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REVIEW

Chemical genetics and its potential in cardiac stem cell therapy

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Over the last decade or so, intensive research in cardiac stem cell biology has led to significant discoveries towards a potential therapy for cardiovascular disease; the main cause of morbidity and mortality in humans. The major goal within the field of cardiovascular regenerative medicine is to replace lost or damaged cardiac muscle and coronaries following ischaemic disease. At present, *de novo* cardiomyocytes can be generated either *in vitro*, for cell transplantation or disease modelling using directed differentiation of embryonic stem cells or induced pluripotent stem cells, or *in vivo* via direct reprogramming of resident adult cardiac fibroblast or ectopic stimulation of resident cardiac stem or progenitor cells. A major bottleneck with all of these approaches is the low efficiency of cardiomyocyte differentiation alongside their relative functional immaturity. Chemical genetics, and the application of phenotypic screening with small molecule libraries, represent a means to enhance understanding of the molecular pathways controlling cardiovascular cell differentiation and, moreover, offer the potential for discovery of new drugs to invoke heart repair and regeneration. Here, we review the potential of chemical genetics in cardiac stem cell therapy, highlighting not only the major contributions to the field so far, but also the future challenges.

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Abbreviations
2-MMB, 2-methoxy-N-(4-methylphenyl) benzamide; ADMET, absorption, distribution, metabolism, excretion and toxicology; BMP2, bone morphogenetic protein 2; BMP4, bone morphogenetic protein 4; c-Myc, myelocytomatosis viral oncogene homologue; CSC, cardiac stem cell; CVD, cardiovascular disease; DMSO, dimethyl sulphoxide; DKK1, dickkopf-related protein 1; EMT, epithelial-to-mesenchymal transition; EPDC, epicardium-derived cells; ES, embryonic stem cells; FDA, Food and Drug Administration; FGF, fibroblast growth factor; iPS cells, induced pluripotent stem cells; Isl1, islet-1; Klf4, Krüppel-like factor 4; LDL, low-density lipoprotein; Lin, lineage; LVEF, left ventricular ejection fraction; MI, myocardial infarction; Oct4, octamer-binding transcription factor 4; RGS4, regulator of G-protein signalling protein 4; SAR, structure-activity relationship; Sca1, stem cell antigen-1; Sox2, sex determining region Y-box 2; SP, side population cells; Tβ4, thymosin β4; TBX18, t-box transcription factor 18; TGFβ, transforming growth factor β; WT1, Wilms' tumour gene 1

Cardiac stem cell therapy: state of the art

Cardiovascular disease (CVD) leading to acute myocardial infarction (MI) and heart failure remains the major cause of morbidity and mortality in the western world and is a growing global health problem. In the UK, someone has a heart attack every 2 min and someone dies from a heart attack every 6 min, which equates to approximately 88 000 people dying in the UK from acute MI per year (British Heart Foundation, 2010). As a result, CVD imposes a great burden on the UK economy with costs estimated at £9.0 billion per year (British Heart Foundation, 2010).

CVD is associated with an acute or gradual loss of functional cardiac muscle cells (cardiomyocytes), compromising cardiac output, leading to pathological remodelling and ultimately heart failure. In adult mammals, the heart is one of

the organs in the body with a reduced capacity for self-repair and regeneration. Consequently, the only long-term solution available for patients is heart transplantation, which is limited by host immune rejection and reduced supply of donor hearts (Augoustides and Riha, 2009). To overcome these current constraints on treatment, there has been intensive effort to develop cell-based strategies for heart repair and regeneration. Such strategies comprise the generation of cardiomyocytes from adult progenitor cells [e.g. cardiac and bone marrow cells (Jackson *et al.*, 2001; Orlic *et al.*, 2001; Toma *et al.*, 2002; Bearzi *et al.*, 2007; Chimenti *et al.*, 2010; Smart *et al.*, 2011)], embryonic stem cells (ES; e.g. Kehat *et al.*, 2001), induced pluripotent stem cells (iPS cells; e.g. Zhang *et al.*, 2009) and, more recently, from reprogrammed fibroblasts (Ieda *et al.*, 2010). So far, these different approaches to promote heart regeneration have had mixed outcomes (recently reviewed by Bollini *et al.*, 2011; Laflamme and Murry, 2011; Vieira and Riley, 2011). For instance, a number of clinical trials have attempted to regenerate heart muscle after MI via the use of bone marrow stem cells (BMSCs), but there is no consensus that these cells can differentiate *in vivo* to give rise to new cardiomyocytes. Indeed, engrafted BMSCs are thought to function in a paracrine fashion to stimulate repair, and perhaps for this reason, the clinical improvement [3–4% increase in left ventricular ejection fraction (LVEF) which is at the margins of clinical detection] has generally been disappointing (Bartunek *et al.*, 2006; Lipinski *et al.*, 2007; Abbate *et al.*, 2008). A more recent phase 1 trial, infusing autologous cardiac stem cells (CSCs) into patients with ischaemic cardiomyopathy, revealed improved functional outcomes up to 1 year post-treatment (Bolli *et al.*, 2011). Specifically, in eight out of 16 patients assessed, LVEF increased by 12.3 ejection fraction units and the infarct size decreased by 30%, when compared with the control group (Bolli *et al.*, 2011). However, the patient numbers in this study were small warranting further phase 2 studies and the mechanism underlying the CSC-induced improvement was unknown (Bolli *et al.*, 2011). Overall, there has been significant variation between trials regarding effects on functional parameters such as LVEF, cardiac wall motion and infarct size, which are widely used to define the success of a clinical trial. However, it should be noted that some studies have recorded improvement in patient outcome despite a relatively small or no improvement on LVEF (Janssens *et al.*, 2006). This illustrates the need for other clinical parameters to be clearly defined to unequivocally assess the success of future trials in improving cardiac function and patient prognosis.

