REVIEW

Cardiac stem cells: translation to human studies

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Abstract The discovery of multiple classes of cardiac progenitor cells in the adult mammalian heart has generated hope for their use as a therapeutic in heart failure. However, successful results from animal models have not always yielded similar findings in human studies. Recent Phase I/II trials of c-Kit (SCIPIO) and cardiosphere-based (CADUCEUS) cardiac progenitor cells have demonstrated safety and some therapeutic efficacy. Gaps remain in our understanding of the origins, function and relationships between the different progenitor cell families, many of which are heterogeneous populations with overlapping definitions. Another challenge lies in the limitations of small animal models in replicating the human heart. Cryopreserved human cardiac tissue provides a readily available source of cardiac progenitor cells and may help address these questions. We review important findings and relative unknowns of the main classes of cardiac progenitor cells, highlighting differences between animal and human studies

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Introduction

Whilst the cardiac regenerative ability of species such as salamanders and zebrafish is now well established, a similar capability of the mammalian heart has only recently been reported (Oberpriller and Oberpriller 1974; Porrello et al. 2011; Poss et al. 2002). The "Holy Grail" is to replicate this in humans. We are not there yet, but we are at a stage where studies using human cardiac tissue have firmly challenged the dogma that the human heart is a terminally differentiated organ.

The adult human heart contains about 3 billion cardiomyocytes, and a large myocardial infarction can eliminate up to 25 % of these cells (Laflamme and Murry 2011). Survival has improved in patients with myocardial infarction and other cardiac diseases. However, despite standard-of-care medical therapy, many patients progress to heart failure, the incidence of which is rising exponentially (Clark et al. 2005). Furthermore, stage IV heart failure carries a grim prognosis with 50 % mortality within 1 year of diagnosis (Cleland 2000). Cardiac regeneration has the potential to permanently improve function in failing hearts. Whilst there are several potential methods by which this could be achieved, these can be broadly grouped into two categories. Firstly, putative stem or progenitor cells can be introduced to generate new cardiomyocytes or other cardiac tissues. Secondly, stimulation of an inherent regenerative capacity

(either stem cell mediated or proliferation of de novo cardiomyocytes) may be possible.

There is an emerging recognition that non-human mammalian hearts have excellent regenerative capacity during development (Porrello et al. 2011). This decreases after birth, but it may be re-activated in post-natal life. Small animal models such as the mouse and rat are popular due to their relative low costs and ease of genetic manipulation (Kooij et al. 2014). Yet, extrapolation to humans is limited by significant differences in proteome, genome, and physiology (Kooij et al. 2014; Siller et al. 2013). Data using murine models of human heart disease are not always applicable (Chong and Murry 2014). Large animal models (including macaque, canine, porcine, and ovine) better approximate human physiology (Dixon and Spinale 2009; Houser et al. 2012) but are expensive to maintain and are more difficult to manipulate genetically. Difficulty in translating promising animal studies into successful therapeutics has led to caution against extrapolation of animal findings to humans (Hackam and Redelmeier 2006; Hartung 2013; Marian 2011). This discrepancy was evidenced when bone marrow mononuclear cells failed to replicate promising animal results in human heart failure (Rehman 2013). More research using large animal models or on human cardiac tissue is needed to bridge this gap. Human cardiac progenitor cells (CPCs) have direct translational significance (Fig. 1), and extension of animal findings to human tissue will aid understanding of their role in human heart failure. However, there are significant hurdles to human research, including difficulty in acquisition of non-diseased specimens, the unpredictability of their availability and rapid degradation (Saha and Hurlbut 2011). In this regard, human heart tissue banks may provide the solution.

Examining human cardiac stem cell sources *within* human cardiac tissue may be the way forward, but first, we need to establish exactly what is a human cardiac stem cell. Here, we review developments in the stem cell field with specific reference to human cardiac stem cells.
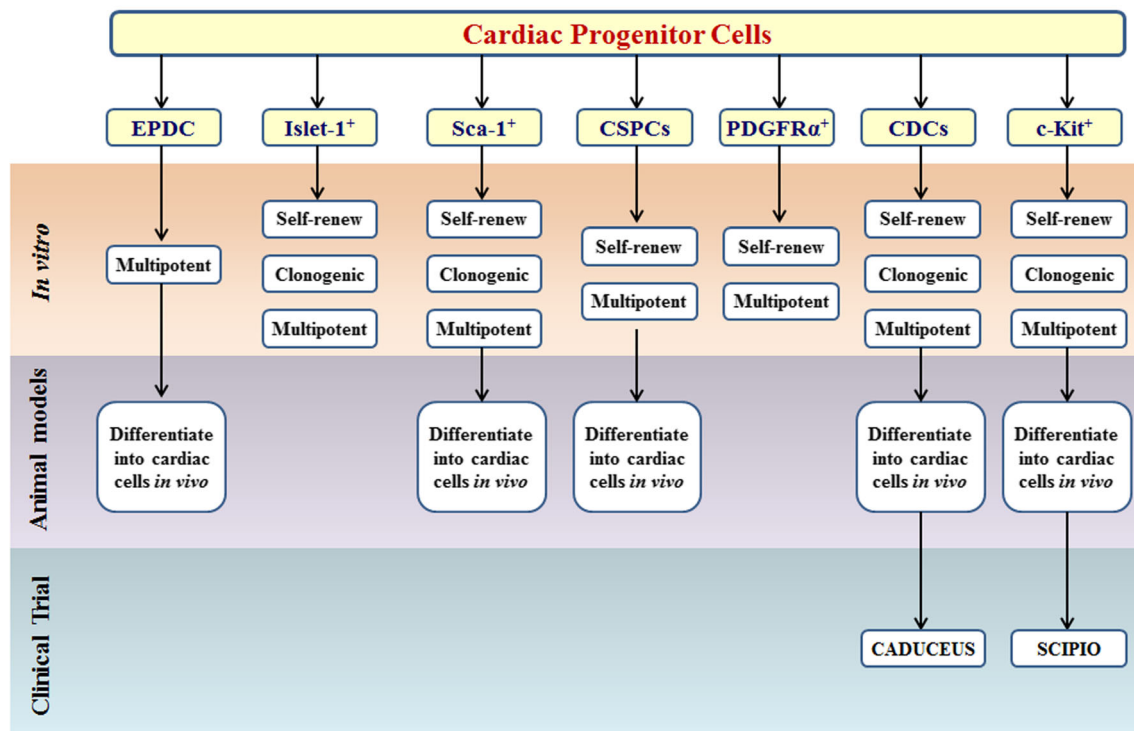
c-Kit[+] cardiac progenitor cells

