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Recent Progress on Chemical Biology of Pluripotent Stem Cell Self-renewal, Reprogramming and Cardiomyogenesis

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Abstract

Pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, hold great promise as a cell source for regenerative therapies to treat many major diseases characterized by an irreversible loss of functional tissues. However, the future clinical application of pluripotent stem cells faces a number of obstacles regarding the safety, efficiency and long-term benefits. Some of these challenges are being addressed by the chemical biology approach using small molecules. In this paper, we review the recent progress and patents on small molecules which promote pluripotent stem cell maintenance, reprogramming, and direct differentiation with a focus on cardiomyogenesis.

Keywords

Cardiomyogenesis; chemical biology; differentiation; iPS cells; pluripotent stem cells; small molecules

INTRODUCTION

Irreversible loss of functional tissue cells is a hallmark of major human diseases, including heart failure, diabetes, Alzheimer's and Parkinson's disease. Currently there are no definitive treatments for these diseases except for organ transplantation. In recent years, stem cell-based regenerative therapy has attracted much attention as a potential alternative approach to treat these diseases by replacing the damaged cells with stem cell-derived healthy ones. Multipotent stem/progenitor cells, such as the bone marrow-derived stem cells (BMSCs) and mesenchymal stem cells (MSCs), which are capable of giving rise to multiple cell lineages, have been extensively evaluated as a potential source of autologous replacement cells. However, beneficial effects of these cells on restoring the damaged organ's functions are often limited and transient, and there is still little evidence that adult stem cells can become myocardial cells [1–7]. By contrast, pluripotent embryonic stem (ES)

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CONFLICT OF INTEREST

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cells, which are capable of indefinite self-renewal and differentiation into any cell type in the body, represent a potentially unlimited reservoir of replacement cells. However, the clinical use of human ES cells faces several obstacles such as ethical/moral concerns, tumorigenicity and immune rejection following transplantation. The recent development of cellular reprogramming to generate induced pluripotent stem cells (iPSCs) from of somatic cells has the potential to revolutionize the field of regenerative medicine since patient-derived iPS cells in principle circumvent the ethical problems and immune rejection associated with human ES cells [8].

Nevertheless, the future clinical translation of pluripotent human ES cells and iPS cells is impeded by numerous hurdles. The most pressing challenges and opportunities for breakthrough are development of 1) well-defined, cost-effective and animal product-free growth conditions to maintain stem cell pluripotency, 2) efficient approaches for directed differentiation of pluripotent stem cells towards desired cell lineages, and 3) more efficient and safer methods for iPS cell reprogramming. Traditionally, self-renewal and directed differentiation of the pluripotent stem cells involve undefined conditions containing animal products and expensive biological reagents which significantly limit culture scalability, often produce variable results and introduce xenoantigens [9]. In contrast, using small chemical molecules to support self-renewal and direct differentiation can offer distinct advantages over the traditional growth methods. First, small molecules are far less expensive than biological reagents, affording greater flexibility in a scale-up production of desired cell types. Second, small molecules provide a high degree of temporal regulation since they can be efficiently delivered to and rapidly removed from cells even in the context of multicellular embryonic bodies. For example, we have used dorsomorphin to narrow down the temporal window for robust cardiac induction in mESCs to initial 24 hours of differentiation, whereas efficient cardiac induction using the BMP antagonist Noggin protein required 5 days of exposure [10]. Third, small molecules often produce more reproducible and robust results than biological reagents, which often exhibit significant batch-to-batch variability in potency and stability. Fourth, by reducing or eliminating the use of animal-derived products, small molecules facilitate the critical translational efforts toward clinical applications. With regard to the cellular reprogramming, the use of small molecules can circumvent the concern for gene disruption and oncogene activation inherent to the current virus-mediated reprogramming methods. Finally, small molecules represent valuable intellectual property (IP) assets crucial for successful development of future regenerative therapies. In this review, we will focus on recent advances and patents in the use of small molecules for pluripotent stem cell maintenance, cellular reprogramming and directed differentiation, with a specific focus on the applications in the cardiovascular field.

CHEMICAL BIOLOGY OF PLURIPOTENT STEM CELLS

Small Molecules for Maintaining Pluripotent Stem Cells

Maintaining pluripotency is one of the most important practical challenges of using pluripotent stem cells. ES cells are traditionally maintained on an inactivated mouse embryonic fibroblast feeder (MEF) layer in medium containing undefined animal serum and exogenous proteins to ensure self-renewal. For example, mouse ES (mES) cells generally require Leukemia Inhibitory Factor (LIF) to inhibit differentiation and promote cell self-renewal. Human ES (hES) cells, however, require Transforming growth factor- β (TGF- β)/Nodal and Basic Fibroblast Growth Factor (bFGF) for maintaining pluripotency [11]. Maintaining pluripotent stem cells with traditional methods presents several important issues. First, the use of undefined serum and feeder layers can complicate understanding the mechanism in cell fate determination and produce variable and heterogeneous results. Second, the use of animal serum products precludes clinical applications due to the risk of xenoantigen introduction [9]. To overcome these obstacles, collective efforts have been

made to understand signaling requirements for the self-renewal of ES cells and develop well-defined culture conditions to maintain pluripotency.

Towards this goal, biological signaling studies have revealed the intrinsic self-renewal circuits in ES cells. Studies have demonstrated that LIF can substitute MEF feeders in maintaining pluripotency of mES cells isolated from a “permissive” inbred mouse 129 strain through activation of Stat3 signaling pathway in the presence of animal serum [12–16]. Another important signaling implicated in mouse ES cell self-renewal is bone morphogenetic protein (BMP) signaling Fig. (1). Ying *et al.* showed that BMP can replace serum to maintain mES cell pluripotency in the presence of LIF by blocking neural differentiation through activation of inhibitors of differentiation (*Id*) genes [17]. The combination of exogenous LIF and BMP4 proteins with other factors can sufficiently maintain a pluripotent state of mES cells derived from “permissive” mouse strains in the absence of MEF and serum. In addition, other serum and feeder replacement medium formulations were also developed for maintaining pluripotent stem cell self-renewal. For instance, the KnockOut Serum Replacement can replace serum for culturing ES and iPS cells [18], and the mTeSR was specifically formulated to maintain hES cells and iPS cells in serum-free and feeder-free conditions [19]. However, both KnockOut Serum Replacement and mTeSR require the use of animal-derived products and recombinant proteins, which increases costs and introduces variability [9, 20]. Thus, developing cost-efficient, animal product-free and more generic growth conditions for maintaining pluripotent stem cells is highly sought. To meet this requirement, many studies have focused on developing defined media with small molecules Table 1.