The use of ES and iPS cells has been relatively successful in terms of generating functional cardiomyocytes *in vitro* but they are coupled with ethical (ES cells) and safety issues (ES and iPS cells) and caveats in progressing towards clinical trials. Indeed, the use of human ES cells in phase 1 studies is severely compromised by immune rejection and risk of teratoma formation. The generation of patient-specific iPS cells by retroviral-mediated nuclear reprogramming of somatic cells eliminates some of the problems associated with ES cells technology, including ethical concerns (iPS cells generation does not involve the destruction of human blastocysts), but despite their autologous origin a recent study has suggested that iPS cells may elicit an immune response (Zhao *et al.*, 2011) and there remains a risk of teratoma or tumour formation due to retroviral integration (Robbins *et al.*, 2010). Moreover, both ES and iPS cell-derived cardiomyocytes display an immature phenotype when compared with adult ventricular cardiomyocytes, in terms of their electrophysiological properties, calcium handling, force generation, contractile protein expression and myofibrillar structure (Dolnikov *et al.*, 2006; Binah *et al.*, 2007). Therefore, the use of these cells in regenerative medicine to replace damaged and/or lost muscle in adult disease could increase the risk of arrhythmia and heart failure. Finally, another limitation associated with the generation of ES or iPS cell-derived cardiomyocytes is the low efficiency of the process. ES cells spontaneously differentiate into embryoid bodies (three-dimensional aggregates) in medium containing fetal serum, but only a small proportion of the constituent cells are cardiomyocytes (Kehat *et al.*, 2001). A better understanding of the biological steps involved in cardiogenic differentiation during development has led to the identification of growth factors that can be used to increase the efficiency of ES cells differentiation into cardiomyocytes, for example, activin A and bone morphogenetic protein 4 (BMP4) (Laflamme *et al.*, 2007). Furthermore, the combination of bone morphogenetic protein 2 (BMP2) with a small molecule inhibitor (SU5402) of fibroblast growth factor (FGF) receptor pathways and pharmacological inhibition of Wnt signalling have been shown to enhance cardiogenic fate of human ES cells (Tomescot *et al.*, 2007; Willems *et al.*, 2011b). Likewise, nuclear reprogramming of somatic cells to produce iPS cells occurs at very low kinetics and frequency [e.g. approximately 0.01–0.1% frequency in iPS reprogramming of mouse fibroblasts (Takahashi and Yamanaka, 2006); also reviewed in Hochedlinger and Plath, 2009]. That said, the use of small molecules has greatly enhanced iPS cell yield (Shi *et al.*, 2008), highlighting the potential of chemical genetics in stem cell therapy. Indeed, by using a small molecule combination of a G9a histone methyltransferase inhibitor (BIX-01294) and an L-channel calcium agonist (BayK8644), Shi and co-workers observed an improved reprogramming of Oct4/Klf4-transduced mouse embryonic fibroblasts (7.7 ± 1.5 colonies per 3.5×10^4 cells vs. 0.5 ± 0.7 colony per 3.5×10^4 cells in no compound treatment control), which do not endogenously express the factors essential for reprogramming (Oct4/Sox2/Klf4/c-Myc) (Shi *et al.*, 2008). iPS cell technology also importantly enables the development of patient-specific cellular models of disease (e.g. Carvajal-Vergara *et al.*, 2010; Moretti *et al.*, 2010; Lahti *et al.*, 2011; Malan *et al.*, 2011), which is a powerful tool for gaining insight into the cellular basis of CVD and may assist in developing drugs to reverse the underlying disease pathology. Of note, the epigenetic changes taking place during nuclear reprogramming are still poorly characterized. Therefore, future research should focus on how aberrant epigenetic remodelling may affect downstream applications of iPS cell technology (Djuric and Ellis, 2010). Taken together, the main utilities of human ES and iPS cell-derived cardiomyocytes would appear to be at the level of *in vitro* drug screening, pharmacological profiling of cardiovascular drug regimens and gaining mechanistic insight into the disease process, rather than viable cell transplantation towards heart repair and regeneration (regenerative medicine).