The population of cardiac progenitor cells that has been most thoroughly characterised is the c-Kit[+] fraction, where c-Kit is the tyrosine kinase receptor for stem cell factor. Beltrami et al. (2003) initially discovered a resident c-Kit[+], lineage negative[1] population within the murine

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[1] Lineage markers are found on mature haematopoietic cells and lineage depletion selects for a subpopulation enriched in early progenitor cells not yet expressing lineage markers

heart that is self-renewing, clonogenic, and differentiates both in vitro and in vivo to cardiomyocytes, endothelial cells and smooth muscle cells. Although the cells do not contract in vitro, cardiomyocytes formed in vivo couple to resident cardiomyocytes and exhibit similar contractile properties to the host myocardium. However, they are small and did not exhibit striations. Similarly, c-Kit[+] CPCs were found in the dog heart and show the greatest cardiomyogenic potential compared to Sca-1^{+} CPCs or CSPCs. Once again, the generated cardiomyocytes are much smaller than that of the host and cannot directly explain the improvement in cardiac function.

The exact mechanism by which c-Kit stem cells alleviate myocardial dysfunction is contentious. Anversa and co-workers (Rota et al. 2008) found that injection of CPCs into rat myocardium 20 days after infarction reduce infarct size by over 40 % through the formation of new arterioles and small-sized cardiomyocytes in the infarct region. However, intracoronary injection of CPCs in a month-old infarct lead to minimal engraftment with the formation of immature cardiomyocytes. Nevertheless, improvements in cardiac function was noted, with paracrine mechanism suggested (Tang et al. 2010). Although bone marrow-derived c-Kit^{+} cells can be found in the heart post-MI (Fazel et al. 2006), they do not form cardiomyocytes or vasculature but exert an indirect effect on angiogenesis (Ellison et al. 2013; Jesty et al. 2012). Ellison et al. used an acute diffuse isoproterenol-induced injury model coupled with fate mapping to demonstrate that c-Kit^{+} cells infused via the tail vein homed to the infarct site differentiate for de novo myocyte formation (Ellison et al. 2013). Although the authors conclude that this proves cardiomyogenesis is the mechanism of cardiac regeneration by c-Kit[+] CPCs (Nadal-Ginard et al. 2014), contrasting studies by Hong et al. show lack of engraftment for CPCs with both intramyocardial (Hong et al. 2013) and intracoronary injection (Hong et al. 2014). Recently, van Berlo and co-workers used a similar fate mapping technique to demonstrated that c-Kit^{+} cells contribute to many cellular fusion events and has little post-natal involvement with cardiomyocyte generation whether in ageing or injury (van Berlo et al. 2014).

The heterogeneity of c-Kit[+] CPCs is one reason why it is so difficult to define the exact role of c-Kit cells in the heart (Table 1). Bearzi et al. characterised two separate multipotent c-Kit[+] populations in the human heart: those expressing c-Kit and myocyte transcription factors but not vascular endothelial growth factor receptor-2 (VEGFR-2) were classified as myocardial progenitor cells that gave rise to more cardiomyocytes and improved function when injected in a rat MI model (Bearzi et al. 2007). Vascular progenitor cells were c-Kit[+]/VEGFR-2^{+} and differentiate in vitro primarily into endothelial and smooth muscle

Fig. 1 Characteristics of various cardiac progenitor cell populations. *EPDC* epicardium-derived cell, *CPC* cardiac progenitor cell, *CSPC* cardiac side population cell, *CDC* cardiosphere-derived cell, *CADUCEUS* cardiosphere-derived autologous stem cells to reverse ventricular dysfunction, *SCIPIO* stem cell infusion in patients with ischemic cardiomyopathy

cells. When injected into dogs with stenosed coronary arteries, they formed coronary vessels up to 1.5 mm in diameter (Bearzi et al. 2009). c-Kit^{+} cells co-expressing CD29 and CD105 differentiate to give cardiac cells but also osteoblasts and adipocytes (Gambini et al. 2011). Indeed, within the same study, He et al. found c-Kit^{+}/VEGFR-2^{+}/CD31^{+} cells in some patients but not others, and suggested that these differences may be due to discordant medical history (He et al. 2011).

Alterations in CPC content and function are also seen with ageing. c-Kit[+] CPCs can be isolated from the developing mouse embryo (Wu et al. 2006) and decline in numbers postnatally such that only a few are seen in niches of the adult heart (Tallini et al. 2009). These embryonic CPCs differentiate into all three cardiac lineages and, unlike their adult counterparts, exhibit spontaneous contraction (Tallini et al. 2009). Early embryonic c-Kit cells negative for cardiac markers were identified from as early as 6 days post-coitus, and divide both symmetrically and asymmetrically in vitro (Ferreira-Martins et al. 2012). This suggests they are a primitive population contributing to early cardiogenesis. Adult CPCs are quiescent and occupy niches found in greater numbers in the atria and apex and especially lowered in the left ventricle (Saravanakumar and Devaraj 2013; Urbanek et al.

