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Small Molecules for Cell Reprogramming and Heart Repair: Progress and Perspective

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Abstract

Regenerative medicine for heart failure seeks to replace lost cardiomyocytes. Chemical approaches for producing ample supplies of cells, such as pluripotent stem cells and cardiomyocytes, hold promise as practical means to achieve safe, facile cell-based therapy for cardiac repair and regenerative medicine. In this review, we describe recent advances in the application of small molecules to improve the generation and maintenance of pluripotent stem cells. We also describe new directions in heart repair and regeneration in which chemical approaches may find their application.

PLURIPOTENT STEM CELLS FOR CARDIAC REGENERATIVE MEDICINE

Heart failure is usually accompanied with severe loss of cardiomyocytes, the beating cells of heart tissue.[1] Cell transplantation might be a way to rebuild damaged heart tissue but it requires ample sources of cells.[2] Pluripotent stem cells (PSCs) differentiate into any cell type, including cardiomyocytes, and thus hold tremendous promise for regenerative medicine and heart repair.[3] The therapeutic potential of pluripotent, human embryonic stem cells (ESCs) has long been recognized.[4] Their derivation, however, inevitably involves manipulation of human embryos and thus is controversial.

Takahashi and Yamanaka began a new era of stem cell biology with their revolutionary reprogramming technology. They demonstrated that murine somatic cells can be "reprogrammed" into induced pluripotent stem cells (iPSCs) with a specific set of transcription factors (TFs), namely Oct4, Sox2, Klf4 and c-Myc (OSKM).[5] The same strategy was soon proven applicable to reprogram human somatic cells and the human iPSCs thus generated can differentiate into cells in the three germ layers.[6, 7] The emergence of iPSC technology circumvented the ethical and political controversies associated with human ESCs and provides an exciting potential autologous cell source for cell-based regenerative therapy.[8] Notably, human iPSCs have started to take root in disease modeling and drug development. [9, 10]

Despite its groundbreaking success, the TF-based method to generate iPSCs has significant drawbacks that limit its application in therapies. The involvement of oncogenic TFs and genetic modifications imposes clinically unacceptable risks such as carcinogenicity and genomic instability of iPSCs.[11] In addition, the efficiency and speed of cell reprogramming must be significantly improved to render the process more useful in practice.

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Small molecules are appealing substitutes for genetic materials. The former can exert their cellular effects in a transient and dose-dependent manner, and allow the timing and the magnitude to be precisely controlled and fine-tuned. The essentially unlimited possibilities for structural variations in small molecules allow for ample opportunities to improve their potencies, selectivities, and pharmacological properties. Bioactive small molecules with high specifities can potentially serve as valuable chemical probes to investigate biological processes.[12] In addition, those advantages also renders small molecules particularly suitable for translational development of drugs.

The search of small molecules to improve and/or enable cell reprogramming towards pluripotency has been most fruitful. Progress in this approach has been comprehensively reviewed elsewhere.[13, 14, 15] In this review, we want to focus on the efforts to replace TFs with small molecules to generate iPSCs from somatic cells. We will highlight the insights drawn from the most recent, significant advances in murine and human cell reprogramming. Special attention will be paid to the connections between the molecular functions of small molecules and their roles in establishing pluripotency, as such knowledge will eventually lead to the realization of chemically induced, therapeutically useful human PSCs (hPSCs). The development of chemically defined conditions to maintain hPSCs will also be summarized. Another focus of the review is the applications of small molecules in cardiac regenerative therapy. Chemical approaches to boost the generation and transplantation of cardiac cells derived from PSCs will be highlighted. Potential opportunities for small molecule-based strategies in *in situ* heart repair will also be discussed.

Inducing PSCs with Small Molecules

Although they share essentially identical genomes, PSCs differ from somatic cells most distinctively in gene expression. The identities of the PSCs and all cells are largely established by their gene expression and epigenetic signatures.[16, 17] During reprogramming, somatic cells must undergo significant epigenetic changes (i.e., histone modifications and DNA methylation) to adopt the ESC-like patterns.[18, 19] On the other hand, epigenetic modifications allow for proper changes of the chromatin structure and thus influence the expression of genes crucial for cell reprogramming.[20] Small molecules modulating activities of enzymes involved in epigenetic modifications can, therefore, exert profound effects on cell reprogramming.