In a high-throughput chemical screen, Chen *et al.* identified a novel compound, Pluripotin/SC1, which can maintain long-term self-renewal of mES cells in the absence of a feeder layer, serum, leukemia inhibitory factor (LIF) or BMP4 [21, 22]. Pluripotin was found to maintain mES pluripotency by inhibiting extracellular signal-regulated kinase-1 (ERK1) and activating the phosphoinositide-3 kinase (PI3K) pathway through blocking RasGAP Fig. (1) [23–25]. Collective evidence has demonstrated that activating PI3K/AKT signaling and inhibiting the mitogen activated protein kinase (MAPK)/ERK signaling are crucial for mESC self-renewal [24–26]. For example, expression of a constitutively active variant of AKT dramatically slowed the differentiation of mES cells, and inhibiting PI3K/AKT signaling with PI3K inhibitors such as LY294002 induced mES cell differentiation even in the presence of LIF [27, 28]. LIF withdrawal, on the other hand, triggered ERK activation. Moreover, Erk2-null mES cells were found to differentiate inefficiently [29], and ERK inhibitor PD98059 enhanced mES cell self-renewal [24]. Finally, BMP signaling promoted mES cell self-renewal in part by inhibiting MAPK/ERK signaling [23].

Interestingly, in contrast to what has been observed in mES cells, inhibiting BMP signaling with the BMP antagonist Noggin promoted long-term self-renewal of hES cell in serum-free, chemically defined conditions [30, 31]. Consistent with these results, dorsomorphin and DMH1, small molecule BMP inhibitors previously identified in our lab, promoted long-term self-renewal of hES cells, presumably by inhibiting BMP induced extraembryonic lineage differentiation [32–34]. These exciting results indicate that inexpensive chemically defined media will soon be widely available for long-term maintenance of human pluripotent stem cells.

Although small molecules that inhibit MAPK/ERK pathway can attenuate ES cell differentiation, they are not sufficient to support mES cell clonal propagation. The Glycogen synthase kinase-3 (GSK-3) inhibitor, CHIR99021, which represents another important small molecule for maintaining mES cell self-renewal, can restore mES cell clonogenicity [35, 36]. Combination of MEK inhibitor PD0325901 with CHIR99021 (known as 2i) can

efficiently maintain long-term self-renewal of mES cells in the absence of exogenous cytokines [35, 37]. Increasing evidence supports that improvement of mES cell self-renewal by inhibiting GSK-3 is through Wnt/ β -catenin cascade Fig. (1) [38–43], although some studies indicated that GSK3 is likely to have β -catenin independent effects in ES cells [35, 44]. Other small molecules which have been used to maintain mES cells in a pluripotent state include PD98059 (a MEK inhibitor), 6-Bromoindirubin-3'-oxime (BIO, a GSK-3 inhibitor) and IQ-1 (a modulator of Wnt/CBP signaling) [45–49].

It was previously impossible to derive ES cells from “non-permissive” mouse strains until small molecule-containing media were developed. Serum-free and feeder-free N2B27 medium supplemented with a MEK inhibitor PD184352, LIF and BMP4 was successfully used to derive ES cells from mouse strains previously considered refractory to ES cell derivation [50]. In addition, the combination of MEK inhibitor PD0325901 with CHIR99021 and LIF (called 2i+LIF) makes it possible to derive ES lines from recalcitrant strains such as NOD mice and even rats [37, 51–53]. These studies indicate that mechanisms controlling ES cell pluripotency are not mouse specific, but might be conserved in rodents and even in other mammals. Recent studies demonstrated that conventional hES cells do not represent the “ground or naïve state” of stemness, but rather a more developmentally mature “primed state” resembling mouse epiblast stem cells (mEpiSCs) found in post-implantation, pre-gastrulation stage embryos [54–59]. When supplemented with other factors (such as Forskolin), the same medium (2i+LIF) which was used to derive and maintain ES cells from “non-permissive” mouse strains and rats can efficiently convert hES cells into a ground or “naïve” state that closely resembles mES cells [54, 55, 57, 60–63]. This medium can maintain long term hES cell self-renewal at the naïve state.

Small Molecules for Reprogramming

Development of iPS cells has boosted tremendous enthusiasm for regenerative medicine as these cells engineered from a patient's own cells could circumvent the ethical and immune rejection problems associated with hES cells. Furthermore, iPS cells hold promise not only for replacement of damaged tissues with functional healthy ones, but also for development of new cellular models to study the pathogenesis of human diseases [8, 64–68]. Presently, practical application of iPS cells faces several major hurdles such as low cellular reprogramming efficiency (0.001 to 0.005%), and oncogenic risks associated with the original virus-based reprogramming strategies [65, 66, 69]. Nonetheless, based on recent reports of plasmid-based, protein-based and modified RNA-based strategies for virus- and integration-free cellular reprogramming [70–73], we anticipate rapid continual advances in cellular reprogramming methodologies.

