Title
Chemical Approaches to Stem Cell Biology and Therapeutics

Journal
Cell Stem Cell, 13(3)

ISSN
1934-5909

Authors
Li, Wenlin
Li, Ke
Wei, Wanguo
et al.

Publication Date
2013-09-01

DOI
10.1016/j.stem.2013.08.002

Peer reviewed

Published in final edited form as:
Cell Stem Cell. 2013 September 5; 13(3): 270–283. doi:10.1016/j.stem.2013.08.002.

Chemical approaches to stem cell biology and therapeutics

Wenlin Li[1], **Ke Li**[2], **Wanguo Wei**[3], and **Sheng Ding**[2,*]

[1]Department of Cell Biology, Second Military Medical University, Shanghai 200433, China

[2]Gladstone Institute of Cardiovascular Disease, Department of Pharmaceutical Chemistry, University of California, San Francisco, 94158, USA

[3]Stem Cell and Regenerative Medicine Center, Shanghai Advanced Research Institute, Chinese Academy of Science, Shanghai 201210, China

SUMMARY

Small molecules that modulate stem cell fate and function offer significant opportunities that will allow the full realization of the therapeutic potential of stem cells. Rational design and screening for small molecules have identified useful compounds to probe fundamental mechanisms of stem cell self-renewal, differentiation, and reprogramming, and have facilitated the development of cell-based therapies and therapeutic drugs targeting endogenous stem and progenitor cells for repair and regeneration. Here, we will discuss recent scientific and therapeutic progress, as well as new perspectives and future challenges for using chemical approaches in stem cell biology and regenerative medicine.

Keywords

stem cells; small molecules; differentiation; self-renewal; reprogramming

INTRODUCTION

Small molecules modulating specific targets involved in signaling, metabolic, transcriptional, or epigenetic mechanisms have emerged as valuable tools for probing basic stem cell biology and manipulating stem cell fate, state, or function *in vitro* and *in vivo* (Schugar et al., 2008; Xu et al., 2008). Compared to genetic manipulations, small molecules have a number of distinct advantages: they are more convenient to use, provide a higher degree of temporal (e.g., effects are rapid and reversible) and spatial (e.g., effects confined to different cell or tissue compartments) control over protein function, and their effects can be fine-tuned by varying their concentrations and combinations. While the specificity of small molecules often presents a challenge for using them and interpreting their effects, their

*Corresponding author: Sheng Ding, Ph.D., Gladstone Institute of Cardiovascular Disease, Department of Pharmaceutical Chemistry, University of California, San Francisco, 1650 Owens Street, San Francisco, CA 94158, Phone (415) 734-2717, Fax: (415) 355 0141, sheng.ding@gladstone.ucsf.edu.

Author Contributions: Wenlin Li, Ke Li and Wanguo Wei: manuscript writing, Sheng Ding: manuscript writing, conception and design.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

polypharmacological mechanisms can also be exploited for desirable outcomes. Rational design and/or screening of small molecules to modulate specific targets or stem cell phenotypes have led to the generation and validation of useful compounds for enhancing cell-based therapy and/or facilitating the development of therapeutic drugs targeting endogenous stem and progenitor cells to treat degenerative diseases, cancer, and injuries (Figure 1). As a nascent field, stem cell research will continue to benefit from its crossover with chemistry. In this review, we discuss some of the recent developments in applying chemical approaches to stem cell biology and regenerative medicine.

Small molecules modulating stem cell maintenance

Pluripotent stem cells

Pluripotent stem cells (PSCs) are unique in that they can indefinitely self-renew and give rise to all cell types in the body. The two most-studied PSC types are the classic murine embryonic stem cells (mESCs) and human ESCs (hESCs), which represent two different pluripotency states (*i.e*., naive *vs*. primed pluripotency states) and consequently display significant differences in gene expression and cell behaviors (Li and Ding, 2011). Identified small molecules that modulate these two types of PSCs have changed the way they are practically manipulated in culture as well as our understanding of pluripotency.

mESCs possess an intrinsic ability to self-renew and do not depend on exogenous pathway activation, a conceptual advance led by the discovery and characterization of pluripotin (a novel synthetic small molecule, 1 in Figure 2) from a high-throughput phenotypic screen (Chen et al., 2006). Pluripotin can maintain the long-term self-renewal of mESCs in the absence of feeder cells, serum products and typically used cytokines (e.g., leukemia inhibitory factor (LIF) and bone morphogenetic protein 4 (BMP4)) by dual inhibition of two endogenous differentiation-inducing proteins, Ras GTPase activating protein (RasGAP) and extracellular signal-regulated kinase-1 (ERK1), and does so without activating essential self-renewal pathways, including LIF-STAT3 (signal transducer and activator of transcription 3), BMP4-Smad, and Wnt-β-catenin. Subsequent studies showed that 2i, the combination of a Mitogen-activated protein/ERK kinase (MEK) inhibitor PD0325901 (2 in Figure 2) and a glycogen synthase kinase-3 (GSK3) inhibitor CHIR99021 (3 in Figure 2) (Ying et al., 2008), and other combinations of factors that inhibit differentiation signaling pathways in conjunction with LIF treatment can support the derivation and long-term self-renewal of mESCs (especially from refractory strains) (Hanna et al., 2009; Yang et al., 2009), rat ESCs and induced pluripotent stem cells (iPSCs) (Buehr et al., 2008; Kawamata and Ochiya, 2010; Li et al., 2008; Li et al., 2009a), and hPSCs that resemble a naive pluripotent state (Hanna et al., 2010; Li et al., 2009a). The approach of using small molecules to maintain PSC self-renewal by inhibiting differentiation mechanisms can also be applied to expand adult stem cell populations or establish pluripotency through reprogramming (Cao et al., 2013; Li et al., 2011; Shi et al., 2008b; Silva et al., 2008).

In line with stem cells' intrinsic self-renewal ability, specific endogenous small molecules derived from stem cells themselves (e.g., metabolites of cellular essential nutrients and other building blocks) regulate cell type–specific signaling, metabolic, transcriptional and epigenetic mechanisms by participating in protein modifications, or serving as endogenous receptor ligands or enzyme cofactors (Folmes et al., 2012; Zhang et al., 2012). For example, mass spectrometry–based untargeted metabolomic profiling of undifferentiated mESCs and their differentiated neural and cardiac derivatives (Yanes et al., 2010) revealed that undifferentiated mESCs were characterized by the low oxidative state of their metabolomes (i.e., enriched with highly unsaturated metabolites), but when differentiation was induced, the level of unsaturated metabolites decreased drastically, thereby increasing the percentage of new hydrogenated and oxygenated metabolites in differentiated populations. This

phenomenon parallels cell plasticity with endogenous “chemical plasticity”. Significantly, either the addition of certain cell stage–specific metabolites or modulation of the corresponding metabolic pathways consistently promoted stem cell self-renewal or induced their specific differentiation, thereby demonstrating how metabolites and metabolic mechanisms control cell fate. Furthermore, this study illuminated how a switch from glycolysis in ESCs to oxidative metabolism in differentiated cells orchestrates the stem cell regulatory network.

Recent studies on hESC survival shed light on how cell adhesion mechanisms control stem cell fate. In contrast to mESCs, dissociated single hESCs survive poorly, which is problematic for routine large-scale culture and clonal selection. By screening a small collection of kinase inhibitors, Watanabe *et al*. initially found that the Rho-associated Kinase (ROCK) inhibitor Y27632 could promote the survival of dissociated hESCs (Watanabe et al., 2007). To gain a better understanding of the molecular mechanisms governing hESC survival, we performed a high-throughput phenotypic screening of 50,000 novel synthetic compounds to identify small molecules that promote hESC survival after trypsin dissociation (Xu et al., 2010). From the primary screening and subsequent structure-activity relationship (SAR) study, two distinct potent compounds—a 2,4-disubstituted thiazole (named thiazovivin/Tzv, in Figure 2) and a 2,4-disubstituted pyrimidine (named Pyrintegrin/Ptn)—were identified to significantly increase single-cell survival with enhanced self-renewal. Further characterization uncovered that Tzv can promote cell survival in both adherent and suspension culture conditions, while Ptn promotes cell survival only in adherent culture conditions. Affinity pull-down experiments using a Tzv-immobilized matrix (along with other studies) revealed that Tzv enhances E-cadherin stability and cell-cell interactions through the inhibition of ROCK, and Ptn regulates cell-ECM interactions to indirectly enhance E-cadherin. Remarkably, at the cell surface, the E-cadherin-mediated cell-cell interactions and integrin-mediated cell-ECM interactions form a positive feedback regulatory loop via inhibition of Rho-ROCK signaling pathways to regulate the survival of hESCs. Concurrent studies also revealed that hESC dissociation (breakdown of E-cadherin interaction) led to a long-lasting apoptotic cellular contraction due to myosin hyperactivation in a Rho/ROCK dependent manner. Inhibition of ROCK or myosin heavy chain ATPase (by blebbistatin) diminished actomyosin contraction and rescued the apoptosis of dissociated hESCs (Chen et al., 2010a; Ohgushi et al., 2010; Walker et al., 2010).