In contrast to cell transplantation, an alternative paradigm exists via the stimulation of resident CSCs or progeni-

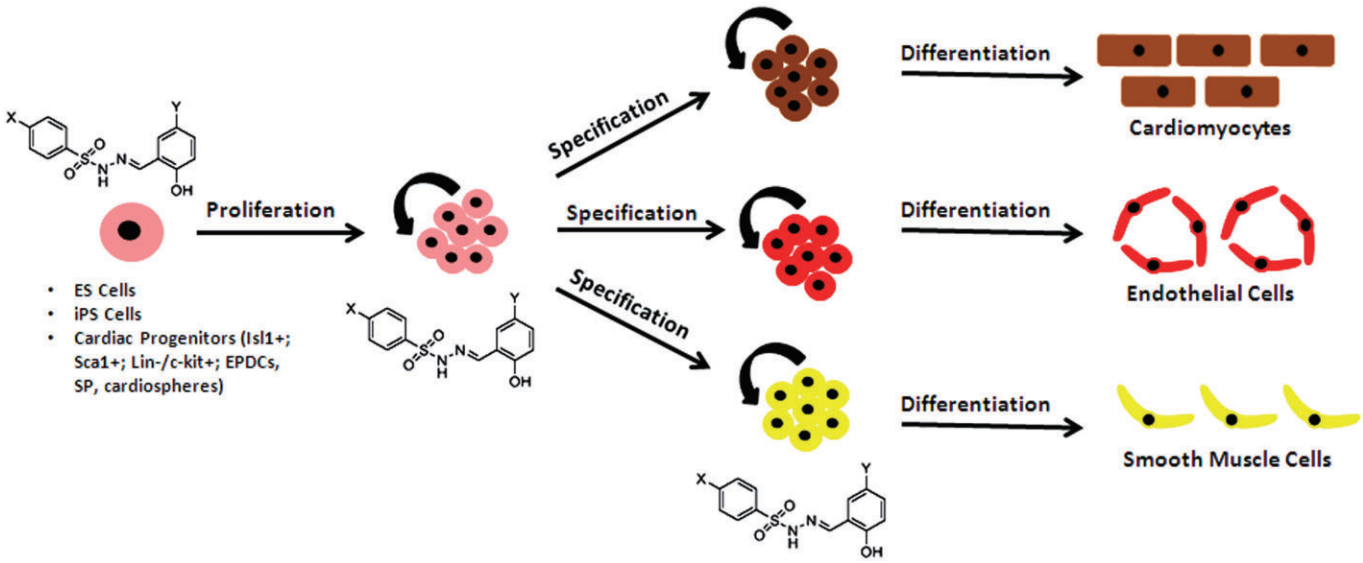

Figure 1

Chemical genetics potential in cardiac stem therapy. ES, iPS and cardiac progenitor's cells are at the present the favoured cell source for replacement of lost or damaged cardiac muscle and coronary vasculature post-ischaemic disease. These cells constitute a perfect platform for small molecule high-throughput screening experiments. Ideally, HTS studies can lead to the identification of small molecules that efficiently stimulate proliferation of these stem/progenitor cells and their self-renewal, specification and differentiation into the different cell lineages required for heart repair, including cardiomyocytes (in brown), endothelial cells (in red) and smooth muscle cells (in yellow).