Posttranslational modifications to histones are one of the most common epigenetic features. Acetylated histones have generally been associated with transcriptional activation.[21] Histone deacetylase (HDAC) inhibitors presumably help to maintain a high level of acetylation of histones and thus facilitate the expression of pluripotency-related genes crucial for the reprogramming process.[22] As an HDAC inhibitor, valproic acid (VPA) was demonstrated to enhance reprogramming of mouse embryonic fibroblasts (MEFs) [23] in the absence of exogenously expressed c-Myc, which has been known to recruit multiple histone acetylase complexes to the genome and thus presumably converts the chromatin structure of somatic cells to an opened, active state[24] characteristic of PSCs.[25] Although viable, reprogramming under c-Myc-free conditions was inefficient.[26, 27] VPA significantly improved the efficiency of this sluggish process. VPA was also reported to promote the reprogramming of human fibroblast in the absence of Klf4 and c-Myc.[28] Small molecules modulating histone and/or DNA methylations were also used to replace TFs in the reprogramming of somatic cells. BIX-01294, an inhibitor of the H3K9 histone methyltransferase G9a,[29] when used in conjunction with either Bayk-8644 (an L-type calcium channel agonist) or RG108 (a DNA methyltransferase inhibitor), enabled Oct4/Klf4 (OK)-mediated reprogramming of MEFs.[30] BIX-01294 could even compensate the absence of ectopic Oct4 in the Sox2/Klf4/Myc (SKM)-mediated conversion of neural progenitor cells to iPSCs.[31]

During TF-mediated reprogramming, the coordinating orchestra of signal transduction pathways is crucial to establish pluripotency.[32] Small molecules modulating signaling pathways have been identified to enhance reprogramming efficiency or even functionally replace TFs in iPSC reprogramming. Activation of the transforming growth factor-β (TGF-β) signaling pathway inhibits mesenchymal-to-epithelial transition (MET), a cellular process indispensible for induction of pluripotency. [33] During the early stage of reprogramming, murine fibroblasts typically undergo MET and are characterized with the adoption of epithelial-like morphology, upregulation of epithelial genes, such as E-cadherin, and simultaneous downregulation of mesenchymal genes, such as Snail. E-cadherin is also expressed at high levels in human ESCs that resemble epithelial cells.[2] Blocking TGFβ signaling with small molecules may facilitate reprogramming towards pluripotency.[34] SB431542 (a TGFβ signaling inhibitor) and PD0325901 (PD) (an inhibitor of MEK) significantly accelerated the rate and enhance the efficiency of human iPSCs generation. E-616542, a small-molecule inhibitor of TGFβ signaling, can functionally replace Sox2 in MEF reprogramming (Table 1 and 2).[35] TGFβ signaling inhibitors can functionally replaced c-Myc as well as Sox2.[36] More recently, iPSCs were generated from MEFs transduced with Oct4 and treated with small molecules A83-01, a TGFβ receptor inhibitor, and AMI-5, a protein methyltransferase inhibitor (Table 1 and 2).[37]

Manipulation of other signaling pathways has also been beneficial to cell reprogramming. Wnt signaling is important for maintaining pluripotency of ESCs and self-renewal of adult stem cells.[38] CHIR99021 (CHIR), a glycogen synthase kinase 3β (GSK3B) inhibitor, activates Wnt signaling and significantly improve the efficiency of reprogramming of MEFs in the absence of Sox2 and cMyc. [39] Notably, combining CHIR with Parnate, an inhibitor of lysine-specific demethylase 1, they converted human keratinocytes to iPSCs upon ectopic expression of Oct4 and Klf4 (Table 1 and 2). In another study, kenpaullone, a GSK3B inhibitor, functionally replaced Klf4 in the reprogramming of MEFs (Table 1 and 2).[40]

Using cocktails of functionally diverse small molecules to synergistically improve cell reprogramming has been highly fruitful. Zhu *et al.* established an optimized combination of small molecules to accomplish the reprogramming of human adult keratinocytes transduced with Oct4 only. [41] PD0325901 and A83-01 induce pluripotency in neonatal human keratinocytes transduced with Oct4 and Klf4. Further screening identified two compounds, sodium butyrate (NaB) and PS48, that allow Klf4 to be omitted (Table 1 and 2). NaB turned out to be superior to VPA as an HDAC inhibitor. Interestingly, mechanistic characterization of PS48 in reprogramming revealed the metabolic switching from mitochondrial oxidation to glycolysis as a fundamental process during reprogramming. Finally, Parnate and CHIR were included in the cocktail to achieve the O-mediated reprogramming of adult human epidermal keratinocytes.[41]

In one recent report, TFs were completely replaced with chemicals.[42] Based on their previous findings that four small molecules (VPA, CHIR, E-616542, Parnate) enabled the reprogramming of MEF under Oct4 only conditions,[43] the authors sought to replace that last TF with chemical compounds. They found that the combination of the compounds with Forskolin enabled the dedifferentiation of MEFs during the early phase of reprogramming, as indicated by the increased expression of E-cadherin, as well as the pluripotency-related genes Sall4 and Sox2. At the late stage, 3-Deazaneplanocin A, a global histone methylation inhibitor,[44] was added to activate Oct4 expression and furnish the fully reprogrammed cells. In this study, 0.2% of the starting MEFs were converted into iPSCs (Table 1 and 2).