The possibility to use small molecules to enhance the efficiency of cellular reprogramming has been extensively investigated in the past several years. Several compounds which can improve iPS cell reprogramming by either modifying epigenetic barriers or regulating signal transduction have been identified Table 2. For example, three small molecule histone deacetylase (HDAC) inhibitors, suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA) and valproic acid (VPA), were shown to greatly improve iPS cell reprogramming efficiency from MEF [74]. VPA, the most potent of the three, led to > 100-fold improvement of reprogramming efficiency over the controls. It is particularly interesting that VPA can also enable efficient induction of iPS cells (50-fold improvement) without introduction of the c-Myc, a tumorigenic transcript factor [74, 75]. By targeting the epigenetic machinery, Shi *et al.* showed that a G9a histone methyltransferase inhibitor, BIX01294, can substantially improve iPSC reprogramming efficiency from Sox2-expressing mouse neural progenitor cells (NPCs) transduced with Oct4 and Klf4 [76, 77]. In a subsequent screen, the same group discovered that RG108 (a DNA methyltransferase inhibitor) and BayK8644 (a L-type calcium channel agonist) could synergize with

BIX01294 to substantially enhance the reprogramming of mouse embryonic fibroblasts transduced with only two transcription factors, Oct4 and Klf4 [76, 77]. In another screening to replace Sox2, a small molecule TGF- β signaling inhibitor called RepSox was identified [78, 79]. This compound was shown to promote iPSC reprogramming via inducing expression of Nanog, a critical transcription factor for ES cell self-renewal [78, 79].

Chemical approaches for improving mouse iPSC reprogramming efficiencies have been explored in human iPS cell reprogramming as well. A combination of three small molecules, SB431542 (an ALK5 inhibitor), PD032-5901 (a MEK inhibitor) and thiazovivin (promoting hES cell survival), was reported to improve human iPS cell reprogramming efficiency by 200-fold [76, 80]. In another study, CHIR99021 (GSK-3 inhibitor) and Parnate (an inhibitor of lysine-specific demethylase, also named tranlylcypromine), were shown to induce the reprogramming of human primary keratinocyte transduced with the two factors, Oct4 and Klf4 [81].

The concept of improving iPS cellular reprogramming with small molecules has also been applied to cell dedifferentiation, a reprogramming process in which terminally differentiated cells or lineage-restricted cells are reverted back to a less-differentiated or multipotent progenitor cell stage. In a screen for small molecules capable of dedifferentiation, an Aurora kinase inhibitor named reversine was shown to enable reversion of lineage-committed myoblasts to a more primitive multipotent state at the clonal level [82–84]. Subsequently, it was shown that reversine can also dedifferentiate both murine and human dermal fibroblasts into myogenic competent cells *in vitro* and *in vivo* [85]. Therefore, inhibiting Aurora kinase seems to be essential for cell dedifferentiation. This concept is further supported by studies of other Aurora kinase inhibitors, such as hesperadin, VX-680 and MLN-8054, which are also able to dedifferentiate lineage-committed myoblasts [86].

The importance of small molecules for reprogramming is being increasingly recognized due to their ability to bypass gene integration and increase efficiency and safety in cellular reprogramming. It is conceivable that chemical approaches will lead to better understanding of the fundamental mechanism of cellular reprogramming and epigenetic modifications in reprogramming methodologies.

Small Molecules for Cardiomyogenesis of Pluripotent Stem Cells

Differentiation of pluripotent stem cells toward desired cell lineages is another important challenge for realizing regenerative stem cell therapy. As undifferentiated pluripotent stem cells can potentially form teratomas *in vivo*, pre-differentiating stem cells to specific cell types prior to transplantation is essential. As we discussed previously, small molecules offer distinct advantages over biological reagents in many aspects, so developing small molecules that direct differentiation of pluripotent stem cells toward the desired cell lineages has attracted significant attention. In recent years, many studies using small molecules to direct stem cell differentiation have been reported. Here we will only focus on small molecules capable of promoting cardiomyocyte formation from pluripotent stem cells Table 3.

High-throughput screening is a general approach for identifying compounds that direct differentiation of stem cells toward cardiac cell lineages. In a screening, ES cells engineered to express luciferase or a fluorescent protein under a cardiac-specific promoter are generally used to identify procardiogenic compounds based on the readouts of luciferase activity or fluorescence during differentiation. For instance, using mES cells stably transfected with a cardiac-specific α -cardiac myosin heavy chain (MHC) promoter-driven enhanced green fluorescent protein (EGFP), Takahashi, *et al.* identified that ascorbic acid (also known as vitamin C) markedly increased the number of EGFP-positive cells with expression of cardiac genes including GATA4, α -MHC, and β -MHC [87, 88]. This effect of ascorbic acid

on cardiac differentiation cannot be mimicked by other antioxidants such as N-acetylcysteine, Tiron, or vitamin E under identical conditions, suggesting that ascorbic acid's improvement of cardiomyogenesis in mES cells is not through its well-known antioxidant activity, but rather an unknown function. Cardiogenols represent another important group of procardiogenic small molecules identified in a high-throughput screening using a mouse embryonic carcinoma cell line P19 stably transfected with cardiac specific atrial natriuretic factor (ANF) promoter driven luciferase [89]. Cardiogenol C, one of the most potent cardiogenols, can lead to > 90% of cells expressing GATA-4 and > 50% of cells expressing the α -MHC at day 7 of differentiation [90]. In a similar way, Schneider and colleagues recently identified a group of small molecules called Shz (sulfonyl-hydrazones) in a large scale screen [91, 92]. The Shz compounds potently induced cardiomyogenesis in numerous types of stem cells and progenitor cells. It is particularly interesting that transplanting human mobilized peripheral blood mononuclear cells (M-PBMCs) pretreated with the Shz compounds into injured rat hearts has led to better engraftment of human M-PBMCs and significant enhancement in injured rat myocardial repair. In summary, the high-throughput screening is an important approach to identify small molecules for directing stem cell cardiomyogenesis. However, a significant drawback of this approach is that underlying cardiogenic mechanisms of the identified small molecules in stem cells often remain unknown.

