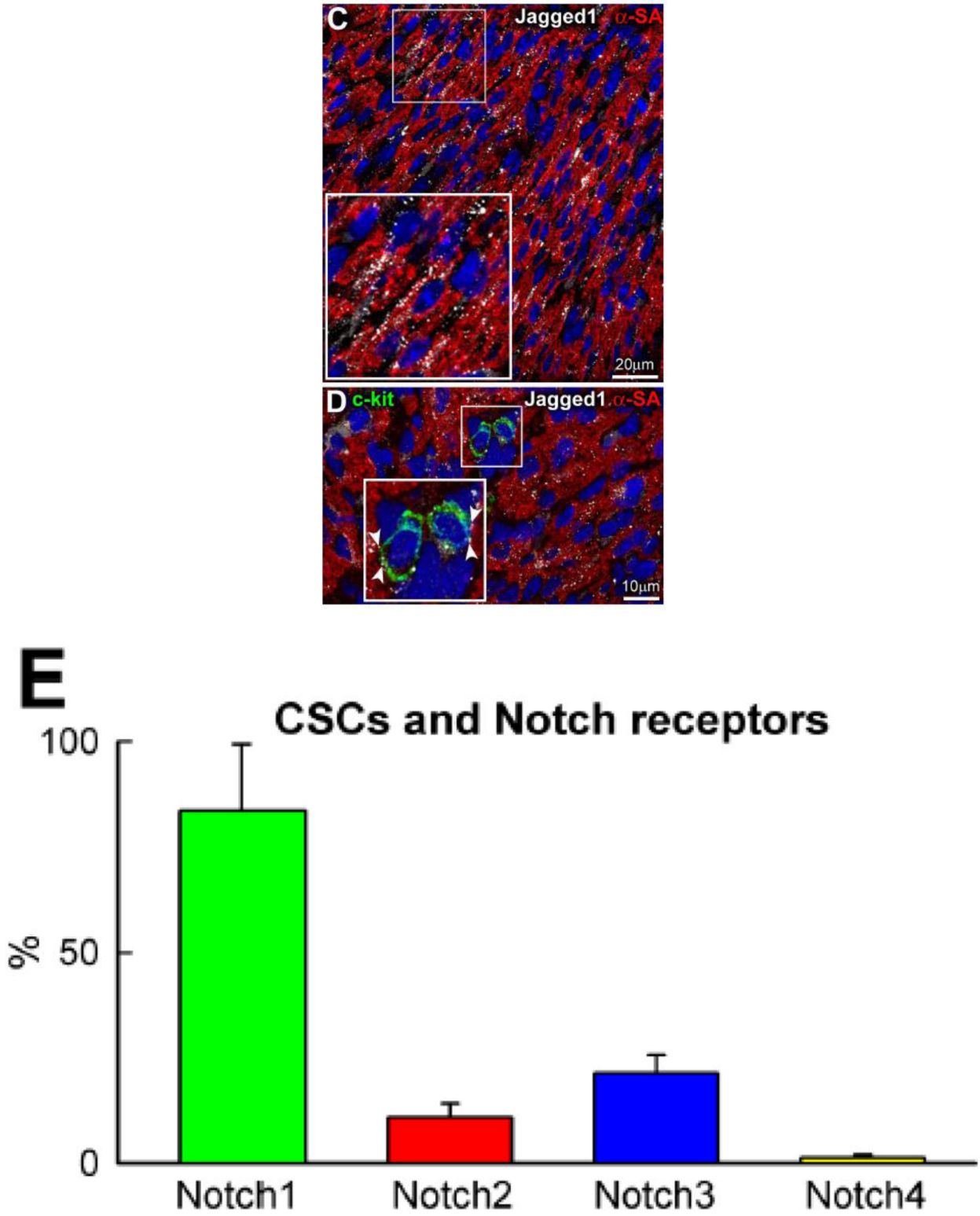


Figure 3. Notch receptors in CSCs

A, LV myocardium at 6 days after birth. The CSC, located in the endocardium and included in the square is shown at higher magnification in the lower panels. CSCs are positive for c-

kit (green) and Notch1 (red). Myocytes are recognized by the white fluorescence of α-sarcomeric actin. **B,** Two examples of CSCs positive for c-kit (green) and Notch1 (red) shown at higher magnification. **C,** LV myocytes display the Notch ligand Jagged1 (white). Jagged1 localization is more apparent in the inset. **D,** c-kit-positive CSCs are in contact with myocytes, and Jagged1 is distributed at the interface between cardiomyocytes and CSCs (insets, arrowheads). Myocytes are recognized by the white fluorescence of α-sarcomeric actin (α-SA). **E,** CSCs express predominantly the Notch1 receptor isoform. Fraction of ckit-positive CSCs expressing the extracellular domains of Notch1, Notch2, Notch3 and Notch4. Results are shown as mean±SD. (From Urbanek et al., 2010).