tors. A number of stem/progenitor populations have been identified within the adult mammalian heart including islet-1 (Isl1)-positive progenitors, stem cell antigen-1 (Sca1)-positive and lineage (Lin)-negative/stem cell growth factor receptor c-kit-positive stem cells, side population (SP) cells and cardiospheres (reviewed by Martin-Puig *et al.*, 2008; Figure 1). In addition to these, the epicardium, a mesothelial cell layer, which covers the surface of the heart, has recently attracted significant attention in terms of regenerative potential (Christoffels, 2011). Epicardium-derived cells (EPDCs) play an essential role during heart development by contributing to cardiac interstitial fibroblasts, vascular smooth muscle cells, and a subset of coronary endothelial cells and cardiomyocytes (Moore *et al.*, 1999; Cai *et al.*, 2008; Zhou *et al.*, 2008) (recently reviewed by Olivey and Svensson, 2010). In the adult heart, the epicardium is mainly quiescent and many genes characteristic of fetal epicardium, such as the *Wilms' tumour gene* (*WT1*) and the *t-box transcription box 18* gene (*TBX18*), are inactive. However, myocardial injury combined with ectopic stimulation by the G-actin monomer binding protein thymosin β4 (Tβ4) re-activates the fetal epicardial gene programme promoting epicardial expansion, EPDC epithelial-to-mesenchymal transition (EMT) and subsequent differentiation into coronary endothelial and smooth muscle cells stimulating neovascularization of the injured hearts (Smart *et al.*, 2010). Remarkably, a small subpopulation of Tβ4-stimulated EPDCs also differentiates into cardiomyocytes, suggesting a potential for heart repair and regeneration (Smart *et al.*, 2011). While a recent study in the mouse, following global knockout of Tβ4, suggested that it is not crucial for survival (Banerjee *et al.*, 2011), this is countered by a previous *in vivo* RNAi knock-down model, which revealed an essential role for Tβ4 in coronary vascular development (Smart *et al.*, 2007), illustrating the more severe instantaneous loss of function with RNAi versus targeting through the germline, which itself can be subject to compensation. That said gain of function, via the ectopic administration of synthetic Tβ4, clearly establishes a potential role for Tβ4 in heart repair. Accordingly, in the absence of Tβ4 stimulation, adult EPDCs adopt a default cell fate and differentiate into mesenchymal cells expressing markers of fibroblasts, myofibroblasts and smooth muscle cells, but not cardiac muscle cells or coronary endothelium (Zhou and Pu, 2011; Zhou *et al.*, 2011a). Moreover, the timing of Tβ4 stimulation post-myocardial injury seems to affect the fate of adult EPDCs, as Tβ4 treatment after MI does not alter epicardial cell fate to include the cardiomyocyte lineage (Zhou *et al.*, 2011b). Of note, EPDCs have been poorly characterized in terms of their antigenic markers and how they relate to other adult cardiac resident stem/progenitor cells (see above). The adult epicardium is thought to be a heterogeneous lineage with the suggestion that approximately 25% of EPDCs in mouse and human are positive for the stem cell marker c-kit (Limana *et al.*, 2007; 2010). Consequently, future studies should aim to rigorously define the inactive versus active EPDCs and to delineate marker signature(s), which underpin lineage potential.

In summary, despite recent advances in regenerative medicine, the (re)generation of cardiomyocytes is an inefficient process in the context of repairing the injured myocardium following ischaemic heart disease and, moreover, clinical trials thus far are not underpinned by direct differentiation to give rise to new muscle in CVD patients. Therefore, further research is a key to gain insight into the molecular pathways that might promote or block tissue regeneration. A chemical genetic approach represents a means to both iden-

tify critical rate limiting steps in cardiomyocyte differentiation and to unlock the regenerative potential of resident cardiac stem or progenitor cells (Figure 1). In the following sections, we will review the current status of chemical genetics with a particular focus on its potential to facilitate cardiovascular regenerative medicine.

Chemical genetics

The term 'chemical genetics' refers to the study of biological systems using small molecules as tools. It can be divided into two categories: forward and reverse chemical genetics (recently reviewed by O'Connor *et al.*, 2011).

Forward chemical genetics is similar to forward genetics, in which researchers go from 'phenotype to genotype', but employs small molecules to assess their potential effect on gene activity, cellular mechanisms or organismal behaviour. The small molecules that produce a phenotype of interest (e.g. a compound that induces gene activation or repression) are then selected, and their structure can be altered in order to enhance their biological response [also known as structure-activity relationship (SAR) studies]. Finally, the critical step in a forward chemical genetic approach is the identification of the biological target responsible for the phenotype of interest (Lokey, 2003). Forward chemical genetics has proved a valuable tool to the field of regenerative medicine; for example, it has been used to unravel tissue regeneration pathways (Mathew *et al.*, 2007) and to screen for new factors with cardiogenic potential (Sadek *et al.*, 2008; Willems *et al.*, 2011b). In contrast, reverse chemical genetics shares similarities with reverse genetics, which operates from 'genotype to phenotype' (O'Connor *et al.*, 2011), whereby the focus is on a specific protein and the identification of compounds from a library that bind and modulated its function. A typical example is the identification of small molecule inhibitors that target specific protein kinases such as 2,6,9-trisubstituted purines, which target the adenosine triphosphate-binding site of the human cyclin-dependent kinase 2, a key regulator of the cell cycle (Gray *et al.*, 1998). The histone deacetylase inhibitor, suberoylanilide hydroxamic acid that targets all 11 human known class I and class II histone deacetylases (Kelly and Marks, 2005), is a further example and one, which has been approved by the US Food and Drug Administration (FDA) for treatment of cutaneous T cell lymphoma (Mann *et al.*, 2007). GPCRs represent a common class of reverse chemical genetic targets and 'hits' include methyl *N*-[(4-chlorophenyl)sulfonyl]-4-nitrobenzenesulfinimidoate (CCG-4986) that specifically inhibits regulator of G-protein signalling protein 4 (RGS4), an important downstream target of activated GPCRs (Roman *et al.*, 2007).