An alternative strategy to identify small molecules for promoting cardiomyogenesis involves examining small molecules that selectively modulate key developmental signaling pathways. In principle, the process of *in vitro* cardiomyogenesis in ES cells or iPS cells recapitulates the complex interplay of key signaling pathways during embryonic heart development. Using small molecules that selectively modulate specific pathways during embryonic heart development might be an effective approach to promote stem cell cardiomyogenesis *in vitro*. For example, Activins and BMPs play critical roles in the mesoderm formation and myocardial lineage specification during embryogenesis. Fittingly, modulating Activin and BMP signaling pathways induces formation of cardiac cells in both human and mouse ES cell models [93–99]. By timely inhibiting BMP pathway with a selective small molecular inhibitor called dorsomorphin, we have demonstrated that it can robustly increase cardiomyocyte formation in mES cells [10]. The pro-cardiomyogenic mechanism of dorsomorphin is extremely interesting as dorsomorphin treatment is required only during the first 24 hours of differentiation, 2 days prior to the early mesoderm marker BryT expression. In addition, the strong cardiac induction in ES cells treated with dorsomorphin occurs in the absence of BryT induction, suggesting that dorsomorphin increases cardiomyogenesis by a way of stimulating an unknown progenitor.

Wnt/ β -catenin signaling is another important pathway during *in vivo* embryonic heart development. In zebrafish, it was found that Wnt/ β -catenin signaling promotes cardiac differentiation before gastrulation, but it inhibits heart formation during gastrulation [100]. This biphasic role of Wnt/ β -catenin signaling during *in vivo* heart formation was also evidenced in ES cultures. In both human and mouse ES cells, early Wnt activation stimulates mesoderm induction and increases cardiac differentiation. Conversely, late activation of Wnt signaling inhibits cardiac differentiation [100–102]. Chemical stage specific modulation of Wnt/ β -catenin signaling has been shown to induce cardiomyocyte formation in ES cells. For instance, 6-bromoin-dirubin-3'-oxime (BIO), which can activate Wnt/ β -catenin signaling by inhibiting GSK-3, significantly induced cardiomyocyte formation in mES cells treated at the early stage of differentiation before mesoderm formation [103, 104]. In contrast, XAV939, a selective small molecule inhibitor of Wnt/ β -catenin signaling, markedly induced cardiomyogenesis in mES cells when this inhibitor was administered at day 3, immediately after the formation of mesoderm [105]. XAV939 was

found to promote a nearly 30-fold increase in cardiomyocyte formation at the expense of other mesodermal lineages such as the endothelial and smooth muscle cell lineages [105].

Despite the significant successes in small molecule-inducing cardiomyogenesis in both human and mouse ES cells, transferring the chemical approaches optimized in cardiomyogenesis of ES cells to iPS cells often faces challenges such as significantly low efficiency of cardiomyocyte formation in iPS cells [106, 107]. One of the main reasons for this discrepancy appears to be iPS cell epigenetic memory. The reprogrammed cells tend to preferentially differentiate back to cell types from which they were originally derived [108, 109]. Recent epigenetic studies in iPS cells indicated that methylation patterns near the tips and centers of chromosomes in iPS cells were not reset to an embryo-like state, but resembled those in the adult tissues from which iPS cells had been derived [110]. Identifying novel small molecules that can reset epigenetic states of iPS cells may be an effective approach to promote cardiomyogenesis of iPS cells. An alternative approach to circumvent the low rate cardiomyogenesis of iPS cells is to directly reprogram fibroblasts to become cardiomyocytes without first generating iPS cells. Leda, *et al* recently reported that mouse postnatal cardiac or dermal fibroblasts can be directly reprogrammed into cardiomyocytes using a combination of three transcription factors (Gata4, Mef2c, and Tbx5) [111]. This transdifferentiation approach has significant therapeutic implications as it can potentially lower the risk of tumor formation by avoiding intermediate iPS cells, and precludes the immune rejection by using individual's own endogenous fibroblasts. Identifying small molecules that can replace these three transcription factors in switching cell fates from fibroblasts to cardiomyocyte would greatly facilitate the future regenerative therapies in live animals.

First, the avoidance of reprogramming to pluripotent cells before cardiac differentiation would greatly lower in the setting of future cell-based therapies. Second, large amounts of an can be grown from a cardiac biopsy or skin biopsy *in vitro* for transduction with the defined factors, followed by delivery of cells to damaged hearts. Third, and most promising, is the potential to introduce the defined factors, or factors that mimic their effects, directly into the heart to reprogram the endogenous fibroblast population,

CURRENT & FUTURE DEVELOPMENTS

Pluripotent stem cells, especially patient-derived iPS cells show great promise as a source of cells for regenerative therapy as well as a platform for drug discovery and patient specific diagnostics. However, the future clinical translation of pluripotent stem cells is impeded by numerous challenges regarding safety, efficacy and lack of long-term benefits. The importance of small molecules as tools to overcome these challenges has been increasingly recognized. Here, we have reviewed recent advances using small molecules that promote stem cell maintenance, cellular reprogramming and cardiomyogenesis.

Despite the truly breathtaking advances in the stem cell field, there remain substantial challenges to developing stem cell-based diagnostic and therapeutic platforms. For example, low reprogramming efficiency and epigenetic memory of iPS cells are significant impediments. However, rapid advances in the fundamental biology of reprogramming, epigenetic memory, tissue and organ development are rapidly revealing new targets for small molecule intervention. For example, while cellular reprogramming remains a poorly understood at the mechanistic level, what has clearly emerged is that this slow and inefficient process dependent on multiple stochastic events and reprogramming factors. Implication of these finding is that a small molecule that can overcome just one of these required events could have a huge log-order scale impact on improving overall reprogramming efficiency [74–77, 79]. Moreover, basic studies have revealed potential rate-

limiting steps to cellular reprogramming, such as the endogenous Oct4 transcriptional activation [74–77, 79]. In this regard, we can easily envision a small molecule or cocktail of small molecules that may one day replace all of the classic Yamanaka factors. Likewise, we envision that, with better understanding of the mechanisms of epigenetic memory, small molecule-based strategies to “erase” it will soon follow.

We can also envision scenarios in which small molecules developed for use in *in vitro* stem cell may have broader utility. For example, small molecule-based approaches for direct transdifferentiation of fibroblasts into desired tissue types may ultimately prove to be useful as alternatives to transplantation of exogenous stem cells. Moreover, small molecules that target key nodes in differentiation of embryonic progenitor cells and pluripotent stem cells may prove to be useful for stimulating endogenous resident stem/progenitor cells to repair damaged tissues. Finally, small molecules that modulate the host tissue microenvironment may be an equally promising future strategy to enhance survival and functional integration of tissues generated from transplanted stem cells and endogenous progenitor cells. Thus, in myriad ways, small molecules could play an important role as a “regenerative pill” to restore function of organs impaired through injury or the toll of aging. In summary, we anticipate that chemical biology will play a transformative role in moving the nascent field of regenerative medicine toward wide range of clinical applications.