Interestingly, these cell adhesion mechanisms are also involved in controlling naive *vs*. primed pluripotency states. mESCs and hESCs exhibit differences in their use of and dependence on E-cadherin- or integrin-mediated cell-adhesion mechanisms for cell maintenance, respectively. This is in part due to differences in E-cadherin expression and stability in the two pluripotency states, which are controlled by the different cell culture signaling environments. Combinations of overexpressing pluripotency factors (*e.g*., Oct4, Klf4, Nanog, E-cadherin etc.) with signaling and metabolic modulation (e.g., treatment with inhibitors of MEK, transforming growth factor β (TGFβ) receptor, GSK3, or ROCK, or compounds promoting glycolytic metabolism) have been shown to promote transition of primed pluripotency toward naïve pluripotency (Chou et al., 2008; Guo et al., 2009; Hanna et al., 2010; Xu et al., 2010; Zhou et al., 2010). Consequently, cells transitioned toward the naïve state rely more heavily on E-cadherin to mediate cell adhesion and grow in a more three-dimensional architecture, which again feeds back to control cell maintenance. The auto-regulatory networks consisting of cell signaling, metabolic, transcriptional and adhesion mechanisms therefore reveal a robustness and coordination of cell fate/state transition. They also suggest the existence of a wide range of self-renewal states created by combined differential utilization of those mechanisms, which exhibit differences in survival, self-renewal and differentiation propensity. Practically speaking, creating and maintaining

human cells in the naive pluripotency state may allow for more robust cell growth and survival, cell engineering (e.g., enhanced gene targeting efficiency due to more open chromatin structure and better cell survival under clonal selection), and a better translation of knowledge gained on mESCs toward hPSCs. In addition, although recent studies demonstrated that mEpiSCs readily generated chimeras with contributions to all somatic tissues including primordial germ cells when transplanted to post-implantation embryos in whole embryo culture (Huang et al., 2012), it is technically more challenging, if not impossible, to generate genetically manipulated animals using EpiSCs that cannot efficiently incorporate into ICM of pre-implanted embryos (Brons et al., 2007; Tesar et al., 2007). Derivation of authentic ICM-stage-like PSCs from species other than rodents, including non-human primates, are highly valuable and remains to be accomplished.

Self-renewal of tissue-specific stem and progenitor cells

Because of their endogenous functions, tissue-specific stem cells have been long pursued for therapeutic purposes. One important example is the transplantation of hematopoietic stem cells (HSCs) from cord blood, bone marrow or mobilized peripheral blood for treating a variety of blood disorders (e.g., leukemia). However, in contrast to PSCs, it remains a significant challenge to expand most types of tissue-specific stem and progenitor cells *in vitro*. For example, one of the main bottlenecks of HSC transplantation is the difficulty in obtaining sufficient numbers of immunologically matched HSCs. These deficiencies reflect our limited understanding of adult stem cell regulation, especially within their *in vivo* microenvironment (also called the stem cell niche) (Watt and Hogan, 2000).

A high-content chemical library screen to examine compounds that affect CD34 and CD133 expression in primary human $CD34^+$ cells identified a synthetic purine derivative, StemRegenin 1 (SR1, 5 in Figure 2), which promotes HSC self-renewal in conjunction with HSC expansion cytokines *in vitro* (Boitano et al., 2010). SR1 treatment led to a 50-fold increase in cells expressing CD34 and a 17-fold increase in cells that retained the ability to engraft immunodeficient mice. Transcriptome analyses of SR1-regulated gene expression identified a mechanism whereby SR1 promoted $CD34^+$ cell expansion through direct binding and inhibition of the aryl hydrocarbon receptor. It is of significant interest to continue exploring and characterizing the clinical utilities of either SR1-expanded cells or SR1 itself as a therapeutic agent *in vivo*.

As HSCs reside within a low-perfusion, hypoxic niche in the bone marrow with restricted nutrient supply, inhibition of mTOR, a key nutrient-sensing regulator, may likely mimic this *in vivo* HSC micro-environment. High dependence on glycolysis for energy supply is another fundamental characteristic of LT-HSCs. During glycolysis, glucose is converted to pyruvate and then anaerobically to lactate or aerobically to acetyl-CoA for use in mitochondrial metabolism. Pyruvate dehydrogenase (PDH) catalyzes the conversion of pyruvate to acetyl-CoA. LT-HSCs express high level glycolytic enzymes, including PDH kinase, which inhibits PDH activation and maintains glycolytic flow by suppressing the influx of glycolytic metabolites into mitochondria. Recently, Takubo *et al*. demonstrated that hypoxia-inducible factor-1α (HIF-1α)-mediated PDH kinase activation is essential to maintain LT-HSC cell cycle quiescence, stemness and hematopoietic repopulation potential after transplantation (Takubo et al., 2013). In addition, 1-aminoethylphosphinic acid (1-AA), a PDH inhibitor, was shown to maintain LT-HSCs, but not short term HSCs and multipotent progenitors for 4 weeks during *in vitro* culture under a standard cytokine condition. 1-AA treatment inhibited cell proliferation, but preferentially maintained LT-HSC frequency, suggesting metabolic control by PDH kinase may represent a promising strategy to modulate HSC cell cycle and maintenance (Takubo et al., 2013).

Because it is practically difficult to non-invasively isolate most types of adult stem cells from specific tissues, the derivation and expansion of tissue-specific stem cells from PSCs represent an attractive alternative approach. A recent example highlights how novel combinations of small molecules could be developed for expanding primitive neural stem cells (pNSCs) from hESCs in culture (Li et al., 2011). It was found that under chemically defined conditions, combining a GSK3 inhibitor (i.e., CHIR99021) with TGFβ and Notch signaling pathway inhibitors induced an efficient conversion of monolayer-cultured hESCs into homogenous primitive neuroepithelia within one week. Remarkably, combination of LIF, CHIR99021 and the TGFβ-receptor inhibitor SB431542 (6 in Figure 2) effectively captured and enabled these pNSCs to stably self-renew over the long-term following serial passages. The underlying mechanisms of this strategy mainly involve the synergy of 1) TGFβ/Activin pathway inhibition that induces neutralization of hESCs by destabilizing pluripotency and blocking mesendoderm differentiation (Pitchford et al., 2009), and 2) GSK3 inhibition that promotes neural stem cell self-renewal (Kim et al., 2009; Mao et al., 2009). The generated pNSCs represent pre-rosette-stage neuroepithelia, which are distinct from typical basic fibroblast growth factor (bFGF)-dependent neural precursor cells that exhibit glial differentiation propensity and restricted response to morphogens. Notably, the long-term expanded pNSCs cultured in the appropriate small molecule/LIF treatment conditions maintain their high neurogenic differentiation propensity, remain highly plastic to instructive regional patterning cues toward midbrain and hindbrain neuronal subtypes, and exhibit appropriate *in vivo* engraftment. Clearly, applying such a strategy to other lineages and developmental stages is a novel area of research that could lead to the development of new cell-based therapies.

Small molecules that induce stem cell differentiation

hPSCs will not be directly utilized for most applications. Typically, hPSCs would be first differentiated into a desirable cell population. Ongoing challenges in developing differentiation paradigms are (1) the need for improved understanding of the developmental mechanisms underlying the generation of target cell types, so that lineage specification can be better recapitulated *in vitro* via precise manipulation of differentiation signals, (2) better methods to isolate, capture, and expand intermediate precursor cell populations to promote controlled step-wise differentiation, and (3) better methods to promote the maturation of differentiated cells to match the adult phenotype.

Chemical approaches have been particularly useful for more conveniently and precisely tuning differentiation signals, allowing for improved synchronization and acceleration of differentiation, increased differentiation efficiency, and for normalizing the various differentiation propensities of diverse hPSC lines. For example, one strategy to synchronize differentiation from each step and reduce significant heterogeneity in the final desired differentiated cell population is to expand PSC-derived stage-specific stem and progenitor cells by small molecules during the differentiation process (Li et al., 2011). Such strategy provides a "check-point" to generate more homogenously differentiated functional cell types by skipping PSCs and using pure intermediate cells as a starting population, thereby removing some of the differentiation steps required.

Small-molecule modulators of key developmental pathways, including Wnt, FGF, Hedgehog, Notch and BMP/TGFβ, have been shown to play essential roles in hPSC differentiation. For example, based on known mechanisms of neural development and hESC neuralization, Chambers *et al* showed that the combination of Noggin protein (a BMP antagonist, which can be replaced by LDN193189, a small-molecule inhibitor of the BMP receptor) with SB431542 rapidly and efficiently promotes the neural induction of hPSCs (>80%) in monolayer (Chambers et al., 2009). These two inhibitors appear to function

synergistically to destabilize hPSC self-renewal, promote neural induction, and block cell differentiation toward trophectoderm, mesoderm and endoderm lineages (where BMP and TGFβ signaling have inductive effects). Based on this dual-Smad inhibition protocol, they further demonstrated that floor plate and midbrain dopamine neurons could be more effectively generated with additional instructive cues, such as the Hedgehog and Wnt signaling small-molecule agonists purmorphamine and CHIR99021, respectively (Fasano et al., 2010; Kriks et al., 2011). More recently, Chambers *et al*. also devised a screening of combinations of small molecules with known functions in regulating essential neural developmental pathways, including TGFβ, BMP, Wnt, FGF, VEGF, Notch, and Hedgehog. They found that the combination of five small molecules (including SB431542, LDN-193189, CHIR99021, SU5402 inhibiting FGFR and VEGFR, and DAPT inhibiting γ-secretase) induced differentiation of hPSCs into nociceptor neurons in a much accelerated manner with >75% efficiency within 10 days (Chambers et al., 2012). These studies suggest that directed PSC differentiation towards a specific lineage can be achieved by deliberately combining inductive signals that promote the desired cell lineage with inhibitory signals that block PSC self-renewal and differentiation toward undesired lineages.