Facilitating hPSC Maintenance

hPSCs can potentially supply an unlimited number of cardiomyocytes for cell-based therapy.[45] Ever since hESCs were first established in 1998,[2] tremendous efforts have been

dedicated to improving the culture conditions for these delicate cells. [46] In the early developed culture conditions, sophisticated media containing mouse feeder cells and/or xenogeneic components were used.[2] Subsequently, modified conditions still involved ill-defined, expensive human feeders and/or serum derived-components.[47] Better-defined, feeder free conditions were later developed.[48, 49] hPSCs are intrinsically prone to apoptosis upon cell dissociation, representing a major hurdle for their preparation and manipulation. hESCs could be maintained in a healthier state by treatment with Rho-associated protein kinase (ROCK) inhibitors, such as Y-7632 and Fasudil.[50] Vitamin A promotes self-renewal of human ESCs in feeder-free conditions.[51] Studies from this lab further unveiled an adhesion signaling pathway regulating hPSC survival and pluripotency. [52] By high-throughput phenotypic screening, two novel small molecules were identified that promoted hESC survival after trypsin dissociation, namely, Thiazovivin (Tzv) and Pyrintegin (Ptn). Target identification revealed that Tzv inhibits ROCK and thus stabilizes E-cadherin and enhances cell-cell interaction. ROCK inhibitors have been incorporated into simplified, chemically defined conditions for the culture of hPSCs.[53, 54]

CARDIAC DIFFERENTIATION AND GRAFTS

Improving Cardiac Differentiation

The *bona fide* cardiomyogenic differentiation potential of human PSCs has long been recognized.[55, 56] Human PSCs differentiate into cardiomyocytes only when guided by appropriate extrinsic influences. Substantial efforts have been made to develop conditions that induce cardiac differentiation from human PSCs in an efficient, reproducible and simple manner. Approaches using small molecules will be discussed in the following sections. Phenotypic cell-based screening has been applied to discover small molecules that promote cardiac differentiation of mouse or human PSCs. Ascorbic acid was among the earliest chemicals identified to increase cardiogenic differentiation of ESC (Table 3).[57] It also rescues cell line–dependent cardiogenic deficiency of iPSCs.[58] The applications of high-throughput screening also led to discoveries of other cardiogenic small molecules, including cardiogenols,[59] isoxazolyl-serine-based agonists of peroxisome proliferator-activated receptors (PPARs), [60] verapamil, [61] SB203580, [62] sulfonylhydrazones,[63] and cinchona alkaloid derivatives (Table 3).[64] While active, cardiogenic small molecules discovered in phenotypic screening assays will continue to serve as immensely useful tools, efforts to elucidate their cellular targets, although challenging, will shed more light on the biology underlying cardiac differentiation.

Aiming to recapitulate embryonic cardiac development, chemical approaches that systematically target the core signaling pathways involved in each step of cardiogenesis turned out to be extremely successful. Embryonic cardiac development is a well-organized process, involving the sequential formation of mesoderm, cardiac progenitors and cardiomyocytes.[3] This stepwise process is finely regulated by multiple signaling pathways. Precise signaling control with appropriate timing using small molecules is critical to the success of chemically guided cardiac differentiation. A number of small molecules that selectively target BMP,[65] TGFβ,[66, 67] and Wnt, [68, 69, 70] when applied at appropriate time-window, enabled the efficient cardiac differentiation of PSCs, especially those of the human origin. Knowledge gained from these discoveries, in turn, enriches our understandings about the logic of cardiac development.

Chemically Defined Conditions for Cardiac Differentiation

The identification of robust cardiogenic chemicals that increase differentiation efficiency and replace complex, ill-defined components (i.e., serum, growth factors, hormones, and extracellular matrix) has allowed for a simple, reliable and cost-effective protocol to