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References

1. Murry CE, Reinecke H, Pabon LM. Regeneration gaps: Observations on stem cells and cardiac repair. *J Am Coll Cardiol*. 2006; 47(9):1777–85. [PubMed: 16682301]
2. Hansson EM, Lindsay ME, Chien KR. Regeneration next: Toward heart stem cell therapeutics. *Cell Stem Cell*. 2009; 5(4):364–77. [PubMed: 19796617]
3. Silva GV, Litovsky S, Assad JA, Sousa AL, Martin BJ, Vela D, et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation*. 2005; 111(2):150–6. [PubMed: 15642764]
4. Wollert KC. Cell therapy for acute myocardial infarction. *Curr Opin Pharmacol*. 2008; 8(2):202–10. [PubMed: 18243051]
5. Gneocchi M, He H, Liang OD, Melo LG, Morello F, Mu H, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med*. 2005; 11(4):367–8. [PubMed: 15812508]
6. Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S, et al. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation*. 2004; 109(12):1543–9. [PubMed: 15023891]
7. Uemura R, Xu M, Ahmad N, Ashraf M. Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. *Circ Res*. 2006; 98(11):1414–21. [PubMed: 16690882]
8. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126(4):663–76. [PubMed: 16904174]
9. Sakamoto N, Tsuji K, Muul LM, Lawler AM, Petricoin EF, Candotti F, et al. Bovine apolipoprotein B-100 is a dominant immunogen in therapeutic cell populations cultured in fetal calf serum in mice and humans. *Blood*. 2007; 110(2):501–8. [PubMed: 17395779]
10. Hao J, Daleo MA, Murphy CK, Yu PB, Ho JN, Hu J, et al. Dorsomorphin, a selective small molecule inhibitor of BMP signaling, promotes cardiomyogenesis in embryonic stem cells. *PLoS One*. 2008; 3(8):e2904. [PubMed: 18682835]

11. Vallier L, Alexander M, Pedersen RA. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. *J Cell Sci*. 2005; 118(Pt 19):4495–509. [PubMed: 16179608]
12. Smith AG, Heath JK, Donaldson DD, Wong GG, Moreau J, Stahl M, et al. Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature*. 1988; 336(6200):688–90. [PubMed: 3143917]
13. Williams RL, Hilton DJ, Pease S, Willson TA, Stewart CL, Gearing DP, et al. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature*. 1988; 336(6200):684–7. [PubMed: 3143916]
14. Niwa H, Burdon T, Chambers I, Smith A. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. *Genes Dev*. 1998; 12(13):2048–60. [PubMed: 9649508]
15. Matsuda T, Nakamura T, Nakao K, Arai T, Katsuki M, Heike T, et al. STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. *EMBO J*. 1999; 18(15):4261–9. [PubMed: 10428964]
16. Raz R, Lee CK, Cannizzaro LA, d'Eustachio P, Levy DE. Essential role of STAT3 for embryonic stem cell pluripotency. *Proc Natl Acad Sci USA*. 1999; 96(6):2846–51. [PubMed: 10077599]
17. Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell*. 2003; 115(3):281–92. [PubMed: 14636556]
18. Cheng J, Dutra A, Takesono A, Garrett-Beal L, Schwartzberg PL. Improved generation of C57BL/6J mouse embryonic stem cells in a defined serum-free media. *Genesis*. 2004; 39(2):100–4. [PubMed: 15170695]
19. Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, et al. Derivation of human embryonic stem cells in defined conditions. *Nat Biotechnol*. 2006; 24(2):185–7. [PubMed: 16388305]
20. Mallon BS, Park KY, Chen KG, Hamilton RS, McKay RD. Toward xeno-free culture of human embryonic stem cells. *Int J Biochem Cell Biol*. 2006; 38(7):1063–75. [PubMed: 16469522]
21. Chen S, Do JT, Zhang Q, Yao S, Yan F, Peters EC, et al. Self-renewal of embryonic stem cells by a small molecule. *Proc Natl Acad Sci USA*. 2006; 103(46):17266–71. [PubMed: 17088537]
22. Chen, S.; Ding, S.; Yan, F.; Schultz, P. Compounds that maintain pluripotency of embryonic stem cells. US20100234400. 2010.
23. Qi X, Li TG, Hao J, Hu J, Wang J, Simmons H, et al. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. *Proc Natl Acad Sci USA*. 2004; 101(16):6027–32. [PubMed: 15075392]
24. Burdon T, Stracey C, Chambers I, Nichols J, Smith A. Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells. *Dev Biol*. 1999; 210(1):30–43. [PubMed: 10364425]
25. Paling NR, Wheadon H, Bone HK, Welham MJ. Regulation of embryonic stem cell self-renewal by phosphoinositide 3-kinase-dependent signaling. *J Biol Chem*. 2004; 279(46):48063–70. [PubMed: 15328362]
26. Storm MP, Bone HK, Beck CG, Bourillot PY, Schreiber V, Damiano T, et al. Regulation of Nanog expression by phosphoinositide 3-kinase-dependent signaling in murine embryonic stem cells. *J Biol Chem*. 2007; 282(9):6265–73. [PubMed: 17204467]
27. Welham MJ, Storm MP, Kingham E, Bone HK. Phosphoinositide 3-kinases and regulation of embryonic stem cell fate. *Biochem Soc Trans*. 2007; 35(Pt 2):225–8. [PubMed: 17371244]
28. Watanabe S, Umehara H, Murayama K, Okabe M, Kimura T, Nakano T. Activation of Akt signaling is sufficient to maintain pluripotency in mouse and primate embryonic stem cells. *Oncogene*. 2006; 25(19):2697–707. [PubMed: 16407845]
29. Kunath T, Saba-El-Leil MK, Almousaillekh M, Wray J, Meloche S, Smith A. FGF stimulation of the Erk1/2 signalling cascade triggers transition of pluripotent embryonic stem cells from self-renewal to lineage commitment. *Development*. 2007; 134(16):2895–902. [PubMed: 17660198]
30. Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. *Nat Methods*. 2005; 2(3):185–90. [PubMed: 15782187]