Chemical screening approaches have also been useful to identify novel small molecules that influence stem cell differentiation. For example, phenotypic screens have identified IDE compounds (7 in Figure 2) that could induce definitive endoderm (DE) differentiation of ESCs (Borowiak et al., 2009), and indolactam V (8 in Figure 2), a compound that could induce Pdx1 expression from hESC-derived DE cells (Chen et al., 2009). Cardiac differentiation from hPSCs exhibits significant variation in efficiency among different hPSC lines, especially in monolayer culture. To address this challenge, screening and application of small molecules that can enhance cardiac differentiation have proved to be particularly useful. It had been reported that temporal modulation of the canonical Wnt signaling during development and ESC differentiation plays a key role in cardiac specification: activation of the Wnt signaling promotes cardiac differentiation at early developmental stages while inhibits it later (Naito et al., 2006; Ueno et al., 2007). Consistent with the earlier findings, several studies more recently identified or devised conditions with Wnt signaling small molecule modulators for more efficient and robust cardiac differentiation from hPSCs. For example, an αMHC-eGFP reporter-based screen in monkey ESCs identified a new small molecule (KY02111, 9 in Figure 2) that could promote cardiac induction when applied on day 4–8 of differentiation after hPSCs were first treated with CHIR99021 for 3 days. Initial characterization suggested KY02111 acted downstream of GSK3β to suppress Wnt signaling, but its precise target remains unknown (Minami et al., 2012). Similarly, temporal and sequential use of CHIR99021 and IWR-1endo (10 in Figure 2, a Tankyrase inhibitor that inhibits Wnt signaling through stabilizing axin) (Gonzalez et al., 2011) or CHIR99021 and IWP compounds (11 in Figure 2, Porcupine inhibitors that inhibit Wnt signaling through blocking Wnt protein palmitylation and secretion) were developed to enhance cardiac differentiation of hPSCs (Lian et al., 2012). These studies suggest that modulation of Wnt signaling in a precise time window (*i.e*., first activate to initiate mesoderm induction and then inhibit to facilitate cardiac specification), is essential for efficient cardiac differentiation.

For *in vivo* applications of PSC-derived lineage-specific cells, residual undifferentiated PSCs in the population may result in tumor formation. Aiming to discover pluripotent cell-specific inhibitors, a high throughput screening of cell viability using hESCs was carried out against 52,448 compounds, and then primary hits were counter screened using hESC derivatives and human cancer cell lines. Among fifteen confirmed hits (named as PluriSIns), nine compounds share a common structure moiety (phenylhydrazine) and the most potent and selective one, PluriSIn#1 (12 in Figure 2), was further characterized. It was revealed that PluriSIn#1 inhibits stearoyl-CoA desaturase-1 (SCD1) and specifically induces endoplasmic

reticulum stress and cell apoptosis in pluripotent cells, but not in somatic cell types. Pretreatment of hESCs with PluriSIn#1 for 48 hours deprived their ability to form teratoma after transplanted into immunodeficient mice. Not only would PluriSIn #1 be useful for eliminating PSCs when transplanting their derivatives *in vivo*, as SCD1 is a key enzyme in oleic acid biosynthesis, this study also revealed the unique dependence of pluripotent state on oleate (Ben-David et al., 2013).

Small molecules that modulate reprogramming

Induced pluripotency

The discovery of small molecules that could induce and enhance the reprogramming of cells to the pluripotent state would offer several significant advantages over TF-based approaches, including reducing the risks of genetic manipulation with the oncogenic reprogramming factors, enhancing reprogramming efficiency, improving the qualities of generated iPSCs, and changing the process to be directed/deterministic. A prevailing logic and practical strategy to develop small molecule-based reprogramming has been first to identify small molecules that can replace each as well as combinations of these reprogramming TFs in different cellular reprogramming contexts (e.g., using different starting somatic cell types that endogenously express some of the reprogramming TFs, with different ectopically expressed TFs, and/or with added small molecules and growth factors), and then to optimize sequential and combinatorial treatments with those small molecules to enable reprogramming without any other genetic factors. In the past several years, significant progress have been made in that a number of small molecules and their combinations that functionally substitute for various reprogramming TFs were identified. In addition, they were shown to significantly enhance reprogramming efficiency and accelerate reprogramming speed. Most importantly, characterizations of those small molecules revealed basic mechanisms underlying the reprogramming process. Based on their mechanisms of action, these small-molecule reprogramming compounds broadly fall into several classes, including those that modulate (1) epigenetic protein activity, (2) signal transduction pathways, (3) transcription factor activity, and (4) cell metabolism.

Using phenotypic screens with Oct4-GFP expression and colony morphology as selection criteria, BIX-01294 (13 in Figure 2), a small-molecule inhibitor of the H3K9 histone methyltransferase G9a (Kubicek et al., 2007), was identified to enable reprogramming with ectopic expression of only Oct4 and Klf4 in somatic cells. When BIX-01294 was combined with a DNA methyltransferase inhibitor RG108 (14 in Figure 2) or an L-type calcium channel agonist Bayk-8644, fibroblasts could be as efficiently reprogrammed using only Oct4 and Klf4 as with the original four TFs (Shi et al., 2008a; Shi et al., 2008b). Remarkably, BIX-01294 could substitute for Oct4 when treating neural progenitor cells transduced with Sox2, Klf4 and c-Myc. This effect is consistent with previous findings revealing that repressive H3K9 methylation is associated with the silencing of pluripotency genes (such as Oct3/4 and Rex1) during differentiation (Feldman et al., 2006), and that histone methylation is dynamic and regulated by histone methyltransferases and demethylases. BIX-01294 may therefore function to facilitate an epigenetic shift toward pluripotency by unsilencing Oct4 to promote active transcription.

Acetylated histones are associated with active gene expression. Similarly, histone deacetylase inhibitors (including valproic acid (VPA) and sodium butyrate), which had been shown in other cellular contexts to de-repress silenced genes, were found to promote mouse and human somatic cell reprogramming (Huangfu et al., 2008a; Huangfu et al., 2008b; Mali et al., 2010). In particular, VPA enabled human fibroblasts to be reprogrammed with two factors (Oct4 and Sox2) (Huangfu et al., 2008b), and facilitated the reprogramming of mouse fibroblasts into iPSCs using recombinant protein forms of the original

reprogramming TFs (Zhou et al., 2009). In addition, parnate (an inhibitor of the H3K4/9 histone demethylase LSD1, 15 in Figure 2) and EPZ004777 (16 in Figure 2, an inhibitor of DOT1-like, histone H3 methyltransferase) were identified to promote the reprogramming of human cells (Li et al., 2009b; Onder et al., 2012). Interestingly, a recent study suggested that vitamin C promoted reprogramming in part by modulating Jhdm1a/1b, two known vitamin C–dependent H3K36 histone demethylases that are potent regulators of reprogramming (Wang et al., 2011a). Consistently, Jhdm1b activation was shown to accelerate cell cycle progression and suppress cellular senescence by repressing the INK4/ARF locus. Another study demonstrated that aberrant epigenetic silencing of the imprinted Dlk1-Dio3 gene cluster, which was observed in some generated iPSC lines, could be prevented with vitamin C treatment through indirectly interfering with Dnmt3a-mediated hypermethylation of the Dlk1-Dio3 locus. The presence of vitamin C during reprogramming allowed to generate high fidelity iPSCs even from mature B cells with the capacity to generate all iPSC-mice through tetraploid complementation (Stadtfeld et al., 2012).

Modulation of cell signaling offers another way to induce and/or enhance reprogramming. Wnt signaling has been shown to play important roles in promoting cell proliferation and stem cell self-renewal (Reya and Clevers, 2005). Among its downstream effectors, β-catenin and c-Myc were shown to have positive effects on reprogramming. Consistent with these findings, Wnt3a protein was shown to enhance the reprogramming of mouse fibroblasts in the absence of c-Myc (Marson et al., 2008). We found that CHIR99021—an inhibitor of GSK3, which in turn can activate Wnt-β-catenin signaling—enabled the reprogramming of mouse fibroblasts in the absence of Sox2 and c-Myc (Li et al., 2009b). When combined with parnate, CHIR99021 facilitated the reprogramming of human keratinocytes in the absence of Sox2 and c-Myc. A key feature of reprogramming fibroblasts into iPSCs entails a dramatic phenotypic change from a spindle mesenchymal-like to a compact epithelial-like morphology, with concomitant upregulation of E-cadherin (which is highly expressed in pluripotent cells). This mesenchymal-to-epithelial-transition (MET) is required for, but may also represent a barrier to, successful iPSC reprogramming. Therefore, small molecules that facilitate the MET process were hypothesized to enhance reprogramming. Indeed, small molecules that target three known MET mechanisms, including inhibition of TGFβ receptors (by SB431542), MEK (by PD0325901), or ROCK (by thiazovivin) significantly enhanced reprogramming efficiency and accelerated reprogramming speed when added individually or in combination, by derepressing the epithelial phenotype via upregulation and stabilization of E-cadherin expression (Lin et al., 2009). This MET mechanism was further characterized in three subsequent studies, where additional small molecules that inhibit the TGFβ pathway or upregulate E-cadherin were used (Chen et al., 2010b; Ichida et al., 2009; Maherali and Hochedlinger, 2009). It was shown that TGFβ receptor inhibition by small molecules alone was sufficient to replace Sox2 during mouse fibroblast reprogramming by facilitating the activation of *Nanog* transcription (Ichida et al., 2009; Maherali and Hochedlinger, 2009). More recently, another TGFβ receptor inhibitor, A-83-01, combined with a protein arginine methyltransferase inhibitor, AMI-5, enabled the reprogramming of mouse fibroblasts transduced with Oct4 alone (Yuan et al., 2011). Additional signaling pathway modulators, including a cAMP pathway activator, 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP) (17 in Figure 2), and inhibitors of Src family kinases were shown to induce and promote reprogramming as well (Staerk et al., 2011; Wang and Adjaye, 2011).