chemically induce cardiac differentiation from PSCs. Gonzalez *et al*. designed a stepwise protocol to generate cardiomyocytes from hESCs (>50% efficiency) in serum and growth factor-free conditions. [71] By systematic screening of 300 known signal transduction modulators, they identified IWR-1-endo (Wnt antagonist), purmorphamine (Sonic Hedgehog signaling agonist), and SB431542 as small molecules that promote differentiation of hESCs into cardiomyocytes (Table 3). Although the system they used was not completely chemically defined (i.e., using MEF conditioned medium and Matrigel), this study demonstrates that exogenous growth factors can be replaced by small molecules for efficient cardiac differentiation. Recently, Lian *et al.* developed a chemically defined cardiac differentiation system. [72] They showed that timely modulation of Wnt signaling (activated by CHIR during the first 24 hours and then blocked by IWP-2 or IWP-4 during days 3–5) is sufficient and necessary for efficient cardiac induction of hPSCs (up to 98% efficiency) under defined, growth factor–free conditions (Table 3). Similarly, Minami *et al.* identified a potential Wnt inhibitor KY02111 that, when used in combined with other Wnt modulators, induced robust cardiac differentiation of hPSCs (up to 98% efficiency) in a xeno-free medium devoid of serum, recombinant cytokines or hormones (Table 3).[73] While the robustness and reproducibility of these protocols need to be tested on more cell lines and may require further modifications, these significant advancements paved the way to safe, efficient and cost-effective protocols for *de novo* cardiomyocyte production from hPSCs on a clinically relevant scale.

Reducing Heterogeneity of Cardiomyocytes

Despite the great progress in developing efficient and defined methods for cardiac differentiation of PSCs, methods are still lacking that enrich a specific subtype cardiomyocyte, such as atrial-, ventricular-, or nodal-like cells. Common hPSC differentiation methodologies give rise only to a mixture of all three major subtypes of cardiomyocytes.[3] A heterogeneous cellular composition, unfortunately, hampers its utilization in medical research and cell-based therapies. In recent years, several breakthroughs in the field of selective cardiac differentiation conditions were made using the pharmacologic approaches. Kleger *et al.* found that 1-ethyl-2-benzimidazolinone (1-EBIO), an agonist of Ca^{2+}-activated potassium channels, induces cardiogenesis of murine ESCs and strongly enriches nodal-like cells (from 7.2 to 57.8%) (Table 3).[74] By using direct action potential phenotyping, activation of genetic label, and subtype-specific marker expression, Zhu and colleagues demonstrated that NRG-1β/ERBB signaling regulates the ratio of nodal- to ventricular-type cells in hESC-derived cardiomyocytes. Inhibition of NRG-1β/ERBB signaling by its antagonist AG1478 significantly enriched the nodal-like cells (21 to 52%) (Table 3).[75] Similarly, Zhang *et al.* found that retinoic acid (RA) signaling regulates atrial versus ventricular specification during the cardiac differentiation of hESCs. When the RA receptor antagonist BMS-189453 was added to cultures, 83% of the cardiomyocytes showed ventricular-like features, whereas 94% of the cells displayed atrial-like phenotypes when RA was applied (Table 3).[76] Overall, these findings highlight the potential of small-molecule-based approaches in directed differentiation of PSCs into specific cardiac subtypes. To efficiently and selectively generate cardiomyocytes of high qualities, it is necessary to further elucidate the mechanisms of cardiac subtype specification and to identify additional chemicals that further improve these methods.

Improving Cardiac Grafts

Besides the production of sufficient amounts of cardiomyocytes of high quality, the integration of transplanted cells into tissues imposes a challenge of no less significance. Several encouraging studies have engrafted and integrated hESC-derived cardiomyocytes into rodent and pig hearts.[77, 78] Nevertheless, most cardiomyocytes were lost shortly after transplantation. A large proportion of the cells remaining in the infarcted myocardium also

underwent programmed cell death soon afterwards.[79, 80] The poor survival of transplanted cells represents a major obstacle for the delivery of long-term value of PSC-derived cardiomyocytes to regenerative therapy.

Attempts with small-molecule approaches to improve graft survival of cardiomyocytes have been conducted. Survival improved in transplanted cells pre-treated with diazoxide, a drug that opens mitochondrial ATP-dependent potassium channels (mitoKATP) in a myocardial infarction (MI) model (Table 3).[81] A small molecule pioglitazone, an activator of PPAR-γ signaling, significantly enhanced the viability of transplanted mesenchymal stem cell-derived cardiomyocytes in experimental animals (Table 3).[82] Moreover, small-molecule inhibitors of the Rho-associated kinase[83] and p38 MAPK[84] improve the survival rate of cells before and/or after transplantation.

Besides small molecules, growth factors such as IGF1,[85] TGFβ2,[86] and erythropoietin[87] were also brought into play to protect transplanted cells. Laflamme *et al.* designed a 'pro-survival cocktail' consisting of Matrigel, IGF1, a Bcl XL peptide (to block mitochondrial death pathways), pinacidil (to open mitoKATP), peptide cyclosporin A (to attenuate cyclophilin D–dependent mitochondrial pathways) and the caspase inhibitor ZVAD-fmk (Table 3).[77] Employing this combination of multiple pro-survival factors, 7-fold increase in graft size in a rat MI model was achieved.[78] Future efforts to further enhance graft survival will most likely involve searching for novel combinations of small molecules and pro-survival factors as well as other strategies, such as pre-conditioning, immunosuppressing, and bioengineering.