31. Yao S, Chen S, Clark J, Hao E, Beattie GM, Hayek A, et al. Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. *Proc Natl Acad Sci USA*. 2006; 103(18):6907–12. [PubMed: 16632596]
32. Yu PB, Hong CC, Sachidanandan C, Babitt JL, Deng DY, Hoyng SA, et al. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat Chem Biol*. 2008; 4(1):33–41. [PubMed: 18026094]
33. Hao J, Ho JN, Lewis JA, Karim KA, Daniels RN, Gentry PR, et al. *In vivo* structure-activity relationship study of dorsomorphin analogues identifies selective VEGF and BMP inhibitors. *ACS Chem Biol*. 2010; 5(2):245–53. [PubMed: 20020776]
34. Gonzalez R, Lee JW, Snyder EY, Schultz PG. Dorsomorphin promotes human embryonic stem cell self-renewal. *Angew Chem Int Ed Engl*. 2011; 50(15):3439–41. [PubMed: 21384475]
35. Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, et al. The ground state of embryonic stem cell self-renewal. *Nature*. 2008; 453(7194):519–23. [PubMed: 18497825]
36. Perry, J.; Li, L.; Grindley, J. Methods and compositions for stem cell self-renewal. US20100196337. 2010.
37. Smith, A.; Ying, Q. Culture medium containing kinase inhibitors, and uses thereof. US20080014638. 2008.
38. Aubert J, Dunstan H, Chambers I, Smith A. Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. *Nat Biotechnol*. 2002; 20(12):1240–5. [PubMed: 12447396]
39. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med*. 2004; 10(1):55–63. [PubMed: 14702635]
40. Ogawa K, Nishinakamura R, Iwamatsu Y, Shimosato D, Niwa H. Synergistic action of Wnt and LIF in maintaining pluripotency of mouse ES cells. *Biochem Biophys Res Commun*. 2006; 343(1):159–66. [PubMed: 16530170]
41. Kielman MF, Rindapaa M, Gaspar C, van Poppel N, Breukel C, van Leeuwen S, et al. Apc modulates embryonic stem-cell differentiation by controlling the dosage of beta-catenin signaling. *Nat Genet*. 2002; 32(4):594–605. [PubMed: 12426568]
42. Pereira L, Yi F, Merrill BJ. Repression of Nanog gene transcription by Tcf3 limits embryonic stem cell self-renewal. *Mol Cell Biol*. 2006; 26(20):7479–91. [PubMed: 16894029]
43. Takao Y, Yokota T, Koide H. Beta-catenin up-regulates Nanog expression through interaction with Oct-3/4 in embryonic stem cells. *Biochem Biophys Res Commun*. 2007; 353(3):699–705. [PubMed: 17196549]
44. Wray J, Kalkan T, Smith AG. The ground state of pluripotency. *Biochem Soc Trans*. 2010; 38(4):1027–32. [PubMed: 20658998]
45. Miyabayashi T, Teo JL, Yamamoto M, McMillan M, Nguyen C, Kahn M. Wnt/beta-catenin/CBP signaling maintains long-term murine embryonic stem cell pluripotency. *Proc Natl Acad Sci USA*. 2007; 104(13):5668–73. [PubMed: 17372190]
46. Brivanlou, A.; Sato, N.; Meijer, L. Maintenance of embryonic stem cells by the GSK-3 inhibitor 6-bromoindirubin-3'-oxime. US20060030042. 2006.
47. Kahn, M. Serum-free expansion of cells in culture. US20070128669. 2007.
48. Smith, A.; Burdon, T. Propagation and/or derivation of embryonic stem cells. US7371573. 2008.
49. Brivanlou, A.; Sato, N.; Meijer, L. Maintenance of embryonic stem cells by the GSK-3 inhibitor 6-bromoindirubin-3'-oxime. US20060030042. 2006.
50. Batlle-Morera L, Smith A, Nichols J. Parameters influencing derivation of embryonic stem cells from murine embryos. *Genesis*. 2008; 46(12):758–67. [PubMed: 18837461]
51. Nichols J, Jones K, Phillips JM, Newland SA, Roode M, Mansfield W, et al. Validated germline-competent embryonic stem cell lines from nonobese diabetic mice. *Nat Med*. 2009; 15(7):814–8. [PubMed: 19491843]
52. Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, et al. Capture of authentic embryonic stem cells from rat blastocysts. *Cell*. 2008; 135(7):1287–98. [PubMed: 19109897]