Besides regulating epigenetic proteins or signaling pathways for controlling transcription, targeting nuclear receptors (a class of ligand-dependent transcription factors) by small molecules represents a powerful way to directly modulate transcription. An orphan nuclear receptor Nr5a2 was found to replace Oct4 in iPSC generation in the presence of ectopic expression of Sox2/Klf4/cMyc (Heng et al., 2010). Other studies revealed that another orphan nuclear receptor Esrrb plays a key role in ESC self-renewal and reprogramming to

pluripotency. Notably, it was recently found that ectopic expression of retinoic acid receptor (RAR) α/γ and Nr5a2 greatly enhanced reprogramming efficiency and kinetics. Interestingly, both RARα agonist CD 437 and RARγ agonist AM 580 were further shown to significantly enhance reprogramming (Wang et al., 2011b).

Glycolytic metabolism is advantageous for highly proliferative cells and stem cells, as it can more effectively produce various macromolecular precursors to meet metabolic and energy demands while generating fewer reactive oxygen species that can induce oxidative damage. Besides transcriptional rewiring through direct epigenetic and signaling modulation, metabolic switching from mitochondrial oxidation to glycolysis represents another fundamental mechanism operant during iPSC reprogramming. This metabolic reprogramming was mechanistically revealed when characterizing how PS48 (18 in Figure 2), a small-molecule allosteric activator of 3′ phosphoinositide-dependent kinase 1, combined with A-83-01, PD0325901 and sodium butyrate enabled the reprogramming of human adult keratinocytes, umbilical vein endothelial cells, or amniotic fluid-derived cells transduced with Oct4 alone (Zhu et al., 2010). It was shown that PS48 exerts its effects at the early stage of reprogramming by enhancing glycolysis. Consistent with this finding, a number of small molecules that act more directly on metabolic pathways and can promote glycolytic metabolism also enhance reprogramming, including fructose 2,6-bisphosphate (an activator of phosphofructokinase 1), 2,4-dinitrophenol (an oxidative phosphorylation uncoupler), as well as N-oxaloylglycine and Quercetin (which promotes hypoxia-inducible factor-1 activity). In contrast, specific glycolysis inhibitors (e.g., 2-Deoxy-D-glucose and oxalate) inhibit reprogramming without altering cell proliferation, which could potentially be used to eliminate undifferentiated PSCs from differentiated cultures. Another concurrent study confirmed that transition from somatic oxidative metabolism to pluripotent glycolysis accompanied reprogramming process through metabolomic and proteomic analysis. Chemical inhibition of glycolysis or glucose deprivation blunted reprogramming (Folmes et al., 2011). A more recent advance, which bypassed the need for genetic manipulation generated mouse iPSCs solely using a combination of small molecules that target the reprogramming mechanisms discussed above, including VPA, CHIR99021, 616452 (a TGFβ receptor inhibitor), parnate, forskolin (cAMP pathway agonist), 3-deazaneplanocin A (an S-adenosylhomocysteine hydrolase inhibitor, blocking histone methylation) and TTNPB (a RAR agonist), and has further supported the logic of small molecule-based reprogramming (Hou et al., 2013).

Lineage transdifferentiation

While cellular transdifferentiation had a long history in converting cells mostly between closely related lineages, iPSC reprogramming strategies (e.g., by employing multiple cell type–specific TFs and miRNAs) have catalyzed new advances on reprogramming fibroblasts to more distantly related or distinct cell lineages, including functional neurons (Ambasudhan et al., 2011; Caiazzo et al., 2011; Pang et al., 2011; Pfisterer et al., 2011; Vierbuchen et al., 2010; Yoo et al., 2011), neural stem cells (Han et al., 2012), macrophage-like cells (Feng et al., 2008), cardiomyocytes and hepatocytes (Huang et al., 2011; Ieda et al., 2010; Sekiya and Suzuki, 2011). Furthermore, direct transdifferentiation *in vivo* has also been achieved with ectopic expression of lineage-specific TFs *in situ* (Zhou et al., 2008; Qian et al., 2012; Song et al., 2012). Similar to the iPSC process, transdifferentiation induced by somatic lineage-specific TFs is slow and inefficient. Based on the previously reported effects that inhibition of SMAD signaling and GSK3 by small molecules could promote iPSC reprogramming and neural induction of human pluripotent stem cells, Ladewig *et al*. recently showed that the efficiency of Ascl1/Ngn2-induced neuronal conversion of human fibroblasts could be enhanced by the combination of three small molecules that inhibit GSK3 (by CHIR99021),

TGFβ receptor (ALK4/5/7, by SB431542), and BMP receptor (ALK2/3/6, by LDN193189), respectively (Ladewig et al., 2012).

Recently, a new method for transdifferentiation using the Cell-Activation and Signaling-Directed (CASD) lineage-conversion strategy was established, which employs transient overexpression of iPSC-TFs (cell activation, CA) in conjunction with lineage specific soluble signals (signal-directed, SD) to reprogram somatic cells into diverse lineage-specific cell types without entering the pluripotent state. This concept and strategy has been demonstrated by direct conversion of fibroblasts to cardiac, neural, endothelial, or definitive endoderm precursor cells using transient expression of iPSC TFs (e.g., 4 days), followed by treatment with BMP4 (Efe et al., 2011), FGF4 (Kim et al., 2011), bFGF/BMP/VEGF (Li et al., 2013), respectively. In comparison to conventional transdifferentiation, in which different cell specifications are determined by ectopic expression of different sets of lineage-specific TFs, CASD-based transdifferentiation might be advantageous in that a single set of TFs is used for all cell types, and transient gene expression might be more easily replaced with safer and more convenient methods without the risk of genetic modifications

Therapeutic development for regenerative medicine

In addition to conventional cell replacement therapies, emerging directions for regenerative medicine include (1) transplanted cells delivering therapeutic molecules through paracrine signaling to promote the repair/regeneration of target tissue; (2) *ex vivo* modulated cell transplants that have enhanced *in vivo* functions, including better survival, homing, proliferation and differentiation, or secretion of specific functional molecules; (3) conventional therapeutics (e.g., chemical or biologic drugs) that can be taken directly by patients and act *in vivo* to specifically modulate patients' own cells in the target tissue through various mechanisms, such as modulating cell fate (e.g., survival, expansion, differentiation, and reprogramming), cell behavior (e.g., migration and niche interactions), or cell state/function (e.g., quiescence and polarization) (Figure 1). The different therapeutic strategies toward developing regenerative medicine are compared in Table 1. The discussion below will focus on recent proof-of-concept advances for using small molecules in therapeutic applications.

Modulating stem cell trafficking and homing

Modulating the trafficking of endogenous stem and progenitor cells for reparative or regenerative therapies via direct cell replacement or paracrine-based mechanisms represents a fertile ground for developing powerful therapeutic strategies, which will go well beyond the initial success in the hematopoietic system and be expanded and refined in many other tissue contexts (Miller and Kaplan, 2012; Wagers, 2012).