The main components required for a chemical genetic screen are: (i) a large and wide-ranging collection/library of small molecules with both known and unknown biological targets; (ii) an assay to screen the library components; and (iii) a method to trace an active compound to its biological target (Lokey, 2003). The library may include small molecules isolated from natural bioactive products, synthetic natural product-like compounds or a bespoke commercial collection. Significantly, over recent years the majority of drugs approved by the FDA were derived from natural products (reviewed by Newman and Cragg, 2007). Paclitaxel, an approved therapeutic agent for the treatment of lung, breast and ovarian cancer, is a classic example of a drug derived from natural bioactive products and was first isolated from the bark of the Pacific yew tree *Taxus brevifolia* (Wall and Wani, 1996). One important consideration when using a library composed of natural bioactive products in a screen is that the elucidation of the chemical structure (e.g. mass spectrometry, nuclear magnetic resonance spectroscopy, x-ray crystallography) of the compound of interest is fundamental to enable future drug development. In this regard, bespoke commercial collections are advantageous in being composed of high-purity compounds of known structure and function. Furthermore, commercial libraries are typically formatted into multi-well plates, which enables high-throughput screening (HTS) (Lokey, 2003). In general, commercially available libraries include therapeutic drugs approved by the FDA, but can also include compounds that have failed in clinical trials due to toxicity problems. There are approximately 1500 unique small molecules approved by the FDA to date (Knox *et al.*, 2011), which act on approximately 250 known molecular targets (proteins). However, with more than 20 000 protein-coding genes estimated to be present in the human genome, it is clear that the potential 'druggable' targets are much higher in number than previously suspected (Hopkins and Groom, 2002), most likely because a significant number of small molecules have more than one molecular target (Overington *et al.*, 2006). For this reason, the use of commercially available libraries is favoured in chemical genetics and has been adopted in a number of ongoing programmes in drug target development, for example, at the Sanford-Burnham Medical Research Institute at La Jolla, USA (http://www.sanfordburnham.org), or at the Genomics Institute of the Novartis Research Foundation (http://www.gnf.org/discovery/).

Selection of the appropriate small molecule screening method is a further important component of any chemical genetics study. In general, screening assays are based on phenotypic changes and involve the use of living cells or organisms (Lokey, 2003). Cellular phenotypes, potentially affected by small molecules, include cell viability, cell proliferation, cell migration and cell differentiation. Molecular phenotypes can also be assigned in cellular screens such as changes in gene expression. Phenotypes at the whole organism level may include changes in body weight, body size, or tissue remodelling and regeneration. Classically, zebrafish has been used as a model to study tissue regeneration due to their remarkable capacity to regenerate their fins, heart or spinal cord (Poss *et al.*, 2003). Chemical genetics has been a key tool to unravel zebrafish tissue regeneration pathways (Andreasen *et al.*, 2006; 2007; Mathew *et al.*, 2006; 2007), for example, Mathew and co-workers, by screening 2000 biologically active small molecules, identified 17 compounds, including beclomethasone, an agent commonly used for the prophylaxis of asthma, which specifically inhibited tissue regeneration via activation of glucocorticoid receptor signalling (Mathew *et al.*, 2007). Of note, detection of cellular or organismal phenotypic changes is in general time consuming, because it often involves assessment of biological parameters such as measurements of body size (e.g. Mathew *et al.*, 2007). Thus, it is important to consider the automation of phenotypic changes and subsequent high-throughput recording

(reviewed in Stockwell, 2004). Screening by cell imaging can be used at high throughput to assess small molecule-induced changes in cell proliferation, cell size, cell shape, mitotic disruption and protein trafficking (Lokey, 2003; Feng *et al.*, 2009). A frequently used alternative approach is based on small molecule-mediated expression change of reporter gene activity (e.g. luciferase, eGFP or mCherry) under control of a promoter of interest (Sadek *et al.*, 2008; Willems *et al.*, 2011b) (also reviewed by Kawasumi and Nghiem, 2007). The main advantage of this approach is that it facilitates automated screening based on cell imaging and fluorescence (Lokey, 2003). However, reliance on reporter activity to assess the effect on gene expression requires account to be taken of the following: (i) the choice of the promoter region to be used in the reporter construct to faithfully recapitulate the expression of the gene of interest; (ii) the cell type to use in the screening, that is cell line versus primary tissue; and (iii) the origin of the cells used (e.g. rodent vs. human).