2006). Neonatal c-Kit[+] CPCs differentiate to both cardiomyocytes and endothelial cells post-infarct leading to partial regeneration while adult CPCs were only able to adopt a vascular lineage (Jesty et al. 2012).

There is disagreement in the distribution of c-Kit[+] CPCs in both healthy and diseased human hearts (Castaldo et al. 2008; Garbade et al. 2010; Matuszczak et al. 2014; Sandstedt et al. 2014). Phase I study of c-Kit[+] CPCs in patients with post-infarct ventricular dysfunction was reported to show functional improvement (Bolli et al. 2013), but concerns about data integrity have surfaced (The Lancet 2014). Given the current controversy over efficacy of c-Kit CPCs both in animal and human trials, the complex relationships between the various subpopulations, and unclear mechanisms by which they exert their action, validated, reproducible studies need to be performed to bridge the gap between animal models and human physiology. Such experiments will undoubtedly require both donor and diseased human heart tissue.

Epicardium-derived cells

The embryonic proepicardium is a cluster of extra-cardiac coelomic cells that adhere to the myocardium, and gives

Table 1 Summary of human CPC populations

	Populations	Source of human tissue
EPDC		
Limana et al. 2007	CD34+ and c-Kit+	Adult and foetal epicardium
van Tuyn et. al. 2007	CD44+/CD90+/CD105+/CD46+/c-Kit-	Adult epicardium covering right atrial appendage
Bax et al. 2011	WT-1	Adult epicardium covering right atrial appendage
Islet-1 CPC		
Bu et al. 2009	Isl-1+	Whole foetal heart
Serradifalco et al. 2011	Isl-1+/c-Kit+	Foetal and infant ventricles (transmural)
Fuentes et al. 2013	Isl-1+/c-Kit+	Adult and neonatal atria
Sca-1 CPC		
van Vilet et al. 2008	Sca-1+/CD105+/CD45-/CD34-/CD133-	Foetal heart (whole) and adult atria
CSPCs		
Sandstedt et al. 2012	CD45+/c-Kit+/ABCG2-/MDR1-	Adult atria
Emmert et al. 2013	ABCG2+	Adult atria and ventricles
Cardiospheres		
Messina et al. 2004	c-Kit+/CD31+/CD34+/Sca-1+	Infant and adult atria and ventricles
Davis et al. 2009	[Core] c-Kit+/CD34-/CD45- [Periphery] - MDR1+/CD31+/CD90+/ CD105+/CD133+/CD34-/CD45-	Endomyocardium
CDCs		
Smith et al. 2007	CD105+/CD90+/c-Kit+/CD31+/CD34+/ MDR1-/CD133-/CD45-	Adult right ventricle endomyocardium
Chan et al. 2012	CD105+/CD90+/c-Kit(low)/CD31(low)/ CD34 (low)/CD45-	Transmural right atria and left ventricle epicardium
c-Kit CPC		
Bearzi et al. 2007	c-Kit+/VEGFR-2-/CD31-/CD34-/CD45-	Unspecified myocardial specimen
Bearzi et al. 2009	c-Kit+/VEGFR-2+/CD31+/CD34-/CD45-	Unspecified myocardial specimen
Gambini et al. 2011	c-Kit+/CD105+/CD90+/CD44+/CD29+	Auricular fragments
Matuszczak et al. 2014	c-Kit+/CD105+/CD90+/CD31+/CD34+	Adult and paediatric atria, ventricles and intraventricular septa
Sandstedt et al. 2014	c-Kit/CD45-	Adult right atrium
PDGFRα CPC		
Chong et al. 2013	PDGFRα+	Foetal and adult hearts

EPDC epicardium-derived cell, *CPC* cardiac progenitor cell, *CSPC* cardiac side population cell, *CDC* cardiosphere-derived cell

rise to the epicardium (visceral pericardium). The epicardium then undergoes an epithelial to mesenchymal transition (EMT) event to give rise to epicardium-derived cells (EPDCs), which migrate into the subepicardium and contribute to vasculature (Carmona et al. 2010; Krenning et al. 2010), and possibly cardiomyocytes (Christoffels et al. 2009; Zhou et al. 2008). Thus, EPDCs in the embryonic heart are highly enriched for stem cell activity (Wessels and Perez-Pomares 2004).

Myocardial infarction (MI) can induce normally quiescent adult mouse EPDCs to proliferate into mesenchymal cells via paracrine mechanisms, promoting subepicardial angiogenesis (Zhou et al. 2011). When treated with thymosin β-4 (Tβ4), explant culture of adult murine epicardium produces outgrowth of cells that differentiate into fibroblasts, smooth muscle cells, and endothelial cells (Smart et al. 2007). A sub-fraction of the resultant primed cells give rise to de novo cardiomyocytes after MI (Smart et al. 2011). Importantly, these cardiomyocytes are seen to structurally integrate with the resident myocardium via gap junctions and exhibit Ca^{2+} transients synchronous with resident cardiomyocytes, reflecting functional integration. Despite this promising finding, post-MI administration of Tβ4 could not induce EPDCs to adopt cardiac phenotypes or migrate into the subepicardium (Zhou et al. 2012), and its use in MI has not been validated (Smart et al. 2013). Small animal studies are still focused on improving differentiation rates with novel drugs.