HSC transplant requires the infusion of a sufficient number of stem cells able to home to the bone marrow and durably repopulate the hematopoiesis system. At steady state, the vast majority of HSCs reside in a specialized niche within the bone marrow, with only a small number of HSCs circulating throughout the body. However, HSCs can be mobilized into the peripheral circulation upon exposure to various stimuli. Currently, hematopoietic stem and progenitor cells (HSPCs) harvested from peripheral blood through mobilization are a major source of cells for transplantation. Granulocyte colony-stimulating factor (G-CSF) is the most widely used agent for HSPC mobilization, and its effects are mediated, at least in part, through suppression of the C-X-C chemokine receptor type 4 (CXCR-4)/stromal cell-derived factor-1 (SDF-1) retention axis (Petit et al., 2002). However, the use of G-CSF alone results in suboptimal stem cell yields in a significant proportion of patients, thereby precluding autologous transplantation. AMD3100 (1 in Figure 3) is a small-molecule antagonist of CXCR4, and a single subcutaneous dose could result in a maximum 20-fold

increase in $CD34^+$ cell counts nine hours after administration in healthy volunteers (Liles et al., 2003). Additional studies showed that the combination of AMD3100 and G-CSF is superior to G-CSF alone for HSPC mobilization (Flomenberg et al., 2005). Importantly, AMD3100 plus G-CSF can mobilize $CD34^+$ cells in non-Hodgkin's lymphoma, multiple myeloma, and Hodgkin's disease patients who are refractory to G-CSF-stimulated mobilization alone (Calandra et al., 2007). Combined with G-CSF, AMD3100 (designated as Plerixafor) is approved by the FDA for HSC mobilization for autologous transplant in non-Hodgkin's lymphoma and multiple myeloma patients. In addition to the SDF-1/CXCR4 axis, the vascular cell adhesion protein 1 (VCAM-1)/α4 integrin (very late antigen 4, VLA-4) axis mediates another important signaling pathway for HSPC homing and retention in bone marrow. In mice, BIO5192 (2 in Figure 3), a small molecule VLA-4 inhibitor, increases the mobilization of murine HSPCs 30-fold above basal levels. BIO5192 could also synergize with AMD3100 or G-CSF to induce an additive 3-fold or 5-fold increase in stem cell mobilization, respectively. Furthermore, the combination of G-CSF, BIO5192, and AMD3100 has been shown to enhance mobilization by 17-fold compared with G-CSF alone. Although the effects of VLA-4 inhibitors on human HSPC mobilization remains to be studied, these data provide strong evidence that the combination of agents targeting distinct mechanisms is a more powerful strategy for HSC mobilization than single agent alone (Ramirez et al., 2009). Interestingly, Ryan *et al*. recently demonstrated that epidermal growth factor receptor signaling was negatively correlated with G-CSF-mediated HSPC mobilization. They also found that reduction of EGFR activity using genetic approaches or by treatment with the EGFR inhibitor erlotinib increased HSPC mobilization, effects that were correlated with downstream suppression of cell division control protein-42 (Cdc42) (Ryan et al., 2010). These findings revealed a previously unknown mechanism mediating HSPC trafficking.

In addition to mobilizing stem and progenitor cells as a graft source for transplantation, *in situ* mobilized endogenous cells could be recruited to diseased tissue to stimulate therapeutic angiogenesis. SDF-1/CXCR4 axis has been implicated in homing and retention of HSCs as discussed. CD26/dipeptidylpeptidase IV is a membrane-bound extracellular peptidase that cleaves and inactivates SDF-1. Previous studies showed that inhibition or deletion of CD26 enhanced the homing and engraftment of mouse marrow long-term competitive repopulating HSCs in lethally irradiated congenic mice (Christopherson et al., 2004). It is worth noting that the SDF-1/CXCR4 axis represents a general chemotactic mechanism not restricted to bone marrow. For example, in ischemic heart tissue, SDF-1α is a major chemokine attracting endogenous endothelial progenitors that express CXCR4 and home to the injured heart (Askari et al., 2003). Zaruba *et al*. devised a reparative/regenerative strategy for myocardial infarction, whereby enhancing the recruitment of G-CSF-mobilized bone marrow stem/progenitor cells to ischemic heart tissue *in vivo* by chemical inhibition of CD26/DPP4 activity led to improved heart function through neovascularization (Zaruba et al., 2009). In ischemic heart tissue, SDF-1 is a major chemokine attracting endogenous endothelial progenitors expressing its cognate receptor CXCR4 to home and localize to the heart. However, SDF-1 is sensitive to cleavage by a number of proteases (including DPP4). The authors demonstrated that combined intraperitoneal administration of G-CSF and the DPP4 inhibitor, diprotin A (3 in Figure 3), enhanced the recruitment of $CXCR4^+$ stem/progenitor cells (including endothelial progenitors) to the myocardium, improved myocardial function by increasing neovascularization and increased animal survival. Previously, DPP4 activity in donor HSCs had been shown to negatively regulate HSC engraftment after transplantation. Consequently, *ex vivo* treatment of HSCs with a DDP4 inhibitor was demonstrated to enhance HSC homing to the bone marrow niche and increase the efficiency of HSC transplantation through preventing SDF-1 cleavage (Christopherson et al., 2004). Clearly, a better understanding of stem cell trafficking and interaction within the target niche will lead to improved strategies to fully harness the therapeutic potential of

endogenous stem and progenitor cells. A more recent study showed SDF-1 also enhances the expansion of hematopoietic progenitor cells (HPCs), which can be further enhanced by DPP4 inhibition. Unexpectedly, this study showed DPP4 has a more general role in regulating hematopoietic cytokines. DPP4 cleaves within the N-termini of mouse and human GM-CSF, G-CSF and erythropoietin. Truncated cytokines act in a dominant-negative fashion to inhibit the activity of their full-length counterparts. Suppression of DDP4 activity by a FDA approved inhibitor Sitagliptin potentiates the activity of hematopoietic cytokines and accelerates hematopoietic recovery after chemotherapy or radiation in mice (Broxmeyer et al., 2012).

The manipulation of cytokine receptors is another fruitful approach to enhance stem cell homing. Through a phenotypic chemical screen examining HSCs in the zebrafish aorta-gonad-mesonephros region (where the definitive HSC pool initially arises), North *et al*. identified that a number of known small molecules enhancing prostaglandin E2 (PGE2) synthesis, including PGE2 itself, were capable of increasing HSC numbers (Goessling et al., 2009; North et al., 2007). *Ex vivo* temporal treatment of murine and human HSCs with 16,16-dimethyl-PGE2 (dmPGE2, 4 in Figure 3), a more stable analog of PGE2, enhanced their engraftment *in vivo* possibly by inducing the transcription of genes involved in HSC homing, including CXCR4. These findings rapidly led to the design of clinical studies to test whether *ex vivo*, dmPGE2-treated human cord blood cells could improve transplantation outcomes in adult patients with hematologic malignancies.

Modulating endogenous regeneration and cell differentiation

Many lower organisms (e.g., planarian worms and newts) possess remarkable regenerative capacity upon injury (e.g. complete limb regeneration after amputation). While some tissues/organs in mammals exhibit significant epimorphic regeneration upon injury during embryonic and neonatal stages, many adult tissues/organs utilize tissue-specific stem cells to maintain homeostasis and exhibit restricted reparative and regenerative capabilities. Various *in vitro* reprogramming and *in vivo* regeneration studies unambiguously suggest that mammalian systems retain the intrinsic ability to respond to appropriate instructive cues to regenerate. Consequently, studies aimed at identifying these signals and translating them into regenerative therapeutics that can awaken and orchestrate a robust regeneration for each tissue type within their niche drive continued research interests and efforts.

Aged tissues, including their stem cells, do not regenerate upon tissue attrition or damage as efficiently as in a young individual, which is correlated with aging-related diseases and degeneration. Rejuvenation of aged stem cells is therefore an attractive strategy to prevent and treat these disorders. Recent mouse studies showed that multiple stem and progenitor cell types in aged tissues, including the hematopoietic system, display hyperactivation of Cdc42 in comparison with young animals (Wang et al., 2007). Cdc42 is a Rho-family GTPase and plays key signaling roles in cell growth and development, cytoskeletal reorganization, and cell polarity. It was found that constitutively increased Cdc42 activity resulted in aging-related phenotypes in young mouse HSCs, including reduced HSC self-renewal and homing activity, impaired capacity in supporting peripheral blood leukocyte numbers, erythropoiesis, and increased contribution of cells to the myeloid lineage, suggesting a causative role of Cdc42 hyperactivation in HSC aging. Interestingly, young HSCs are polarized cells with asymmetrical distribution of cytoskeletal and polarity proteins together with Cdc42, while aged HSCs are mainly apolar. Down-regulation of Cdc42 activity by the small molecule CASIN (5 in Figure 3) restored polarity and rejuvenated aged HSC functions *in vivo*. CASIN treatment increased histone H4K16 acetylation in LT-HSCs, an epigenetic feature highly enriched in young LT-HSCs (Florian et al., 2012).

Adult neurogenesis takes place in mainly two regions in mammals, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus. Although the functional relevance of adult neurogenesis is still being investigated, evidence showed that neurogenesis is impaired in aging-related cognitive deficits and in disorders such as Alzheimer's disease (AD). Modulating endogenous neurogenesis may therefore hold promise for the treatment of cognitive and neurodegenerative diseases. Pieper *et al*. recently reported a study using an impressive *in vivo* chemical screen to identify new pro-neurogenic small molecules in mice. They tested 1,000 chemicals for their ability to stimulate neurogenesis in the SGZ. Initially, small pools of 10 compounds were infused into the left ventricle of adult mice. The effective pools were further deconvoluted to identify individual active compounds. One of the compounds, designated as P7C3 (pool 7 compound 3, 6 in Figure 3), enhanced the survival of newborn neurons apparently without stimulating neural precursor cell proliferation. *In vivo* administration of P7C3 restored morphological and electrophysiological deficits in the dentate gyrus of mice lacking neuronal PAS domain protein 3 (Npas3), a critical transcriptional factor for neurogenesis. Prolonged treatment of aged rats with P7C3 enhanced neurogenesis and ameliorated cognitive decline. Although the precise target of P7C3 remains unknown, P7C3 inhibited apoptosis of newborn neurons and preserved mitochondrial membrane integrity (Pieper et al., 2010). Subsequent structural optimization studies of P7C3 led to the generation of more potent analogs with improved pharmacokinetic properties (MacMillan et al., 2011), which may enable subsequent pre-clinical and clinical studies for treating neurodegenerative diseases.