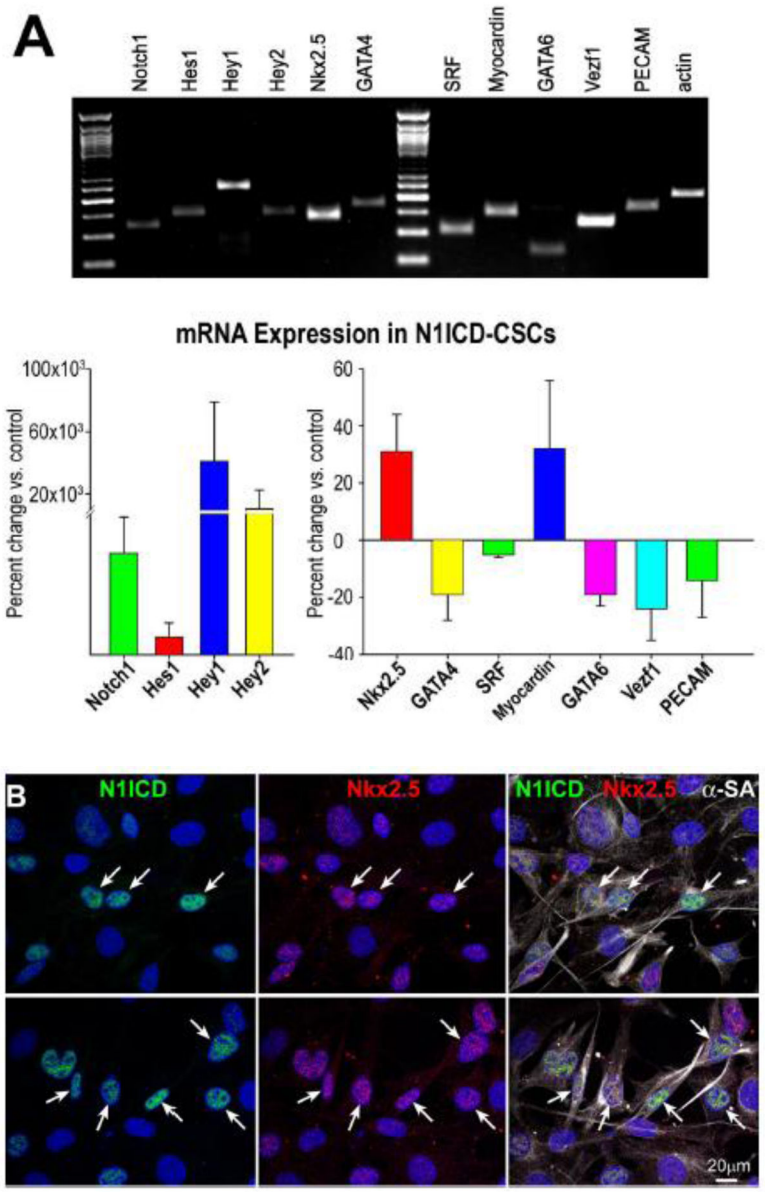


Figure 4. N1ICD overexpression in CSCs promotes the upregulation of myocyte-specific genes

A, Quantitative RT-PCR for target genes of Notch and cardiovascular genes. Expression is shown as change with respect to CSCs infected with EGFP-lentivirus only. **B**, CSCs overexpressing the Notch1 intracellular domain (N1ICD, green) are positive for Nkx2.5 (red) and possess a thin layer of α-SA (alpha-sarcomeric actin, white). (From Urbanek et al., 2010).