Explant culture of human epicardium derived from atrial appendages yields c-Kit⁻/Islet-1⁻ epithelioid cells with a "cobblestone" appearance before transitioning to a spindle shaped appearance in subculture, mimicking the EMT process. These cells demonstrate ability to differentiate into osteoblasts in vitro, but not endothelial cells nor adipocytes, and therefore appear to have limited plasticity (van Tuyn et al. 2007). When treated with bone morphogenic protein-2 or transforming growth factor-β1, they differentiate to form smooth muscle cells with high efficiency (van Tuyn et al. 2007). In contrast, rat EPDCs attain smooth muscle cell phenotype after stimulation with platelet-derived growth factor with less than 5 % efficiency (Wada et al. 2003). It is unknown whether this is due to differences in intrinsic commitment of these progenitor cells or in the signalling molecule, and whether another molecule such as Tβ4 can induce differentiation into cardiomyocytes. Winter et al. (2009) injected human EPDCs with and without human cardiac progenitor cells marked by Sca-1 into infarcted mouse myocardium and documented improved ejection fraction which is most marked with co-injection. The engrafted cells remained undifferentiated but acted through paracrine channels to increase the vascularity of the infarct area. Di Meglio et al. demonstrated that ischaemia causes mesothelial cells to be lost from the epicardium of adult human hearts while promoting relative accumulation of cells positive for mesenchymal gene products M-cadherein and vimentin in the subepicardium. A subset of EPDCs derived from epicardial mesothelial cells were c-Kit⁺ and migration and transition of EPDCs into c-Kit⁺ cardiac progenitor cells (CPCs) is a possible mechanism of endogenous heart regeneration following MI (Di Meglio et al. 2010). A c-Kit⁺/CD34⁺/CD45⁻ group of EPDCs has also been isolated in human epicardium, although it is uncertain whether they represent the same population as myocardial derived populations or those investigated in the epicardium of ischaemic hearts (Limana et al. 2007).

Islet-1⁺ cardiac progenitor cells

Islet-1 (Isl-1) is a transcription factor of the LIM domain family and a marker of the progenitor cells of the second heart field (SHF). The majority of these cells appear to develop from Isl-1⁺ progenitors, since extensive defects of the atria, right ventricle, and outflow tract occur in mice lacking Isl-1 (Cai et al. 2003). In vivo, Isl-1⁺ cells give rise to a variety of cardiac lineages including cardiomyocytes, endothelial cells, smooth muscle cells and also pacemaker cells (Moretti et al. 2006; Sun et al. 2007). Ex vivo culture induced differentiation to all three cardiac lineages (Moretti et al. 2006), with cardiomyocytes exhibiting mature phenotype with

synchronised $[Ca^{2+}]$ transients (Domian et al. 2009; Laugwitz et al. 2005). After MI, reperfusion induced up-regulation of Isl-1 transcripts points to a possible role in cardiac repair (Genead et al. 2012).

In order for Isl-1⁺ cells to play a significant role in cardiac regeneration of the adult heart, they must be present at this stage. Expression of Isl-1 declines progressively, and relatively few are present at birth (Bu et al. 2009; Cai et al. 2003; Genead et al. 2010), and this has been attributed to the loss of the Isl-1 marker with commitment to cardiac lineage. However, Genead et al. detected Isl-1⁺ cells in the outflow tract and its junction with the ventricle, and the inflow tract of the right atrium in young adult rats, some of which actively proliferate into cardiomyocytes (Genead et al. 2010). Subsequently, Khattar et al. characterised in adult mice two different clusters of Isl-1⁺ cells in mitotically quiescent states: one formed cardiac ganglia, while the other contributed to the cardiomyocyte lineage (Khattar et al. 2011). In order to conclusively characterise these cells in the murine heart, Weinberger et al. examined serial cryosections of adult Isl-1-nLacZ knock-in mice between 10 weeks and 18 months old (Weinberger et al. 2012). Isl-1⁺ cells were found in four regions and additionally expressed markers for smooth muscle, parasympathetic ganglia, cardiomyocytes and pacemaker cells, respectively. However, no undifferentiated Isl-1⁺ cells were found. Isl-1⁺ cells were not found in the ventricular myocardium, and MI did not lead to expansion of Isl-1⁺ cells in the infarct area, making their role in regeneration questionable (Sussman 2012; Weinberger et al. 2012).

Early in the discovery of Isl-1 progenitor cells, Laugwitz and co-workers identified these cells as distinct from c-Kit⁺ and Sca-1⁺ cells (Laugwitz et al. 2005), but more recently Isl-1⁺/c-Kit⁺ cells were reported in foetal and adult human hearts (Fuentes et al. 2013; Serradifalco et al. 2011). Distribution of Isl-1⁺ cells in the human foetus (Bu et al. 2009) matched that of the mouse (Cai et al. 2003; Moretti et al. 2006) and concentrated in the right atrium, the outflow tract as well as the left atrium and atrial appendage. This reflected their embryological relationship to the SHF. Pandur et al. recently reviewed the role of Isl-1⁺ progenitors across a number of species and advocated Isl-1⁺ cells as a common cardiac progenitor for both the SHF and the FHF (Pandur et al. 2013). Studies have found that, in both human embryonic and adult hearts, all progenitor cells expressing Isl-1 also expressed c-Kit, suggesting that Isl-1⁺ cells may be a subpopulation of the c-Kit⁺ population (Fuentes et al. 2013; Serradifalco et al. 2011). The relationship between Isl-1 progenitors and Sca-1 is similarly unclear. Interestingly, Sca-1⁺ cardiospheres also show enrichment in Isl-1⁺ cells (Ye et al. 2012).

The central role of Isl-1 in heart development was investigated as a cause of congenital heart disease but with conflicting results (Luo et al. 2014; Stevens et al. 2010; Xue et al. 2012). Isl-1^{+} cardiac progenitor cells are a population worth further investigation, and studies sourcing a similar population from human embryonic stem cells have been performed (Moretti et al. 2010). Fundamental questions about human Isl-1^{+} CPCs have yet to be answered, including their distribution in both healthy and diseased adult hearts, their relationship with other identified CPCs including c-Kit^{+} cells, and ultimately their therapeutic value in heart failure.