In another study, Wang *et al*. showed atypical protein kinase C (aPKC) isoforms could regulate the transition of radial precursors to neurons during embryonic cortical neurogenesis through activating transcriptional coactivator CREB-binding protein (CBP). Knockdown of either aPKCζ or aPKCι reduced the neurogenisis of mouse embryonic cortical radial precursors without influence on their proliferation and apoptosis. As a previous study had demonstrated that an anti-diabetic agent metformin can activate the aPKC-CBP pathway through activation of AMPK in liver (He et al., 2009), they tested whether metformin could enhance neurogenisis through activation of aPKC-CBP pathway. Interestingly, metformin treatment of *in vitro* cultured cortical precursors doubled the percentage of βIII-tubulin-positive neurons in 3 days through activation of aPKC-CBP pathway. More significantly, administration of metformin *in vivo* increased adult olfactory and hippocampal dentate gyrus neurogenesis and enhanced spatial memory formation in adult mice by a water maze experiment (Wang et al., 2012). These data is reminiscent of a positive correlation between dietary restriction and hippocampal neurogenesis (Lee et al., 2000), and provided insights into the energy metabolism and neurogenesis.

In addition to protecting endogenous cells and/or promoting their regeneration, modulating endogenous cell differentiation *in vivo* by small molecule–based therapeutics could also be used for the treatment of degenerative diseases and injuries. To identify such lead compounds, *in vitro* assays using physiologically or pathologically relevant cells with a disease-modifying phenotypic readout could be developed. For example, phenotypic screening using chondrogenesis as a readout identified a novel small molecule, kartogenin (KGN, 7 in Figure 3), that could induce the differentiation of chondrocytes from cultured primary human bone marrow mesenchymal stem cells. Interestingly, KGN could also protect primary bovine articular chondrocytes and cartilage explants cultured in the presence of tumor necrosis factor α and oncostatin M (which induces cytokine-mediated chondrocyte damage). In a mouse joint injury model, intra-articular administration of KGN induced the regeneration of cartilage matrix, reduced blood-borne pathological markers of cartilage damage, and alleviated osteoarthritis-induced pain. The efficacy of KGN is attributed to its chondrocyte-protective effects along with its ability to direct the chondrogenesis of cartilage-resident mesenchymal stem cells. Mechanistic studies revealed that KGN binds to

filamin A, thereby disrupting its interaction with the transcription factor core-binding factor β subunit (CBFβ) in cytoplasm; this leads to the nuclear localization of CBFβ in human mesenchymal stem cells to regulate the CBFβ-RUNX1 transcriptional program. Further development of KGN-like compounds may lead to novel therapies for treating osteoarthritis (Johnson et al., 2012).

Hair cells are postmitotic sensory neurons for the detection of sound in cochlea and cannot regenerate themselves. Hair cell loss induced by noise trauma leads to irreversible deafness. During development, lateral inhibition mediated by Notch signaling has been shown to regulate cell fate determination of prosensory epithelial cells to generate a balanced mixture of sensory hair cells and supporting cells. Notch signaling activation can repress hair cell differentiation factor Atoh1 through its downstream target Hes5. Jeon *et al*. found Notch pathway inhibition by γ-secretase inhibitors induced hair cell differentiation of inner ear stem cells in vitro (Jeon et al., 2011). In a mouse genetic model with specific and acute hair cell damage, a follow-up study showed the inner ear local administration of a potent γ-secretase inhibitor LY411575 (that blocks Notch pathway, 8 in Figure 3) could increase the number of hair cells in the outer hair cell region in organ of Corti. Interestingly, the recovery of hair cells was accompanied by the decrease of supporting cells. Lineage tracing experiments demonstrated the trans-differentiation of supporting cells into hair cells upon LY411575 treatment in the mice with acoustic trauma. It remains to be determined whether the trans-differentiation passes through a stem cell/progenitor stage. Mechanistically, noise exposure can transiently activate Notch pathway and up-regulate Hes5 expression in adult mouse cochlea. LY411575 administration abolishes Hes5 up-regulation and increases the expression of Atoh1. While these data suggested that LY411575 treatment may be only effective in acute acoustic trauma in the transient Hes5 up-regulation window (Mizutari et al., 2013), such studies provided a proof-of-principle validation toward developing hair cell regenerative therapy.

CONCLUSION

The fields of stem cell research and regenerative medicine are thriving with breakthrough discoveries and enormous growth in recent years. In addition, innovative discovery technologies and new advances in other fields have provided unprecedented opportunities to accelerate translation of stem cell biology into therapies. Chemical approaches will continue to play leading roles in guiding therapeutic developments in regenerative medicine. In settings where endogenous repair and regenerative mechanisms are not adequate (e.g., acute and massive tissue damage from disease or injury), cell-based therapies may provide the best treatment option. In addition, cell-replacement therapies using functionally and phenotypically identical cells that are generated to replace cells lost to disease or injury would have superior specificity compared to conventional therapeutics. Enabling and improving the generation of such engraftable cells as well as enhancing their functions *in vivo* by chemical approaches will undoubtedly expand cell-based therapies to address many unmet medical needs. Developing conventional therapeutics to precisely stimulate endogenous repair and regeneration *in vivo* will surely improve convenience and also promises to revolutionize treatments for many devastating injuries and degenerative diseases (Figure 1). Challenges to small molecule–based approaches (e.g., specificity, pharmacokinetic properties, mechanism-based safety issues) will be addressed by combining approaches in conventional drug development with our ever-improving understanding of stem cell biology, and better discovery strategies to identify highly selective and functional cell modulators (e.g., using the high throughput and high definition screening technology based on sequencing, (Li et al., 2012) or delivery strategies (e.g., antibody-drug-conjugates).

Acknowledgments

Sheng Ding is supported by funding from National Institutes of Health, California Institute for Regenerative Medicine, and the Gladstone Institutes. We would like to thank Gary Howard and Anna Lisa Lucido for the editing of this manuscript. The authors apologize to all scientists whose research could not be properly discussed and cited in this review owing to space limitations.

REFERENCES

Ambasudhan R, Talantova M, Coleman R, Yuan X, Zhu S, Lipton Stuart A, Ding S. Direct Reprogramming of Adult Human Fibroblasts to Functional Neurons under Defined Conditions. Cell stem cell. 2011; 9:113–118. [PubMed: 21802386]

Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. The Lancet. 2003; 362:697–703.

Ben-David U, Gan QF, Golan-Lev T, Arora P, Yanuka O, Oren YS, Leikin-Frenkel A, Graf M, Garippa R, Boehringer M, et al. Selective elimination of human pluripotent stem cells by an oleate synthesis inhibitor discovered in a high-throughput screen. Cell Stem Cell. 2013; 12:167–179. [PubMed: 23318055]

Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE, Walker JR, Flaveny CA, Perdew GH, Denison MS, et al. Aryl Hydrocarbon Receptor Antagonists Promote the Expansion of Human Hematopoietic Stem Cells. Science. 2010; 329:1345–1348. [PubMed: 20688981]

Borowiak M, Maehr R, Chen S, Chen AE, Tang W, Fox JL, Schreiber SL, Melton DA. Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell Stem Cell. 2009; 4:348–358. [PubMed: 19341624]

Brons IG, Smithers LE, Trotter MW, Rugg-Gunn P, Sun B, Chuva de Sousa Lopes SM, Howlett SK, Clarkson A, Ahrlund-Richter L, Pedersen RA, et al. Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature. 2007; 448:191–195. [PubMed: 17597762]

Broxmeyer HE, Hoggatt J, O'Leary HA, Mantel C, Chitteti BR, Cooper S, Messina-Graham S, Hangoc G, Farag S, Rohrabaugh SL, et al. Dipeptidylpeptidase 4 negatively regulates colony-stimulating factor activity and stress hematopoiesis. Nat Med. 2012; 18:1786–1796. [PubMed: 23160239]

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. Capture of authentic embryonic stem cells from rat blastocysts. Cell. 2008; 135:1287–1298. [PubMed: 19109897]

Caiazzo M, Dell'Anno MT, Dvoretskova E, Lazarevic D, Taverna S, Leo D, Sotnikova TD, Menegon A, Roncaglia P, Colciago G, et al. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature. 2011; 476:224–227. [PubMed: 21725324]

Calandra G, McCarty J, McGuirk J, Tricot G, Crocker SA, Badel K, Grove B, Dye A, Bridger G. AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. Bone Marrow Transplant. 2007; 41:331–338. [PubMed: 17994119]

Cao N, Liang H, Huang J, Wang J, Chen Y, Chen Z, Yang H-T. Highly efficient induction and long-term maintenance of multipotent cardiovascular progenitors from human pluripotent stem cells under defined conditions. Cell Res. 2013

Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol. 2009; 27:275–280. [PubMed: 19252484]

Chambers SM, Qi Y, Mica Y, Lee G, Zhang X-J, Niu L, Bilsland J, Cao L, Stevens E, Whiting P, et al. Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. Nat Biotech. 2012; 30:715–720.