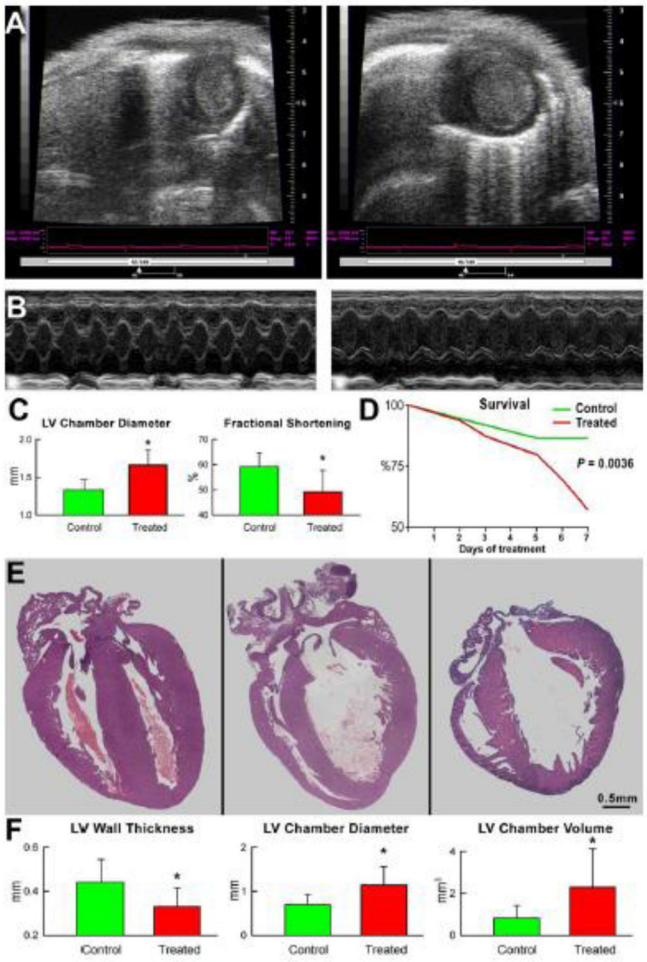


Figure 5. Notch inhibition induces a dilated myopathy

A and **B**, B-mode and M-mode echocardiography of vehicle (left) and γ-secretase inhibitor (right) injected mice. **C and D**, γ-secretase inhibition led to ventricular dilation, depressed fractional shortening, ejection fraction and increased mortality. **E,** With respect to a control heart (left panel), ventricular dilation and wall thinning are apparent in γ-secretase inhibitor-treated mice (central and right panels). **F,** Effects of γ-secretase inhibition on cardiac anatomy. Results are mean±SD. *P<0.05. (From Urbanek et al., 2010).

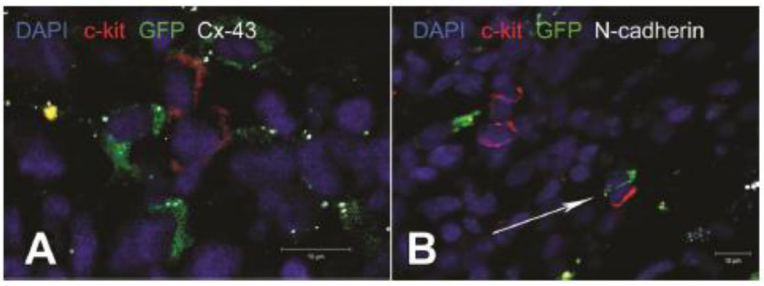


Figure 6. Cell-cell interaction between endogenous and exogenous stem cells

A and **B,** Immature MSCs (green) within the host myocardium are connected to resident c-kit-positive CSCs (red) by connexin 43 (A, white) and N-cadherin (B, white), closely resembling stem cell niches. (Courtesy of Dr. Joshua H. Hare. From Hatzistergos et al., 2010).

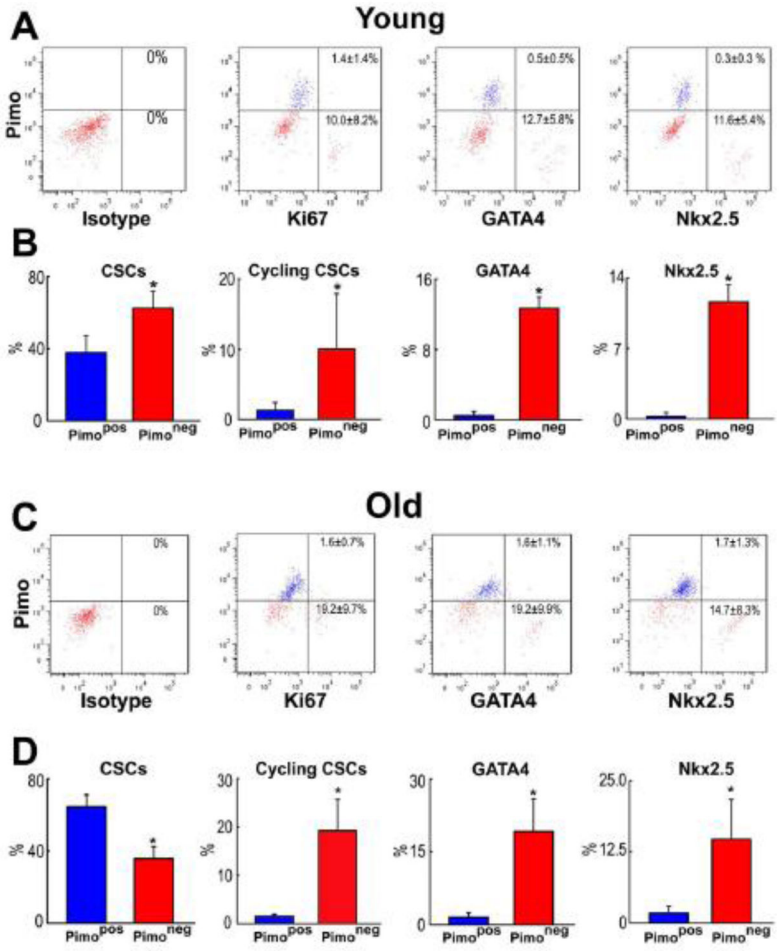


Figure 7. Hypoxic (Pimo^{pos}-CSCs) and normoxic (Pimo^{neg}-CSCs) in the young and old myocardium

A–D, Bivariate distribution of Pimo, Ki67, GATA4 and Nkx2.5 in CSCs from young (A: 3M) and old (C: 30M) hearts. Quantitative results in young (**B**) and old (**D**) are shown as mean±SD. *P<0.05 vs. Pimo^{pos}.