Sca-1^{+} stem cells

Stem cell antigen-1 (Sca-1) is a surface protein of the Ly-6 family (Vanderijn et al. 1989) commonly used to purify mouse haematopoietic stem cells (Holmes and Stanford 2007). Similar to c-Kit, Sca-1 is widely expressed by a range of organs (Holmes and Stanford 2007), and therefore Sca-1 selection enriches the "stemness" of a population without being specific for stem cells. A significant proportion of non-myocytes within the murine heart express Sca-1 (Oh et al. 2003; Rosenblatt-Velin et al. 2005), with a maximal density in the atria and ventricular apex that decreases with age (Saravanakumar and Devaraj 2013). While the original study by Oh et al. (2003) found that Sca-1^{+} progenitor cells were negative for haematopoietic lineage markers CD45, CD34 and c-Kit, different subpopulations were found that were variably positive for CD31, CD38, CD44 (Huang et al. 2011; Meinhardt et al. 2011; Oh et al. 2003; Rosenblatt-Velin et al. 2005), Isl-1 (Takamiya et al. 2011) and even c-Kit (Uchida et al. 2013). Sca-1^{+} CPCs were shown to differentiate in vitro into endothelial cells and also into functional cardiomyocytes after co-culture with cardiomyocytes or oxytocin treatment (Matsuura et al. 2004; Pfister et al. 2005). In vivo, transplanted Sca-1 stem cells differentiate into both endothelial and cardiomyocytes and improve function post-MI (Takamiya et al. 2011; Wang et al. 2006). Subpopulations have differing lineage potential, and Sca-1^{+}/CD31^{−} cells differentiate primarily into cardiomyocytes in vivo (Pfister et al. 2005; Wang et al. 2006), while Sca-1^{+}/CD31^{+} cells do not (Pfister et al. 2005).

Although both infused (Oh et al. 2003) and endogenous (Liu et al. 2013) Sca-1^{+} CPCs homed to the infarct border, in both cases cells show a limited ability to differentiate into cardiomyocytes (Uchida et al. 2013; Wang et al. 2006). Therefore, any improvements in cardiac function conferred by Sca-1^{+} CPCs may occur mainly via a paracrine route (Huang et al. 2011; Matsuura et al. 2009). Treatment with CPCs or CPC-conditioned culture medium prior to MI acutely improved cardiac function, while treatment with differentiated CPCs failed to show any effect. This early phase effect is attributed to preservation of myocardial tissue by reducing apoptosis, although the reported mechanism differs between groups (Huang et al. 2011; Tateishi et al. 2007). Knockdown of Sca-1 expression reduced colony formation and expansion in vitro, and retarded engraftment and proliferation in vivo (Tateishi et al. 2007). Sca-1^{+} cells contribute to myocyte turnover during physiological aging (Uchida et al. 2013) and coordinate differentiation of other precursor cells (Rosenblatt-Velin et al. 2011). The importance of Sca-1^{+} cells in physiological maintenance is underscored by impaired function of c-Kit[+] CPCs, development of dilated cardiomyopathy and impairment of heart function in knock-out mice (Bailey et al. 2012; Rosenblatt-Velin et al. 2011).

No human homolog of Sca-1 exists (Holmes and Stanford 2007). Van Vilet and co-workers used anti-Sca-1 antibody to bind an unknown antigen and identified a group of cells with cardiomyogenic potential from atrial biopsies (van Vliet et al. 2008). They further characterised this population in foetal and adult human hearts and demonstrated more mature phenotype in cardiomyocytes derived from adult Sca-1^{+} cells, which also exhibit less spontaneous contractions than the foetal cells. Adult cells generated more cardiomyocytes and smooth muscle cells, whereas foetal cells preferred endothelial lineage (van Vliet et al. 2010). The method of using Sca-1 to identify human CPCs are still unvalidated. Obvious questions remain as to the exact nature of the epitope target of Sca-1 in humans, and the regenerative potential of these cells.

Side population cells

Side population cells (SPCs) are distinguished by their ability to efflux Hoechst dye due to the presence of membrane ABC transporter proteins (Ding et al. 2010; Zhou et al. 2001). First discovered in the murine haematopoietic system (Goodell et al. 1996), SPCs have since been identified in a number of organs (Ding et al. 2010), including the murine (Hierlihy et al. 2002; Martin et al. 2004; Yoon et al. 2007) and human (Emmert et al. 2013; Meissner et al. 2006; Sandstedt et al. 2012) hearts. SPCs are enriched in Sca-1^{+} cells while being low in CD34, CD45 and c-Kit (Martin et al. 2004; Pfister et al. 2005).

Cardiac side population cells (CSPCs) constitute a complicated mixture of endothelial and smooth muscle cells of the heart, and also promulgate mesenchymal lineage potential (Yamahara et al. 2008). Under co-culture with cardiomyocytes, mouse CSPCs demonstrated myotube formation (Hierlihy et al. 2002), and α-actinin expression (Martin et al. 2004; Pfister et al. 2005), while expression of Nkx2.5 varied with time after isolation and between subpopulations (Martin et al. 2004; Yamahara et al. 2008). Spontaneous contraction in vitro was achieved by some

(Oyama et al. 2007; Pfister et al. 2005), but not others (Yamahara et al. 2008). CD31$^-$/Sca-1$^+$ CSPCs mark a sub-population with cardiomyogenic potential that is the most well studied. Evidence exists for the homing of this subpopulation to the infarct area post-MI, followed by differentiation into all 3 cardiac lineages with limited efficiency (Liang et al. 2010; Oyama et al. 2007). Liang et al. additionally characterized the CD31$^+$/Sca-1$^+$ cells found in cardiac vasculature, and established their capacity to migrate to the ischaemic myocardium and differentiate into endothelial cells (Liang et al. 2011), confirming earlier in vitro studies (Yoon et al. 2007). It is known that, after an MI, there is an acute depletion of CSPCs followed by gradual replenishment by bone marrow-derived SPCs (Mouquet et al. 2005). Whether the small numbers of differentiated cells generated from CSPCs significantly contribute to function is unknown.