Chen G, Hou Z, Gulbranson DR, Thomson JA. Actin-Myosin Contractility Is Responsible for the Reduced Viability of Dissociated Human Embryonic Stem Cells. Cell stem cell. 2010a; 7:240–248. [PubMed: 20682449]

Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol. 2009; 5:258–265. [PubMed: 19287398]

Chen S, Do JT, Zhang Q, Yao S, Yan F, Peters EC, Scholer HR, Schultz PG, Ding S. Self-renewal of embryonic stem cells by a small molecule. Proc Natl Acad Sci U S A. 2006; 103:17266–17271. [PubMed: 17088537]

Chen T, Yuan D, Wei B, Jiang J, Kang J, Ling K, Gu Y, Li J, Xiao L, Pei G. E-Cadherin-Mediated Cell–Cell Contact Is Critical for Induced Pluripotent Stem Cell Generation. STEM CELLS. 2010b; 28:1315–1325. [PubMed: 20521328]

Chou Y-F, Chen H-H, Eijpe M, Yabuuchi A, Chenoweth JG, Tesar P, Lu J, McKay RDG, Geijsen N. The Growth Factor Environment Defines Distinct Pluripotent Ground States in Novel Blastocyst-Derived Stem Cells. Cell. 2008; 135:449–461. [PubMed: 18984157]

Christopherson KW, Hangoc G, Mantel CR, Broxmeyer HE. Modulation of Hematopoietic Stem Cell Homing and Engraftment by CD26. Science. 2004; 305:1000–1003. [PubMed: 15310902]

Efe JA, Hilcove S, Kim J, Zhou H, Ouyang K, Wang G, Chen J, Ding S. Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. Nat Cell Biol. 2011 advance online publication.

Fasano CA, Chambers SM, Lee G, Tomishima MJ, Studer L. Efficient Derivation of Functional Floor Plate Tissue from Human Embryonic Stem Cells. Cell stem cell. 2010; 6:336–347. [PubMed: 20362538]

Feldman N, Gerson A, Fang J, Li E, Zhang Y, Shinkai Y, Cedar H, Bergman Y. G9a–mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. Nature cell biology. 2006; 8:188–194.

Feng R, Desbordes SC, Xie H, Tillo ES, Pixley F, Stanley ER, Graf T. PU.1 and C/EBPalpha/beta convert fibroblasts into macrophage-like cells. Proc Natl Acad Sci U S A. 2008; 105:6057–6062. [PubMed: 18424555]

Flomenberg N, Devine SM, DiPersio JF, Liesveld JL, McCarty JM, Rowley SD, Vesole DH, Badel K, Calandra G. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. Blood. 2005; 106:1867–1874. [PubMed: 15890685]

Florian, Maria C.; Dörr, K.; Niebel, A.; Daria, D.; Schrezenmeier, H.; Rojewski, M.; Filippi, M-D.; Hasenberg, A.; Gunzer, M.; Scharffetter-Kochanek, K., et al. Cdc42 Activity Regulates Hematopoietic Stem Cell Aging and Rejuvenation. Cell stem cell. 2012; 10:520–530. [PubMed: 22560076]

Folmes, Clifford DL.; Dzeja, Petras P.; Nelson, Timothy J.; Terzic, A. Metabolic Plasticity in Stem Cell Homeostasis and Differentiation. Cell stem cell. 2012; 11:596–606. [PubMed: 23122287]

Folmes, Clifford DL.; Nelson, Timothy J.; Martinez-Fernandez, A.; Arrell, DK.; Lindor, Jelena Z.; Dzeja, Petras P.; Ikeda, Y.; Perez-Terzic, C.; Terzic, A. Somatic Oxidative Bioenergetics Transitions into Pluripotency-Dependent Glycolysis to Facilitate Nuclear Reprogramming. Cell metabolism. 2011; 14:264–271. [PubMed: 21803296]

Goessling W, North TE, Loewer S, Lord AM, Lee S, Stoick-Cooper CL, Weidinger G, Puder M, Daley GQ, Moon RT, et al. Genetic Interaction of PGE2 and Wnt Signaling Regulates Developmental Specification of Stem Cells and Regeneration. Cell. 2009; 136:1136–1147. [PubMed: 19303855]

Gonzalez R, Lee JW, Schultz PG. Stepwise Chemically Induced Cardiomyocyte Specification of Human Embryonic Stem Cells. Angewandte Chemie International Edition. 2011; 50:11181–11185.

Guo G, Yang J, Nichols J, Hall JS, Eyres I, Mansfield W, Smith A. Klf4 reverts developmentally programmed restriction of ground state pluripotency. Development. 2009; 136:1063–1069. [PubMed: 19224983]

Han, Dong W.; Tapia, N.; Hermann, A.; Hemmer, K.; Höing, S.; Araúzo-Bravo, Marcos J.; Zaehres, H.; Wu, G.; Frank, S.; Moritz, S., et al. Direct Reprogramming of Fibroblasts into Neural Stem Cells by Defined Factors. Cell stem cell. 2012; 10:465–472. [PubMed: 22445517]

Hanna J, Cheng AW, Saha K, Kim J, Lengner CJ, Soldner F, Cassady JP, Muffat J, Carey BW, Jaenisch R. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. Proceedings of the National Academy of Sciences. 2010

Hanna J, Markoulaki S, Mitalipova M, Cheng AW, Cassady JP, Staerk J, Carey BW, Lengner CJ, Foreman R, Love J, et al. Metastable pluripotent states in NOD-mouse-derived ESCs. Cell Stem Cell. 2009; 4:513–524. [PubMed: 19427283]

He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, Radovick S, Wondisford FE. Metformin and Insulin Suppress Hepatic Gluconeogenesis through Phosphorylation of CREB Binding Protein. Cell. 2009; 137:635–646. [PubMed: 19450513]

Heng J-CD, Feng B, Han J, Jiang J, Kraus P, Ng J-H, Orlov YL, Huss M, Yang L, Lufkin T, et al. The Nuclear Receptor Nr5a2 Can Replace Oct4 in the Reprogramming of Murine Somatic Cells to Pluripotent Cells. Cell stem cell. 2010; 6:167–174. [PubMed: 20096661]

Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature. 2011; 475:386–389. [PubMed: 21562492]

Huang Y, Osorno R, Tsakiridis A, Wilson V. In Vivo Differentiation Potential of Epiblast Stem Cells Revealed by Chimeric Embryo Formation. Cell Reports. 2012; 2:1571–1578. [PubMed: 23200857]

Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol. 2008a; 26:795–797. [PubMed: 18568017]

Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol. 2008b; 26:1269–1275. [PubMed: 18849973]

Ichida JK, Blanchard J, Lam K, Son EY, Chung JE, Egli D, Loh KM, Carter AC, Di Giorgio FP, Koszka K, et al. A Small-Molecule Inhibitor of Tgf-² Signaling Replaces Sox2 in Reprogramming by Inducing Nanog. Cell stem cell. 2009; 5:491–503. [PubMed: 19818703]

Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell. 2010; 142:375–386. [PubMed: 20691899]

Jeon S-J, Fujioka M, Kim S-C, Edge ASB. Notch Signaling Alters Sensory or Neuronal Cell Fate Specification of Inner Ear Stem Cells. The Journal of Neuroscience. 2011; 31:8351–8358. [PubMed: 21653840]

Johnson K, Zhu S, Tremblay MS, Payette JN, Wang J, Bouchez LC, Meeusen S, Althage A, Cho CY, Wu X, et al. A Stem Cell–Based Approach to Cartilage Repair. Science. 2012

Kawamata M, Ochiya T. Generation of genetically modified rats from embryonic stem cells. Proceedings of the National Academy of Sciences. 2010; 107:14223–14228.

Kim J, Efe JA, Zhu S, Talantova M, Yuan X, Wang S, Lipton SA, Zhang K, Ding S. Direct reprogramming of mouse fibroblasts to neural progenitors. Proceedings of the National Academy of Sciences. 2011

Kim W-Y, Wang X, Wu Y, Doble BW, Patel S, Woodgett JR, Snider WD. GSK-3 is a master regulator of neural progenitor homeostasis. Nat Neurosci. 2009; 12:1390–1397. [PubMed: 19801986]

Kriks S, Shim J-W, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G, Antonacci C, Buch A, et al. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson/'s disease. Nature. 2011; 480:547–551. [PubMed: 22056989]

Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, Teodoro ML, Rea S, Mechtler K, Kowalski JA, Homon CA, et al. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Mol Cell. 2007; 25:473–481. [PubMed: 17289593]

Ladewig J, Mertens J, Kesavan J, Doerr J, Poppe D, Glaue F, Herms S, Wernet P, Kogler G, Muller F-J, et al. Small molecules enable highly efficient neuronal conversion of human fibroblasts. Nat Meth. 2012; 9:575–578.

Lee J, Duan W, Long J, Ingram D, Mattson M. Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats. J Mol Neurosci. 2000; 15:99–108. [PubMed: 11220789]

Li H, Zhou H, Wang D, Qiu J, Zhou Y, Li X, Rosenfeld MG, Ding S, Fu X-D. Versatile pathway-centric approach based on high-throughput sequencing to anticancer drug discovery. Proceedings of the National Academy of Sciences. 2012

Li J, Huang NF, Zou J, Laurent TJ, Lee JC, Okogbaa J, Cooke JP, Ding S. Conversion of Human Fibroblasts to Functional Endothelial Cells by Defined Factors. Arteriosclerosis, Thrombosis, and Vascular Biology. 2013

Li P, Tong C, Mehrian-Shai R, Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, et al. Germline competent embryonic stem cells derived from rat blastocysts. Cell. 2008; 135:1299–1310. [PubMed: 19109898]

Li W, Ding S. Human Pluripotent Stem Cells: Decoding the Naïve State. Science Translational Medicine. 2011; 3 76ps10.