Following the initial library screening, compounds producing a phenotype of interest are selected and SAR studies performed to determine if modifications in structure may enhance compound activity and to find whether specific chemical substructures might be associated with a certain biological effect. Also, SAR studies will help to improve the 'drug-likeness' of the selected compounds with regard to their pharmaceutical and pharmacological profile including absorption, distribution, metabolism, excretion and toxicology (ADMET properties). A range of parameters can be used to assess the quality of a compound in terms of its ADMET properties, as proposed in Lipinski's rule of five (Lipinski *et al.*, 2001). Such parameters include calculated properties such as cLogP to estimate lipophilicity, molecular weight, polar surface, ionization (pKa) and solubility to estimate drug absorption (Lipinski *et al.*, 2001).

Subsequently, a secondary screen is performed using the same primary assay. The main aim of these screens is to identify lead 'hits' and to determine any potential toxicity issues (Feng *et al.*, 2009). When performing secondary screens, the selected compounds can be combined with each other to assess possible synergistic actions; likewise, if there are growth factors known to regulate/modulate the phenotype of interest, they can be incorporated into the secondary screens as controls and may also be used in combination with the hits to assess potential modifier functions. Once the phenotypic action of the 'hits' has been confirmed, the next step is the identification of the biological target. Even if the small molecules selected have known function(s) and target(s), it is necessary to determine if the phenotypic event was caused by a known versus previously uncharacterized interaction. Several methods to trace an active compound to its biological target(s) are available (reviewed by O'Connor *et al.*, 2011). Affinity-based techniques, which normally involve immobilization of the small molecule on a glass slide, slide for microarrays, magnetic beads or beads for affinity chromatography, are the most widely employed. The biological target is then purified from a cellular extract or tissue homogenate using the immobilized version of the small molecule and identified by mass spectrometry. As an alternative, small molecules can also be radio-labelled or tagged with biotin, which binds strongly to streptavidin-coated beads, for non-covalent immobilization (O'Connor *et al.*, 2011). Tagging or immobilizing small molecules incurs the risk of affecting the binding to the molecular target; hence, there is a requirement to test the modified small molecule within the primary screen.

Small molecules have proved to be a key in many biological discoveries (Xu *et al.*, 2008); they provide a high degree of temporal control over protein function, inducing rapid activation or inhibition, and their effects are often reversible. The actions of small molecules on biological systems can be finely tuned by varying their concentration, and a single compound can potentially perturb not only a single function of a multifunction protein, but also multiple targets within, or across, protein families and signalling pathways (Xu *et al.*, 2008). In addition, small molecules can disrupt protein-protein interactions and typically function across cell types and species, establishing broad potential for therapeutic development (Kawasumi and Nghiem, 2007). Finally, the observation that several natural and synthetic compounds, including flavonoids and dimethyl sulphoxide (DMSO) have cardiogenic potential has elevated small molecules and chemical genetics as tractable tools for drug discovery within the field of cardiovascular regenerative medicine (Dinsmore *et al.*, 1996; Wu *et al.*, 2004; Willems *et al.*, 2009).

How can chemical genetics help CSC therapy?

The main goal of CSC therapy is to repair and regenerate the human heart. Critical tissue loss following ischaemic injury affects both the cardiac muscle and the coronaries, therefore, stem cell therapy needs to provide not only new cardiomyocytes, but also to stimulate the development of a network of blood vessels to nourish the newly formed muscle (Figure 1). Focusing on muscle regeneration, a number of different strategies have been adopted to generate new cardiomyocytes, including induced differentiation of cardiac progenitors, ES and iPS cells, but with significant caveats around ethics, immunogenicity, low differentiation efficiency and immature derivatives (as reviewed above). An alternative was recently proposed by Ieda and co-workers who generated cardiomyocytes by direct reprogramming of fibroblasts in a more rapid, efficient and stable manner when compared with classic iPS cell reprogramming (20% vs. 0.1%) (Ieda *et al.*, 2010). Instead of using nuclear reprogramming to an iPS cell fate (Takahashi and Yamanaka, 2006), a combination of three master regulator genes (*Gata4*, *Mef2c* and *Tbx5*) was sufficient for direct reprogramming of fibroblasts into cardiac muscle cells (Ieda *et al.*, 2010). These findings still need to be validated in human cells, and the question remains as to whether the reprogramming is efficient enough to replace the 0.5–1 billion cardiomyocytes that are lost on average in the human left ventricle after MI (Murry *et al.*, 2006). On the other hand, the observation that nuclear reprogramming can be achieved with specific cardiac factors suggests that in the future researchers may rely on the use of small molecules, known to activate key molecular pathways centred around these regulators, instead of using retroviral-based approaches, thus circumventing this way the risk of teratoma formation associated with insertional mutagenesis.