There is a gap in the literature with regards to large animal and human studies of CSPCs, and current studies have been limited in their scope. Immunohistochemistry of the human endomyocardial biopsy samples reveal highest levels of CSPCs in the left atria and elevation with ischaemia (Emmert et al. 2013; Sandstedt et al. 2012). Unlike murine CSPCs, these cells did not differentiate into functional cardiomyocytes in vitro. Further research will reveal whether human CSPCs can attain cardiomyogenic potential and improve function in heart failure.

Cardiospheres

Cardiospheres (CSs) can be derived from murine and human hearts as an explant culture of myocardial tissue on poly-D-lysine-coated multiwell plates (Messina et al. 2004). They refer to clusters of small, round, phase bright cells that emerge from the explant and are clonogenic, self-renewing and differentiable into cardiomyocytes, endothelial cells, and smooth muscle cells, both in vitro and in vivo (Davis et al. 2009; Messina et al. 2004). CSs are composed of a mixture of stem cells in their core while progenitor cells, and more differentiated lineage cells aggregate at the periphery to form a niche-like environment (Li et al. 2010). Markers for cardiospheres include those for progenitor cells (c-Kit, Sca-1), endothelial cells (CD31, CD34, CD105, CD133), mesenchymal cells (CD90, CD105) and cardiomyocyte-related proteins (Cx43, Nkx2.5, αMHC) (Davis et al. 2009; Messina et al. 2004; Ye et al. 2012). While murine CSs beat spontaneously soon after formation, human CSs require co-culture with rat cardiomyocyte before they can contract. In ascertaining the origins of CSs, it was posited that contractions may be due to contaminating cardiomyocytes and that CSs form by aggregation rather than expansion (Andersen et al. 2009). These propositions were later refuted by Davis and co-workers, who also

demonstrated that small variations in culture conditions can greatly impact on the phenotype and yield of cardiospheres (Davis et al. 2009).

Smith et al. (2007) used percutaneous biopsies to obtain human myocardial samples and generated cardiosphere-derived cells (CDCs) by plating CSs onto fibronectin-coated plates. CDCs are now commonly used due to the relative ease by which they are produced and expanded (Aminzadeh et al. 2014; Chan et al. 2012; Hsiao et al. 2014; Makkar et al. 2012). CDCs come from a more homogeneous population of cells, mainly of a stromal phenotype with high expression of CD105 along with reduced expression of CD45 (Makkar et al. 2012; Marban and Cingolani 2012). However, after prolonged culture, CDCs also adopt a heterogeneous phenotype (Davis et al. 2009). CDCs can be delivered via an intracoronary route without infarction, whereas CSs require intramyocardial injection (Johnston et al. 2009). They also do not exhibit major histocompatibility class II (MHCII) antigen nor B7 costimulatory molecules, and it is hoped that allogenic CDCs can one day be used as "off the shelf" products in heart failure (Malliaras et al. 2012), a premise investigated in the ongoing ALLSTAR clinical trial (NCT01458405).

Ageing and non-ischaemic cardiac disease impact on the quantity and regenerative potential of CDCs, while transplantation of CDCs may also alleviate the functional decline associated with these conditions. Allogenic transplantation of CDCs in a murine model of dilated cardiomyopathy showed promising results and again hint at the potential of CDCs as an allogenic therapeutic (Aminzadeh et al. 2014). Heart samples from neonatal (<30 days) congenital heart disease patients express three times the level of c-Kit seen in those >2 years of age, with the right atrium showing the greatest proliferative capacity (Mishra et al. 2012). This finding agrees with murine models of ageing, which also show decreased c-Kit, Sca-1, and reduced CDC proliferation, migration and differentiation (Hsiao et al. 2014). Interestingly, Mdx mice, which exhibit some of the phenotype of Duchenne muscular dystrophy, do not experience decline in either quantity or capacity of derived CDCs (Hsiao et al. 2014). In contrast, ventricle-derived CDCs from golden retriever muscular dystrophy (GRMD) dogs have reduced self-renewal and myogenic potential compared to healthy controls (Cassano et al. 2012). Better understanding of the disease process in humans would help resolve these conflicting findings and help transform current treatment.

CSs and CDCs have proven beneficial in ischaemic cardiomyopathy in many murine and porcine studies by increasing cardiac function while minimising adverse remodelling, MI mass and scar size (Johnston et al. 2009; Lee et al. 2011; Shen et al. 2012; Tseliou et al. 2013, 2014; Ye et al. 2012). However, direct differentiation of the stem cells into cardiomyocytes appears to be a minor contributor and generated cardiomyocytes lacked calcium transients (Chimenti