IN SITU HEART REPAIR AND REGENERATION

While addressing challenges that cell transplantation therapy is currently faced with, researchers have sought to develop new strategies for *in situ* heart repair and regeneration. The advantages and drawbacks of a variety of therapeutic strategies for cardiac regenerative medicine are listed in Table 4. Progress in emerging, promising strategies in this direction will be the focus of the following sections. Potential applications of small-molecule approaches in these strategies will be tentatively suggested.

Cardiomyocyte Dedifferentiation and Proliferation

During mammalian embryonic development, the heart grows through the proliferation of cardiomyocytes but switches to hypertrophic growth soon after birth. As cardiomyocytes exit the cell cycle at this point, further increases in cardiac mass are mainly due to the increase in cardiomyocyte size instead of number.[88] In contrast to hearts of some lower organism, such as zebrafish, which have a robust regenerative response upon injury mainly through cardiomyocytes dedifferentiation and proliferation, an adult mammalian heart typically has extremely limited renewal capacity and is incapable of restoring the damaged myocardium after injury. [89] Nonetheless, mammalian cardiomyocytes can slowly self-renew and turnover under physiological condition,[90, 91] presumably through the division of pre-existing cardiomyocytes instead of differentiation of residue progenitor cells,[92] even although this multiplication capacity is clearly not sufficient to repair a damaged heart. Genetic[93, 94] and pharmacological[95] strategies to enhance the intrinsic renewal capacity have improved cardiac function after infarction. With the rapid progress in stem cell biology and high-content screening platform, systematic screenings have been performed to identify chemicals inducing cardiomyocyte proliferation.[96] Future efforts to continually identify novel small molecules that robustly induce dedifferentiation and proliferation of cardiomyocytes will be of great value to fulfill the potential of this regenerative strategy.

Direct Cardiac Conversion of Non-Myocytes

The establishment and advances in iPSC technology have re-galvanized research on direct reprogramming somatic cells from one lineage into another without entering the pluripotent stage, a process conventionally known as transdifferentiation.[14] Transdifferentiation of endogenous or explanted fibroblasts represents a fascinating, novel regenerative approach. Fibroblasts account for up to 50% of all cells in an adult human heart. After cardiac injury, fibroblasts are hyper-proliferated and lead to fibrosis and scar formation within the damaged area. [97] Properly reprogrammed into cardiomyocytes, the ample fibroblasts can serve as an attractive cell source to replenish the myocardial muscle. Proof-of-principle demonstration of the successful cardiac reprogramming of fibroblasts has been achieved by ectopic induction of multiple cardiac-enriched transcription factors (Gata4, Mef2c and Tbx5). [98] On the other hand, Efe *et al.* established the cell-activation and signaling-directed (CASD) cardiac reprogramming, which involves transient expression of the Yamanaka factors in conjunction with cardiogenic signal simulation.[99] The success of CASD lineage conversion reveals a common paradigm for both transdifferentiation and reprogramming towards pluripotency. Cardiogenic transdifferentiation was also accomplished by transfection of cardiac fibroblasts with microRNAs (miR-1, miR-133, miR-208 and miR-499).[100]

Impressively, *in vivo* delivery of reprogramming factors into infarcted mouse hearts regenerated the post-infarcted, damaged myocardial muscle *in situ* by converting resident cardiac fibroblasts into cardiomyocytes.[100, 101, 102] The induced cardiomyocytes generated in their native environment displayed mature, adult-like phenotype and improved heart function.[101, 102] Evidence of electrical coupling to the host myocardial tissue was also observed.[101] Despite the encouraging results observed, several safety concerns need to be addressed, including the viral delivery of transgenes and partially reprogrammed cells that potentially disturb the cardiac rhythm. Small molecules that can avoid the usage of transcription factors and/or enhance the *in situ* transdifferentiation will have tremendous impact on the successful translation of this attractive strategy from bench to beside.