Cardiac differentiation from ES or iPS cells is a stepwise process that involves mesoderm induction, mesoderm patterning, cardiac specification and cardiomyocyte formation (reviewed by Willems *et al.*, 2011a). Significant insight into the natural biological signals controlling cardiac differentiation has been derived from a number of studies investigating the nodal/TGFβ, Wnt and BMP pathways, all of which have been manipulated to derive increased yields of cardiomyocytes (Willems *et al.*, 2011a). However, further characterization is required as to how committed cardiac mesoderm is formed, how it differentiates into cardiomyocytes and, finally, how the cardiomyocytes become fully mature. Small molecules are being used to interrogate these unknowns; for example, a family of sulfonyl hydrazones has been shown to activate cardiac mRNA and protein expression in different embryonic and adult progenitor cells, enhancing their capacity for myocardial repair (Sadek *et al.*, 2008). Moreover, a chemical screening approach using human ES cells has led to the identification of several compounds acting at different levels in the cardiogenic pathway (Willems *et al.*, 2011a). One of these compounds, IWR1, inhibits transduction of canonical Wnt signalling within the cell in a highly efficient fashion, driving human ES cell-derived mesoderm to a cardiac fate (Willems *et al.*, 2011b). Natural Wnt inhibitors [e.g. dickkopf-related protein 1 (DKK1)] are already known to direct cardiogenesis in *Xenopus* and human ES cells (Foley and Mercola, 2005; Kattman *et al.*, 2011), therefore, the identification of this compound confirms that pharmacological inhibition of Wnt signalling might act as a therapeutic target in directing human mesoderm down the cardiomyocyte lineage (Willems *et al.*, 2011b).

Another evolving area where the use of small molecules is likely to be beneficial is in disease modelling through the use of human iPS cells. Cardiomyopathy and cardiac channelopathy are two examples that have recently been studied using patient-specific iPS cell models (Carvajal-Vergara *et al.*, 2010; Moretti *et al.*, 2010; Itzhaki *et al.*, 2011; Lahti *et al.*, 2011; Malan *et al.*, 2011). Cardiomyopathies are characterized by insufficient cardiac muscle function leading to congestive heart failure. Different mutations in key sarcomere or myofilament-related genes have been identified and associated with different forms of cardiomyopathies (Dambrot *et al.*, 2011). Cardiac channelopathies are diseases caused by mutations in cardiac ion channels, which impair ion transport leading to changes in action-potential dynamics and, consequently, heart failure (Dambrot *et al.*, 2011). Due to the lack of adequate *in vitro* models, it has been challenging to understand how specific gene mutations can lead to the phenotypes observed in either disease state. The generation of patient-specific models has led to increased insight into new signalling pathways related to hypertrophic cardiomyopathy in LEOPARD syndrome (Carvajal-Vergara *et al.*, 2010), and also provided improved characterization of electrophysiological features of the long-QT channelopathy, such as specific variations in action-potential dynamics (Moretti *et al.*, 2010; Itzhaki *et al.*, 2011; Lahti *et al.*, 2011; Malan *et al.*, 2011). These patient-specific cardiac disease models are now being used as platforms for small molecule screening and drug sensitivity studies, with the ultimate aim of identifying drugs that ameliorate or reverse the disease phenotype. As an example, the long-QT human iPS-derived cardiac-tissue model was used to evaluate the potency of existing and novel pharmacological agents that may either aggravate (potassium-channel blockers E-4031 and cisapride) or ameliorate (calcium-channel blocker nifedipine, potassium$_{ATP}$-channel opener pinacidil and late sodium-channel blocker ranolazine) the disease phenotype (Itzhaki *et al.*, 2011). Noteworthy, a recent phenotype-driven chemical screen in the context of a whole organism (zebrafish) has identified two compounds (out of 1200 small molecules), flurandrenolide and the novel compound 2-methoxy-N-(4-methylphenyl) benzamide (2-MMB), as small molecules that rescue the phenotype of the zebrafish model of long-QT type 2 syndrome (Peal *et al.*, 2011). These two structurally unrelated compounds function as chemical suppressors of long QT by shortening the ventricular action-potential duration through a mechanism that may be dependent on glucocorticoid signalling pathway, because the canonical pure glucocorticoid dexamethasone can also rescue the long-QT phenotype (Peal *et al.*, 2011). Future pharmacological studies are now required for understanding the mechanism(s) of action and potential toxicology of these compounds. Moreover, it will be critical to assess if the benefits of flurandrenolide and 2-MMB can be translated to human long-QT patients, for instance, by using long-QT human iPSC-derived cardiac-tissue models.