et al. 2010; Malliaras et al. 2013; Shenje et al. 2008; Ye et al. 2012). Instead, paracrine mediators such as VEGF, HGF and IGF1 appear to preserve cardiac function by enhancing cardiomyocyte proliferation, increasing angiogenesis, and decreasing apoptosis (Chimenti et al. 2010; Malliaras et al. 2013; Tseliou et al. 2014). This hypothesis is supported by observations that retention of injected stem cells is less than 1 % at 3 weeks (Aminzadeh et al. 2014; Tseliou et al. 2014). Improving engraftment has been shown to increase functional benefit of stem cells (Cheng et al. 2010). Although CDCs may be more easily prepared, their efficacy compared to CSs have been questioned. A porcine infarct model demonstrated the superiority of SCs over CDCs in improving regional function and ventricular remodelling (Lee et al. 2011). Murine CS culture enhances the expression of c-Kit (taken as a marker of "stemness"), and shows greater improvement in left ventricular ejection fraction (LVEF) than CDCs (Li et al. 2010). The significance of enhanced c-Kit expression in CSs was recently questioned when c-Kit$^+$-purified CDCs exhibited reduced potency both in terms of functional benefit and paracrine secretion compared to the unrefined mixture (Li et al. 2012). The authors suggest that mesenchymal and stromal cells present within the heterogeneous cells present within CSs and CDCs act synergistically with cardiac progenitors cells after exogenous delivery to the injured heart. Therefore, removal of these cells during c-Kit purification leads to decreased paracrine effects of transplanted cells. Nevertheless, CDCs have superior myogenic potency, angiogenesis, and paracrine effects in vitro and in vivo compared to marrow and adipose mesenchymal stem cells, as well as bone marrow-derived mononuclear cells (Li et al. 2012). CDCs from heart failure patients have improved engraftment, and induce greater endogenous repair than equivalent cells from healthy donors (Cheng et al. 2014). Beyond the application of CSs and CDCs, secondary cardiospheres have been cultured from CDCs in 3-dimensional culture and may have an event greater therapeutic effect than CSs (Cho et al. 2012).

The CADUCEUS phase I clinical trial found that intracoronary CDCs transplanted into patients with post-MI ventricular dysfunction significantly reduced scar size but did not improve LVEF or heart failure symptoms. Better understanding of the relative contributions of each subgroup of cells in CS and CDCs will enlighten with regards to their comparative efficacy, and improved outcomes in future studies may result.

PDGFRα^+ progenitor cells

Bone marrow colony-forming unit-fibroblasts (CFU-Fs) are a group of perivascular cells that grow in vitro to form mesenchymal stromal cells (Friedenstein et al. 1970). Perivascular cells resembling CFU-Fs reside in a number of solid organs

(Crisan et al. 2008) and exhibit multipotent differentiation potential, including myogenicity in long-term culture. This population isolated from both murine (Chong et al. 2011) and human (Chong et al. 2013) hearts was enabled by expression of the tyrosine kinase, platelet-derived growth factor receptor α (PDGFRα). Comprehensive lineage tracing and bone marrow transplantation studies have shown these cells to be distinct from their bone marrow-derived counterpart (Slukvin 2011). Interestingly, there is significant overlap in gene expression, differentiation potential and immunophenotype between CFU-Fs from different organs, with important differences related to tissue-specific roles (Pelekanos et al. 2012). It is also possible that cardiac CFU-Fs are a subpopulation of the cardiac fibroblastic pool (Furtado et al. 2014). The distinction between fibroblasts and mesenchymal stromal cells are not well characterised and in vitro they are indistinguishable (Bianco et al. 2008; Hematti 2012). Both cardiac fibroblasts and cardiac CFU-Fs originate from the proepicardium, and undergo EMT before populating the subepicardium and myocardial interstitium where they adopt a perivascular location (Chong et al. 2011; Krenning et al. 2010).

Although murine PDGFRα^+ progenitor cells can differentiate into cardiomyocytes when co-cultured with neonatal rat cardiomyocytes or after transplantation into the infarcted heart, it is unclear whether this cardiomyogenic role is dominant during normal cardiac homeostasis (Chong et al. 2009, 2011). In human hearts, PDGFRα^+ progenitors were predominantly the smooth muscle cells of coronary vessels and interstitial cells throughout the ventricles. Co-expression with troponin-T was rare, suggesting minimal myogenesis (Chong et al. 2013). Interestingly, in hearts from patients with heart failure, PDGFRα^+ cardiomyocytes were more frequent than in hearts from patients without overt heart failure (unpublished data, not shown). Following our previous work, we have recently isolated cardiovascular cells from cryopreserved donor left ventricles from an established tissue bank (see below). Explant culture of this tissue in media containing high serum yields spindle-shaped PDGFRα^+ cells (data not shown). Detailed characterisation of their biological properties and their relationship to other cardiac progenitor cell types is currently ongoing. From a translational perspective, the regenerative potential of these PDGFRα^+ cardiac progenitor cells is currently being tested in small and large animal models of heart failure.

Pluripotent Stem Cells

Another category of stem cells requires mentioning, although detailed discussion is beyond the scope of this review. Human pluripotent stem cells have the ability to form bona fide cardiomyocytes that robustly form spontaneously contracting

syncytia after in vitro differentiation. Resulting cells can be used as a potentially unlimited source of cardiomyocytes for replacement therapy to treat heart failure. Transplantation studies of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) in rodents and non-human primates have shown encouraging results in terms of both regeneration of new myocardium and improvement of cardiac function in infarcted hearts [Shiba et al. 2012; Chong and Murry 2014; Chong et al. 2014]. hESC-CM, however, require further preclinical optimisation and are currently still some time from clinical trials.

Cardiac stem cells and tissue banking

The clinical application of human heart tissue-derived multipotent CPCs requires expansion, cryopreservation, and transportation from the laboratory to the site of cell implantation. Thus, the preferred setting for tissue engineering and regenerative medicine applications is the availability of human samples and cell products. Current studies have relied on the availability of fresh heart samples, often obtained during interventional procedures on diseased hearts. Donor heart tissue is not always available for use and cryopreserved tissue represents an alternative approach. However, there are few published data characterising CPCs isolated from cryopreserved heart tissue.