Activation of Endogenous Cardiac Progenitor Cells

The existence of resident cardiac progenitor cells (CPCs) in the adult rodent and human heart has been well demonstrated over the last decade.[103] Although many efforts have been made on the identification, *in vitro* expansion and subsequent differentiation of these cells, little is known about their roles and behaviors within the naïve heart niche under physiological and pathological conditions. In view of their well-characterized cardiogenesis potential both *in vitro* and *in vivo*,[103] it is reasonable to envisage a CPC-based therapy that allows the proper mobilization of resident CPCs to replace the lost or damaged cells *in situ*, avoiding the problems of limited graft survival, restricted integration to the host tissue, and potential immune rejection. The feasibility of this approach has been established in recent years. Using genetic fate mapping, Loffredo *et al.* demonstrated that exogenously delivered bone marrow–derived cells could stimulate resident CPCs and promote the endogenous cardiomyocyte refreshment.[104] Remarkably, Smart *et al.* described that thymosin β4, a known pro-angiogenic peptide, mobilized an epicardial origin of progenitor population, induced concomitant cardiac differentiation and regeneration of myocardial tissue, and ultimately improved heart function post-infarction.[105] Similarly, Zangi *et al.* found that intramyocardial injection of synthetic modified RNA encoding human VEGF-A resulted in the expansion and directed differentiation of endogenous CPCs, and markedly improved heart function in a mouse MI model.[106] An attempt to target CPCs using small molecules has recently been reported.[107] Russell *et al.* found that a 3,5-disubstituted isoxazole, Isx1, could activate cardiac genes expression in residential, multipotent Notch-activated epicardium-derived cells *in vivo* and induced the generation of CPCs (Table 3).[107] Unfortunately, MI abrogated Isx1's cardiogenic effects and led to fibrosis.[107] Nonetheless,

the possibilities to develop novel small-molecule tools to achieve safe, robust and cost-effective activation of CPCs for heart regeneration are undoubtedly alluring.

CONCLUSIONS AND OUTLOOK

The discovery and improvement of iPSC technology, as well as the development of efficient cardiac differentiation system offer tremendous hope for novel cell replacement therapies to improve cardiac function in compromised individuals. The eventual success of complete small-molecule-based reprogramming through activation of endogenous expressions of genes enabling pluripotency will greatly propel the realization of the clinical potentials of iPSCs.

Multiple concerns still hinder the applications of cardiac cell transplantation therapy, including insufficient quantities and qualities of cardiomyocytes, ineffective delivery and retention, acute graft death and rejection. The tremendous potentials of small molecules to address these issues have been well recognized. Since a broad spectrum of small molecules have been identified that can replace factors during iPSC generation and facilitate the transition of partially reprogrammed cells into ground state pluripotency,[41] it may be possible to eliminate the risk associated with editing of host genome by viral genes, increase the overall efficiency of cardiac reprogramming, and improve the functional integrity of induced cardiomyocytes using pharmacological approaches. The *in vitro* generated cardiomyocytes might be further engineered pharmacologically and serve as suitable materials for direct transplantation. Methods for temporal- and spatial-controllable *in vivo* delivery of small molecule must be developed to achieve their therapy values. Hopefully, drugs that facilitate the transplantation can be developed based on small molecules that improve the survivals and functions of transplanted tissues.

In contrast to the significant achievement of small molecules approaches made on modulating cardiac cell fate and function, their potential in *in situ* heart repair is yet to be explored. The possibilities to convert resident non-cardiomyocytes into myocardium, activate and/or enhance the intrinsic regenerative capacity of cardiac cells by pharmacologic means will provide alternative, fascinating options for regenerative therapy. Towards these ultimate goals, high-throughput screening will continue to serve as a powerful strategy to discover more novel chemicals with desired properties. Applications of combinations of small molecules to garner their synergistic effects have already proven advantageous. Efforts will be continuously made to search for the optimal cocktails for specific therapeutic purposes. Last but not least, better understanding of these cell reprogramming and developmental processes will ultimately benefit stem cell biology as well as regenerative therapy.

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KEY WORDS

Small Molecule	a defined chemical entity, often an organic compound with a molecular weight smaller than 900 Daltons

PSC	pluripotent stem cell, a stem cell possessing the potential to differentiate into all cell types in the body
Cell Reprogramming	the artificial conversion of one particular cell state and/or fate into another, often referring to the generation of stem cells from more differentiated cells
TF	transcription factor, a protein, either on its own or in complex with other proteins, which binds to specific DNA sequences and thereby controls the transcription of genes
Signaling Pathway	the relaying of signals among a group of molecules which ultimately triggers cellular responses
Differentiation	a cellular process in which stem cells become more specialized cell types
Transdifferentiation	the conversion of one type of somatic cells into another cell type without passing through the pluripotent state
Cardiac Regeneration	a process to replenish lost myocardial tissues and restore cardiac function in post-injured hearts