Chemical genetics can be applied to unlocking the regenerative potential of adult cardiac progenitor cells, such as EPDCs (reviewed by Vieira and Riley, 2011). EPDCs play an essential role during heart development contributing to different cardiac cell types (as reviewed above). However, shortly after birth, EPDCs become quiescent with an accompanying loss of regenerative potential. Reactivation of adult EPDCs was recently demonstrated by priming of adult mice with Tβ4 followed by induced MI. This combination restored the embryonic lineage potential manifested in coronary vessel development and formation of myocardium (Smart *et al.*, 2007; 2010; 2011). Although Tβ4 provided a critical window into the plasticity of stimulated adult EPDCs, the incidence of cardiovascular derivatives was very low [e.g. 0.59% frequency of *de novo* cardiomyocytes (Smart *et al.*, 2011)] and the differentiation process from the activated epicardial population inefficient. The fact that Tβ4-mediated activation of EPDCs requires preconditioning/priming represents a significant challenge in translating to human therapy, especially if such pretreatment is generic across all prospective 'drug-like' compounds. However, because it is now possible to not only identify people at risk from acute MI, via genetic predisposition and family history of ischaemic heart disease, but monitor the population at large for major risk factors such as elevated blood pressure (hypertension) and high circulating low-density lipoprotein (LDL) cholesterol, there ought to be opportunities to pretreat such individuals. Moreover, patients presenting at the clinic with the first indications of chest pain (angina) offer a therapeutic window for priming of resident EPDCs prior to the onset of MI. That said, the application of chemical screens to adult EPDCs may identify small molecules which function to activate adult EPDCs *after* MI and represents a tractable approach to identify effective small molecules and/or trophic factors that promote a more optimal EPDC differentiation into cardiac muscle and coronary vasculature as an ideal platform for drug discovery (Figure 1).

Future challenges

Within the pharmaceutical industry, conventional drug development costs have recently been estimated at $1.8 billion per launch and this figure is rising rapidly (Paul *et al.*, 2010). The huge expenditure takes into consideration the high costs of drug development (e.g. clinical trials account for almost 40% of the total cost) as well as the resources lost on failed leads. Of note, only 21.5% of the drugs that started phase I trials are approved for clinical use (Paul *et al.*, 2010). Against the backdrop of a slew of blockbuster medicines about to lose patent protection conventional target-based research and development within pharma is gradually making way for phenotypic HTS as a cost-effective process of interrogating large compound libraries towards drug discovery. In this regard, chemical genetics holds great potential. Moreover, the recent identification of adult CSC and progenitor cell populations, which appear tractable in animal models to genetic manipulation, presents an opportunity to screen against multiple small molecule libraries to gain both valuable insight into the molecular pathways controlling heart repair and regeneration, and also to identify novel therapeutic drugs. The main challenge in the application of chemical genetics to drug discovery is to efficiently accelerate the linear pathway to discovery: biological assay development, primary screening to identify compounds of interest, optimization of hits, secondary screening, target identification and, finally, candidate selection for further drug development. One proposal, as far as big pharmaceutical companies are concerned, is to increasingly outsource early-stage drug development, including the phenotypic screen itself to phase I safety trials, to academia or to small, specialist companies (Cressey, 2011). This would leave pharmaceutical companies to focus on their strengths: running large clinical trials and marketing medicines. For the 'specialists', a methodological solution to prevent the drug pipeline running dry might be through the use of combinatorial chemistry (reviewed by Lehar *et al.*, 2008), involving the systematic testing of multiple perturbations either via chemical agents alone or in combination with genetic manipulation perturbations (Lehar *et al.*, 2008). For example, the programme in drug target discovery currently in progress at the University of Oxford, UK (http://www.tdi.ox.ac.uk/home) combines siRNA-mediated knockdown against the druggable genome with small molecule treatment followed by high-throughput tag sequencing to determine how both perturbations affect cellular transcription at a global level. Alternatively, the use of chemical proteomics combined with recent advances in mass spectrometry could also be beneficial to drug discovery in regenerative medicine (Bantscheff and Drewes, 2011). Two notable chemical compounds, SNX-5422 and GSK1210151A, were recently identified by chemoproteomics and are currently progressing towards potential therapeutics in cancer treatment (Fadden *et al.*, 2010; Dawson *et al.*, 2011). Whether the same successes will be forthcoming in cardiovascular stem cell therapy remains to be determined, but given the global CVD patient market and recent academic advances in cardiovascular regeneration, it ought only to be a matter of time before the potential for drug discovery within the field is realized.

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Conflict of interest

The authors have no conflict of interest to declare.

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