53. Li P, Tong C, Mehrian-Shai R, Jia L, Wu N, Yan Y, et al. Germline competent embryonic stem cells derived from rat blastocysts. *Cell*. 2008; 135(7):1299–310. [PubMed: 19109898]
54. Bao S, Tang F, Li X, Hayashi K, Gillich A, Lao K, et al. Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature*. 2009; 461(7268):1292–5. [PubMed: 19816418]
55. Hanna J, Cheng AW, Saha K, Kim J, Lengner CJ, Soldner F, et al. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. *Proc Natl Acad Sci USA*. 2010; 107(20):9222–7. [PubMed: 20442331]
56. Nichols J, Smith A. Naïve and primed pluripotent states. *Cell Stem Cell*. 2009; 4(6):487–92. [PubMed: 19497275]
57. Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, Smith A. Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biol*. 2008; 6(10):e253. [PubMed: 18942890]
58. Silva J, Nichols J, Theunissen TW, Guo G, van Oosten AL, Barrandon O, et al. Nanog is the gateway to the pluripotent ground state. *Cell*. 2009; 138(4):722–37. [PubMed: 19703398]
59. Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, et al. New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature*. 2007; 448(7150):196–9. [PubMed: 17597760]
60. Pera MF, Tam PP. Extrinsic regulation of pluripotent stem cells. *Nature*. 2010; 465(7299):713–20. [PubMed: 20535200]
61. Tchieu J, Kuoy E, Chin MH, Trinh H, Patterson M, Sherman SP, et al. Female human iPSCs retain an inactive X chromosome. *Cell Stem Cell*. 2010; 7(3):329–42. [PubMed: 20727844]
62. Hanna JH, Saha K, Jaenisch R. Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues. *Cell*. 2010; 143(4):508–25. [PubMed: 21074044]
63. Lengner CJ, Gimelbrant AA, Erwin JA, Cheng AW, Guenther MG, Welstead GG, et al. Derivation of pre-X inactivation human embryonic stem cells under physiological oxygen concentrations. *Cell*. 2010; 141(5):872–83. [PubMed: 20471072]
64. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007; 448(7151):313–7. [PubMed: 17554338]
65. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science*. 2008; 322(5903):949–53. [PubMed: 18845712]
66. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131(5):861–72. [PubMed: 18035408]
67. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, et al. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*. 2007; 448(7151):318–24. [PubMed: 17554336]
68. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318(5858):1917–20. [PubMed: 18029452]
69. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science*. 2008; 322(5903):945–9. [PubMed: 18818365]
70. Cho HJ, Lee CS, Kwon YW, Paek JS, Lee SH, Hur J, et al. Induction of pluripotent stem cells from adult somatic cells by protein-based reprogramming without genetic manipulation. *Blood*. 2010; 116(3):386–95. [PubMed: 20439621]
71. Okita K, Hong H, Takahashi K, Yamanaka S. Generation of mouse-induced pluripotent stem cells with plasmid vectors. *Nat Protoc*. 2010; 5(3):418–28. [PubMed: 20203661]
72. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*. 2010; 7(5):618–30. [PubMed: 20888316]
73. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell*. 2009; 4(5):381–4. [PubMed: 19398399]

74. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol.* 2008; 26(7):795–7. [PubMed: 18568017]
75. Huangfu, D.; Melton, D.; Maehr, R. Efficient induction of pluripotent stem cells using small molecule compounds. US20110014164. 2011.
76. Shi, Y.; Despons, C.; Lin, T.; Li, W.; Zhu, S.; Ding, S. Chemical approaches for generation of induced pluripotent stem cells. US20100267141. 2010.
77. Shi Y, Do JT, Despons C, Hahm HS, Scholer HR, Ding S. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. *Cell Stem Cell.* 2008; 2(6):525–8. [PubMed: 18522845]
78. Ichida, J.; Blanchard, J.; Lam, K.; Rubin, L.; Eggan, K. Efficient induction of pluripotent stem cells using small molecule compounds. WO2010033906. 2010.
79. Ichida JK, Blanchard J, Lam K, Son EY, Chung JE, Egli D, et al. A small-molecule inhibitor of *tgf-Beta* signaling replaces Sox2 in reprogramming by inducing nanog. *Cell Stem Cell.* 2009; 5(5): 491–503. [PubMed: 19818703]
80. Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, Abujarour R, et al. A chemical platform for improved induction of human iPSCs. *Nat Methods.* 2009; 6(11):805–8. [PubMed: 19838168]
81. Li W, Zhou H, Abujarour R, Zhu S, Young Joo J, Lin T, et al. Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. *Stem Cells.* 2009; 27(12):2992–3000. [PubMed: 19839055]
82. Chen S, Zhang Q, Wu X, Schultz PG, Ding S. Dedifferentiation of lineage-committed cells by a small molecule. *J Am Chem Soc.* 2004; 126(2):410–1. [PubMed: 14719906]
83. Chen, S.; Ding, S.; Schultz, P. Compositions and methods for inducing cell dedifferentiation. US20050176707. 2005.
84. Lee EK, Bae GU, You JS, Lee JC, Jeon YJ, Park JW, et al. Reversine increases the plasticity of lineage-committed cells toward neuroectodermal lineage. *J Biol Chem.* 2009; 284(5):2891–901. [PubMed: 19015271]
85. Anastasia L, Sampaulesi M, Papini N, Oleari D, Lamorte G, Tringali C, et al. Reversine-treated fibroblasts acquire myogenic competence *in vitro* and in regenerating skeletal muscle. *Cell Death Differ.* 2006; 13(12):2042–51. [PubMed: 16729034]
86. Amabile G, D'Alise AM, Iovino M, Jones P, Santaguida S, Musacchio A, et al. The Aurora B kinase activity is required for the maintenance of the differentiated state of murine myoblasts. *Cell Death Differ.* 2009; 16(2):321–30. [PubMed: 18974773]
87. Takahashi T, Lord B, Schulze PC, Fryer RM, Sarang SS, Gullans SR, et al. Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes. *Circulation.* 2003; 107(14):1912–6. [PubMed: 12668514]
88. Passier, R.; Mummery, C. Differentiation of human embryonic stem cells and cardiomyocytes and cardiomyocyte progenitors derived therefrom. US20080254003. 2008.
89. Wu, X.; Ding, S.; Schultz, P. Compositions and methods for inducing cardiomyogenesis. US20050209231. 2005.
90. Wu X, Ding S, Ding Q, Gray NS, Schultz PG. Small molecules that induce cardiomyogenesis in embryonic stem cells. *J Am Chem Soc.* 2004; 126(6):1590–1. [PubMed: 14871063]
91. Sadek H, Hannack B, Choe E, Wang J, Latif S, Garry MG, et al. Cardiogenic small molecules that enhance myocardial repair by stem cells. *Proc Natl Acad Sci USA.* 2008; 105(16):6063–8. [PubMed: 18420817]
92. Olson, E.; Frantz, D.; Hsieh, J.; Mcknight, SL.; Schneider, J. Stem cell differentiating agents and uses therefor. US20090076103. 2009.
93. Moore CS, Mjaatvedt CH, Gearhart JD. Expression and function of activin beta A during mouse cardiac cushion tissue formation. *Dev Dyn.* 1998; 212(4):548–62. [PubMed: 9707328]
94. Nakajima Y, Yamagishi T, Hokari S, Nakamura H. Mechanisms involved in valvuloseptal endocardial cushion formation in early cardiogenesis: Roles of transforming growth factor (TGF)-beta and bone morphogenetic protein (BMP). *Anat Rec.* 2000; 258(2):119–27. [PubMed: 10645959]