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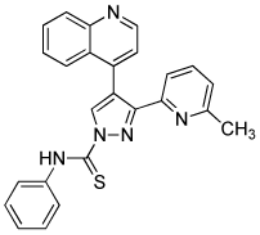
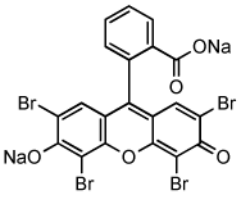
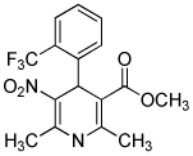
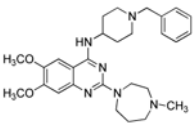
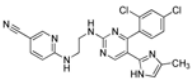
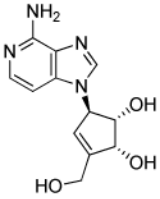
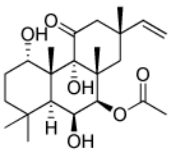
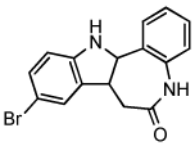
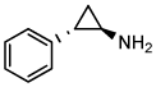
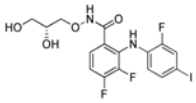
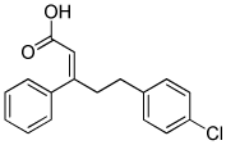
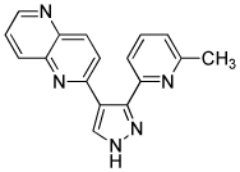
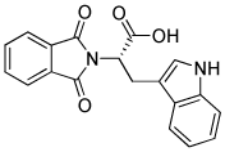
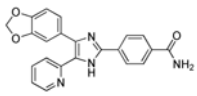
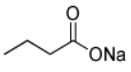
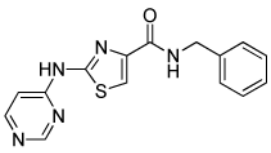
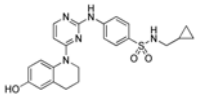
Table 1

Representative Small Molecules Used to Reprogram Cells and Maintain hPSCs

Name	Structure	Known function(s)
A83-01		TGFβ receptor ALK4/5/7 inhibitor
AMI-5		Protein arginine N-methyltransferase inhibitor
(±)BayK 8644 (BayK)		L-type calcium channel agonist
BIX-01294 (BIX)		Histone methyltransferase G9a inhibitor
CHIR99021 (CHIR)		Glycogen synthase kinase 3β inhibitor
3-Deazaneplanocin A (DZNep)		Histone methylation inhibitor
Forskolin		PKA activator

Name	Structure	Known function(s)
Kenpaullone		GSK3 and CDK1/cyclin B inhibitor
Parnate		Lysine specific demethylase 1 inhibitor
PD0325901 (PD)		MEK inhibitor
PS48		PDK1 activator
E-616452		TGFβ Receptor I kinase inhibitor
RG108		DNA methyltransferase inhibitor
SB431542		Activin receptor-like kinase 4/5/7 inhibitor
Sodium butyrate (NaB)		Histone deacetylase inhibitor
Thiazovivin (Tzv)		ROCK inhibitor
Pyrintegrin (Ptn)		Unknown

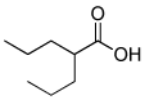
Name	Structure	Known function(s)
Valproic acid (VPA)		Histone deacetylase inhibitor

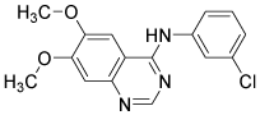
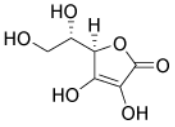
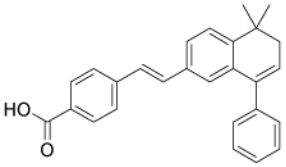
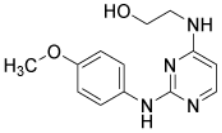
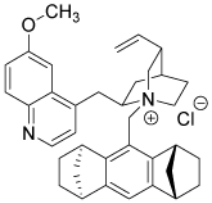
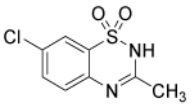
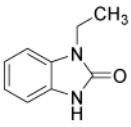
Table 2

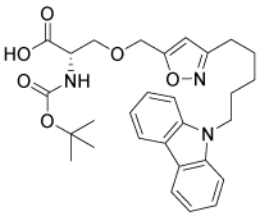
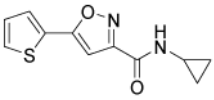
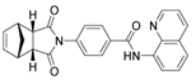
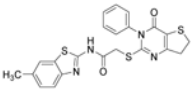
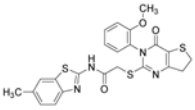
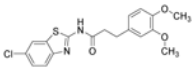
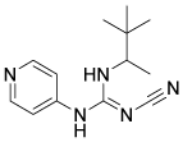
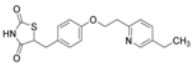
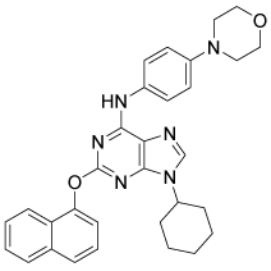
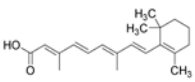
Small Molecules Replacing TFs in the Reprogramming of Somatic Cells