The approach to isolation and preparation of stem cells with cryopreserved tissue has been successfully demonstrated

in various tissues including human periodontal ligament, cord and adipose tissue (Choudhery et al. 2013, 2014; Seo et al. 2005). In these studies, mesenchymal stem cells (MSCs) isolated from fresh and frozen tissue were capable of differentiating into adipogenic, chondrogenic, osteogenic and neurogenic lineages, and no significant functional differences were observed. Furthermore, the cryopreservation and thawing process of MSCs did not alter the fundamental characteristics (growth properties, phenotypes and gene expression patterns, as well as similar differentiation potential in vitro) of these cells (Mamidi et al. 2012). In contrast to fresh tissue sources, harvesting CPCs from frozen tissue is a non-invasive procedure and poses no risk to the donor.

In order to facilitate utilisation of cryopreserved heart tissue, human bio-repositories containing a sufficient range of failing hearts (dilated, hypertrophic and ischaemic cardiomyopathies) and non-failing hearts is required. These are from hearts procured but not used for orthotopic heart transplantation. The broad spectrum of pre-phenotyped tissue will expedite pre-clinical biological studies. Non-diseased cryopreserved cardiac tissue will provide a readily available source of cardiac progenitor cells for allogeneic cell transplantation albeit with necessary implications of immune suppression. Success using this human cardiac tissue for regenerative studies relies on there being no significant degradation between the last heartbeat and the start of an experiment. All tissues should be frozen within minutes and maintained at liquid nitrogen temperature (−196 °C) to preserve tissue quality
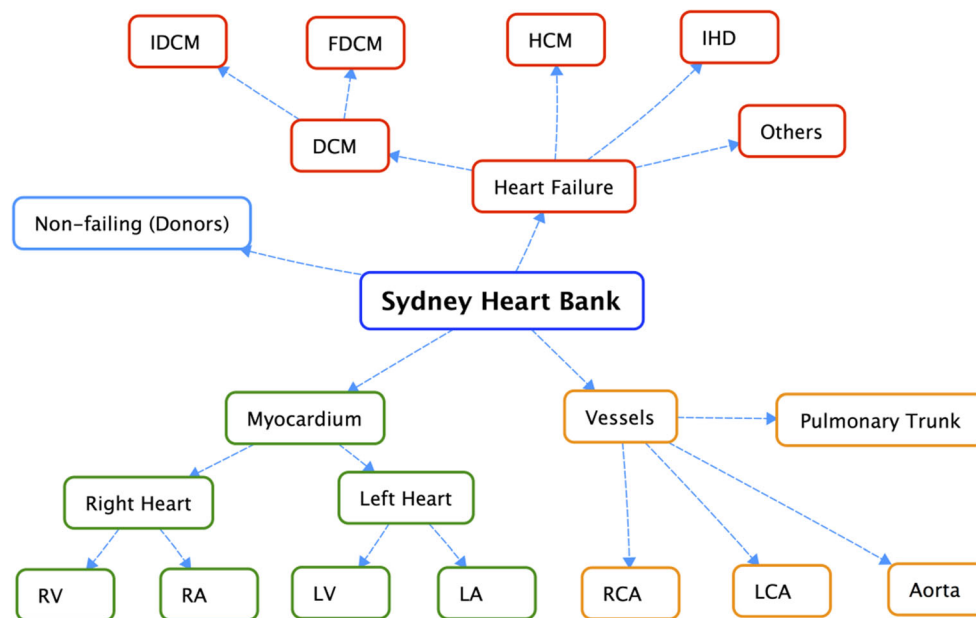
Fig. 2 The Sydney Heart Bank currently contains about 20,000 tissue samples from approximately 600 human hearts. Of these, 190 are from healthy organ donors and the remainder are from failing hearts. The *upper cluster* of the figures shows the donor hearts (*blue*) and the major subsets of failing hearts (*red*). The *lower cluster* provides more detail about the types of tissue (myocardium, vessels) obtained from these hearts. *IDCM* idiopathic dilated cardiomyopathy, *FDCM* familial dilated cardiomyopathy, *DCM* dilated cardiomyopathy, *HCM* hypertrophic cardiomyopathy, *IHD* ischaemic heart disease, *RV* right ventricle, *RA* right atrium, *LV* left ventricle, *LA* left atrium, *RCA* right coronary artery, *LCA* left coronary artery

(intact mRNA, no proteolysis). Under these conditions, our experience (Fig. 2) is that the quality of the samples does not deteriorate even after 20 years of storage. In contrast, tissue stored at −80 °C appears to undergo slow degradation over a period of 6–12 months. Given the merits of such an approach and the large number of successful collaborative projects sourcing from the Sydney Heart Bank (Li et al. 2013), we believe that cryopreserved heart tissue will prove an invaluable resource for future stem cell-mediated therapies.

Conclusion

Although the existence of resident cardiac stem cells has now been validated in a large number of animal and human studies, many questions remain as to their origin, developmental relationships, and influence in heart regeneration. Variation with both age and disease and interindividual diversity poses particular challenge to the resolution of these issues. It is essential that these be addressed as they have direct therapeutic implications.

Over the last decade, attempts to treat patients with various cardiac stem cells from peripheral blood, bone marrow (Fisher et al. 2014) and skeletal myoblasts (Menasche et al. 2008) have produced varied results with no conclusive evidence of clinically significant regeneration. More recently, encouraging results from clinical trials using human cardiac progenitors such as c-Kit^{+} cells (Bolli et al. 2013) and CDCs (Makkar et al. 2012; Takehara et al. 2012) have emerged. An alternative approach to exogenous cell is to stimulate intrinsic mechanisms of regeneration. Biological insights into the varied cardiac progenitor populations will be required for meaningful progress in either of these approaches.

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