95. Jones CM, Lyons KM, Hogan BL. Involvement of Bone Morphogenetic Protein-4 (BMP-4) and Vgr-1 in morphogenesis and neurogenesis in the mouse. *Development*. 1991; 111(2):531–42. [PubMed: 1893873]
96. Winnier G, Blessing M, Labosky PA, Hogan BL. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev*. 1995; 9(17):2105–16. [PubMed: 7657163]
97. Johansson BM, Wiles MV. Evidence for involvement of activin A and bone morphogenetic protein 4 in mammalian mesoderm and hematopoietic development. *Mol Cell Biol*. 1995; 15(1):141–51. [PubMed: 7799920]
98. Gadue P, Huber TL, Paddison PJ, Keller GM. Wnt and TGF-beta signaling are required for the induction of an *in vitro* model of primitive streak formation using embryonic stem cells. *Proc Natl Acad Sci USA*. 2006; 103(45):16806–11. [PubMed: 17077151]
99. Klaus A, Saga Y, Taketo MM, Tzahor E, Birchmeier W. Distinct roles of Wnt/beta-catenin and Bmp signaling during early cardiogenesis. *Proc Natl Acad Sci USA*. 2007; 104(47):18531–6. [PubMed: 18000065]
100. Ueno S, Weidinger G, Osugi T, Kohn AD, Golob JL, Pabon L, et al. Biphasic role for Wnt/beta-catenin signaling in cardiac specification in zebrafish and embryonic stem cells. *Proc Natl Acad Sci USA*. 2007; 104(23):9685–90. [PubMed: 17522558]
101. Naito AT, Shiojima I, Akazawa H, Hidaka K, Morisaki T, Kikuchi A, et al. Developmental stage-specific biphasic roles of Wnt/beta-catenin signaling in cardiomyogenesis and hematopoiesis. *Proc Natl Acad Sci USA*. 2006; 103(52):19812–7. [PubMed: 17170140]
102. Paige SL, Osugi T, Afanasiev OK, Pabon L, Reinecke H, Murry CE. Endogenous Wnt/beta-catenin signaling is required for cardiac differentiation in human embryonic stem cells. *PLoS One*. 2010; 5(6):e11134. [PubMed: 20559569]
103. Tseng AS, Engel FB, Keating MT. The GSK-3 inhibitor BIO promotes proliferation in mammalian cardiomyocytes. *Chem Biol*. 2006; 13(9):957–63. [PubMed: 16984885]
104. Koshimizu, U.; Tanaka, T.; Kawashima, K.; Kadokura, M. Method for inducing differentiation of pluripotent stem cells into cardiomyocyte. US20090325288. 2009.
105. Wang H, Hao J, Hong CC. Cardiac induction of embryonic stem cells by a small molecule inhibitor of wnt/beta-catenin signaling. *ACS Chem Biol*. 2011; 6(2):192–7. [PubMed: 21077691]
106. Mauritz C, Schwanke K, Reppel M, Neef S, Katsirntaki K, Maier LS, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation*. 2008; 118(5):507–17. [PubMed: 18625890]
107. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res*. 2009; 104(4):e30–41. [PubMed: 19213953]
108. Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010; 467(7313):285–90. [PubMed: 20644535]
109. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol*. 2010; 28(8):848–55. [PubMed: 20644536]
110. Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011; 471(7336):68–73. [PubMed: 21289626]
111. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*. 2010; 142(3):375–86. [PubMed: 20691899]

Fig. (1).
Signaling pathways involved in pluripotent stem cell self-renewal, reprogramming and differentiation.

Table 1

Patents of Small Molecules Capable of Maintaining Stem Cell Self-Renewal.

Patent No	Structure	Description
US20100234400		Pluripotin Dual inhibitor of RasGAP/ERK1
US20100196337		CHIR99201 GSK3 inhibitor
US20080014638		PD0325901 MEK inhibitor
US20060030042		BIO (6-bromoindirubin-3'-oxime) GSK3 inhibitor
US7371573		PD98059 MEK inhibitor
US20070128669		IQ-1 Phosphatase PP2A inhibitor
Pending		Dorsomorphin ALK2 inhibitor (BMP pathway)

Table 2

Patents of Small Molecules Capable of Promoting Cell Reprogramming.

Patent No	Structure	Description
US20100173414		SAHA Histone deacetylase (HDAC) inhibitor
US20100173414		TSA Histone deacetylase (HDAC) inhibitor
US20110014164		Valproic acid (VPA) Histone deacetylase (HDAC) inhibitor
US20100267141	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>A</p> </div> <div style="text-align: center;"> <p>B</p> </div> </div>	(A) BayK8644 L-type calcium channel agonist (B) BIX01294 Histone methyltransferase inhibitor
US20100267141		RG108 DNA methyltransferase inhibitor
US20100267141		SB431542 ALK5 inhibitor
WO2010033906		RepSox A TGFβ R1 inhibitor

Patent No	Structure	Description
US20100267141		Parnate Lysine-specific demethylase inhibitor
US20050176707		Reversine Aurora kinase inhibitor
Pending		Hesperadin Aurora kinase inhibitor
Pending		VX-680 Aurora kinase inhibitor
Pending		MLN-8054 Aurora kinase inhibitor

Table 3

Patents of Small Molecules Capable of Inducing Stem Cell Cardiomyogenesis.

Patent No	Structure	Description
US20080254003		Ascorbic acid Unknown target
US20050209231		Cardiogenol C Unknown target
US20090076103	 R= H, CH ₃ or Cl; X= Br or Cl, Y=OH	Sulfonyl hydrazones Unknown target
US20090325288		Bio(6-bromoindirubin-3'-oxime) GSK3 inhibitor
Pending		Dorsormorphin ALK2 inhibitor (BMP pathway)
Pending		XAV939 Tankyrase inhibitor (Wnt/ β -catenin pathway)