Small molecules combination	Starting cells	TFs required	Ref.
VPA	MEFs	OSK	Huangfu *et al.*, 2008
BIX, BayK or RG108	MEFs	OK	Shi *et al.*, 2008
Kenpaullone	MEFs	OSM	Lyssiotis *et al.*, 2009
E-616542	MEFs	OKM	Ichida *et al.*, 2009
A83-01, AMI-5	MEFs	O	Yuan *et al.*, 2011
VPA, CHIR, E-616542, Parnate	MEFs	O	Li *et al.*, 2011
VPA, CHIR, E-616542, Parnate, Forskolin, DZNep	MEFs	none	Hou *et al.*, 2013
VPA	Primary human fibroblast	OS	Huangfu *et al.*, 2008
CHIR, Parnate	Human keratinocytes	OK	Li *et al.*, 2009
NaB, A83-01, PS48, PD	Neonatal human epidermal keratinocytes	O	Zhu *et al.*, 2010

Table 3

Representative Small Molecules Enhancing Cardiac Differentiation, Graft Integration, and Heart Regeneration

Name	Structure	Molecular Function(s)	Ref.
AG1478		EGFR tyrosine kinase inhibitor	Zhang *et al.*, 2011
Ascorbic acid		Multiple	Takahashi *et al.*, 2003 Cao *et al.*, 2012
BMS-189453		Pan-retinoic acid receptor antagonist	Zhang *et al.*, 2011
Cardiogenol C		Unknown	Wu *et al.*, 2004
Cinchona alkaloid derivative		Unknown	Berkessel *et al.*, 2010
Diazoxide		ATP-sensitive activator K^+ channel	Niagara *et al.*, 2007
1-EBIO		Agonist of Ca^{2+}-activated K^+ channels	Kleger *et al.*, 2010

Name	Structure	Molecular Function(s)	Ref.
Isoxazolyl-serine derivative		PPAR agonist	Wei *et al.*, 2004
Isx1 (isoxazole)		Cardiac muscle gene activator. Neuronal reporter genes activator.	Russell *et al.*, 2013
IWR-1-endo		Wnt inhibitor	Gonzalez *et al.*, 2011
IWP-2		Wnt inhibitor	Lian *et al.*, 2012
IWP-4		Wnt inhibitor	Lian *et al.*, 2012
KY02111		Wnt inhibitor	Minami *et al.*, 2012
Pinacidil		ATP-dependent K^+ channel opener	Laflamme *et al.*, 2007
Pioglitazone		PPAR-γ activator	Shinmura *et al.*, 2011
Purmorphamine		Sonic Hedgehog signaling agonist	Gonzalez *et al.*, 2011
RA		Natural ligand of RA receptors	Zhang *et al.*, 2011

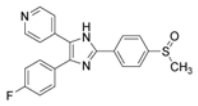
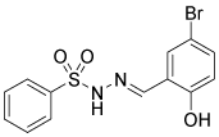
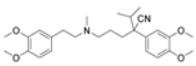
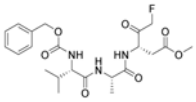
Name	Structure	Molecular Function(s)	Ref.
SB203580		p38 MAPK inhibitor	Graichen *et al.*, 2008
Shz-1 (sulfonylhydrazone)		Activator of gene *Nkx2.5*	Sadek *et al.*, 2008
Verapamil		L-type Ca^{2+} channel blocker	Sachinidis *et al.*, 2006
ZVAD-fmk		Caspase inhibitor	Laflamme *et al.*, 2007

Table 4

Comparison between PSC-based Cardiac Cell Therapy and *in situ* Heart Regeneration

Issues	**PSC-based Cardiac Cell Therapy**	***in situ* Heart Regeneration**
Therapeutic mechanisms	Replacing the damaged myocardium through transplantation of *in vitro* generated cardiac cells into the heart	Modulating the heart's own regenerative response by simulating or reprogramming endogenous cells
Cell sources	Theoretically unlimited amounts. Well-controlled cell type and quality.	Cell type, quality and amounts typically restricted and context-dependent.
In vitro bioengineering	Applicable	Not applicable
Cellular maturation	Fetal or neonatal cardiomyocytes-like features	Often adult cardiomyocyte-like features
Risk of tumor formation	Possible due to residue pluripotent cells	Possible due to modifying host genome by transgenes and uncontrollable transgene expression
Risk of immune rejection	Possible but ameliorable with iPS technology	Unlikely
Risk of arrhythmias	Possible due to autorhythmicity, immaturity and inorganization of graft cells	Possible due to potentially unpredictable and incomplete reprogramming
Graft survival and host-graft integration	Challenging	Not necessary
Ease of implementation	Low	Relatively high
Cost	Relatively high	Relatively low