Li W, Sun W, Zhang Y, Wei W, Ambasudhan R, Xia P, Talantova M, Lin T, Kim J, Wang X, et al. Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors. Proceedings of the National Academy of Sciences. 2011

Li W, Wei W, Zhu S, Zhu J, Shi Y, Lin T, Hao E, Hayek A, Deng H, Ding S. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell. 2009a; 4:16–19. [PubMed: 19097958]

Li W, Zhou H, Abujarour R, Zhu S, Young Joo J, Lin T, Hao E, Scholer HR, Hayek A, Ding S. Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. Stem cells (Dayton, Ohio). 2009b; 27:2992–3000.

Lian X, Hsiao C, Wilson G, Zhu K, Hazeltine LB, Azarin SM, Raval KK, Zhang J, Kamp TJ, Palecek SP. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. Proceedings of the National Academy of Sciences. 2012; 109:E1848–E1857.

Liles WC, Broxmeyer HE, Rodger E, Wood B, Hübel K, Cooper S, Hangoc G, Bridger GJ, Henson GW, Calandra G, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. Blood. 2003; 102:2728–2730. [PubMed: 12855591]

Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, Abujarour R, Lin X, Hahm HS, Hao E, Hayek A, et al. A chemical platform for improved induction of human iPSCs. Nat Meth. 2009; 6:805–808.

MacMillan KS, Naidoo J, Liang J, Melito L, Williams NS, Morlock L, Huntington PJ, Estill SJ, Longgood J, Becker GL, et al. Development of Proneurogenic, Neuroprotective Small Molecules. Journal of the American Chemical Society. 2011; 133:1428–1437. [PubMed: 21210688]

Maherali N, Hochedlinger K. Tgf² Signal Inhibition Cooperates in the Induction of iPSCs and Replaces Sox2 and cMyc. Current biology : CB. 2009; 19:1718–1723. [PubMed: 19765992]

Mali P, Chou B-K, Yen J, Ye Z, Zou J, Dowey S, Brodsky RA, Ohm JE, Yu W, Baylin SB, et al. Butyrate Greatly Enhances Derivation of Human Induced Pluripotent Stem Cells by Promoting Epigenetic Remodeling and the Expression of Pluripotency-Associated Genes. STEM CELLS. 2010; 28:713–720. [PubMed: 20201064]

Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, et al. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. Cell. 2009; 136:1017–1031. [PubMed: 19303846]

Marson A, Foreman R, Chevalier B, Bilodeau S, Kahn M, Young RA, Jaenisch R. Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell stem cell. 2008; 3:132–135. [PubMed: 18682236]

Miller, Freda D.; Kaplan, David R. Mobilizing Endogenous Stem Cells for Repair and Regeneration: Are We There Yet? Cell stem cell. 2012; 10:650–652. [PubMed: 22704501]

Minami I, Yamada K, Otsuji Tomomi G, Yamamoto T, Shen Y, Otsuka S, Kadota S, Morone N, Barve M, Asai Y, et al. A Small Molecule that Promotes Cardiac Differentiation of Human Pluripotent Stem Cells under Defined, Cytokine- and Xeno-free Conditions. Cell Reports. 2012; 2:1448–1460. [PubMed: 23103164]

Mizutari K, Fujioka M, Hosoya M, Bramhall N, Okano Hirotaka J, Okano H, Edge Albert S.B. Notch Inhibition Induces Cochlear Hair Cell Regeneration and Recovery of Hearing after Acoustic Trauma. Neuron. 2013; 77:58–69. [PubMed: 23312516]

Naito AT, Shiojima I, Akazawa H, Hidaka K, Morisaki T, Kikuchi A, Komuro I. Developmental stage-specific biphasic roles of Wnt/β-catenin signaling in cardiomyogenesis and hematopoiesis. Proceedings of the National Academy of Sciences. 2006; 103:19812–19817.

North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature. 2007; 447:1007–1011. [PubMed: 17581586]

Ohgushi M, Matsumura M, Eiraku M, Murakami K, Aramaki T, Nishiyama A, Muguruma K, Nakano T, Suga H, Ueno M, et al. Molecular Pathway and Cell State Responsible for Dissociation-Induced Apoptosis in Human Pluripotent Stem Cells. Cell stem cell. 2010; 7:225–239. [PubMed: 20682448]

Onder TT, Kara N, Cherry A, Sinha AU, Zhu N, Bernt KM, Cahan P, Marcarci BO, Unternaehrer J, Gupta PB, et al. Chromatin-modifying enzymes as modulators of reprogramming. Nature. 2012; 483:598–602. [PubMed: 22388813]

Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Sudhof TC, et al. Induction of human neuronal cells by defined transcription factors. Nature. 2011; 476:220–223. [PubMed: 21617644]

Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, Ponomaryov T, Taichman RS, Arenzana-Seisdedos F, Fujii N, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol. 2002; 3:687–694. [PubMed: 12068293]

Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Bjorklund A, Lindvall O, Jakobsson J, Parmar M. Direct conversion of human fibroblasts to dopaminergic neurons. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:10343–10348. [PubMed: 21646515]

Pieper AA, Xie S, Capota E, Estill SJ, Zhong J, Long JM, Becker GL, Huntington P, Goldman SE, Shen C-H, et al. Discovery of a Proneurogenic, Neuroprotective Chemical. Cell. 2010; 142:39–51. [PubMed: 20603013]

Pitchford SC, Furze RC, Jones CP, Wengner AM, Rankin SM. Differential Mobilization of Subsets of Progenitor Cells from the Bone Marrow. Cell stem cell. 2009; 4:62–72. [PubMed: 19128793]

Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature. 2012; 485:593–598. [PubMed: 22522929]

Ramirez P, Rettig MP, Uy GL, Deych E, Holt MS, Ritchey JK, DiPersio JF. BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. Blood. 2009; 114:1340–1343. [PubMed: 19571319]

Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005; 434:843–850. [PubMed: 15829953]

Ryan MA, Nattamai KJ, Xing E, Schleimer D, Daria D, Sengupta A, Kohler A, Liu W, Gunzer M, Jansen M, et al. Pharmacological inhibition of EGFR signaling enhances G-CSF-induced hematopoietic stem cell mobilization. Nat Med. 2010; 16:1141–1146. [PubMed: 20871610]

Schugar RC, Robbins PD, Deasy BM. Small molecules in stem cell self-renewal and differentiation. Gene Ther. 2008; 15:126–135. [PubMed: 17989703]

Sekiya S, Suzuki A. Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature. 2011; 475:390–393. [PubMed: 21716291]

Shi Y, Desponts C, Do JT, Hahm HS, Scholer HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell stem cell. 2008a; 3:568–574. [PubMed: 18983970]

Shi Y, Do JT, Desponts C, Hahm HS, Scholer HR, Ding S. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. Cell Stem Cell. 2008b; 2:525–528. [PubMed: 18522845]

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:e253. [PubMed: 18942890]

Song K, Nam YJ, Luo X, Qi X, Tan W, Huang GN, Acharya A, Smith CL, Tallquist MD, Neilson EG, et al. Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature. 2012; 485:599–604. [PubMed: 22660318]

Stadtfeld M, Apostolou E, Ferrari F, Choi J, Walsh RM, Chen T, Ooi SSK, Kim SY, Bestor TH, Shioda T, et al. Ascorbic acid prevents loss of Dlk1-Dio3 imprinting and facilitates generation of all-iPS cell mice from terminally differentiated B cells. Nat Genet. 2012; 44:398–405. [PubMed: 22387999]

Staerk J, Lyssiotis CA, Medeiro LA, Bollong M, Foreman RK, Zhu S, Garcia M, Gao Q, Bouchez LC, Lairson LL, et al. Pan-Src Family Kinase Inhibitors Replace Sox2 during the Direct Reprogramming of Somatic Cells. Angewandte Chemie International Edition. 2011; 50:5734–5736.

Takubo K, Nagamatsu G, Kobayashi Chiharu I, Nakamura-Ishizu A, Kobayashi H, Ikeda E, Goda N, Rahimi Y, Johnson Randall S, Soga T, et al. Regulation of Glycolysis by Pdk Functions as a Metabolic Checkpoint for Cell Cycle Quiescence in Hematopoietic Stem Cells. Cell stem cell. 2013; 12:49–61. [PubMed: 23290136]

Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, Gardner RL, McKay RD. New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature. 2007; 448:196–199. [PubMed: 17597760]

Ueno S, Weidinger G, Osugi T, Kohn AD, Golob JL, Pabon L, Reinecke H, Moon RT, Murry CE. Biphasic role for Wnt/β-catenin signaling in cardiac specification in zebrafish and embryonic stem cells. Proceedings of the National Academy of Sciences. 2007; 104:9685–9690.

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. Nature. 2010; 463:1035–1041. [PubMed: 20107439]

Wagers, Amy J. The Stem Cell Niche in Regenerative Medicine. Cell stem cell. 2012; 10:362–369. [PubMed: 22482502]

Walker A, Su H, Conti MA, Harb N, Adelstein RS, Sato N. Non-muscle myosin II regulates survival threshold of pluripotent stem cells. Nat Commun. 2010; 1:71. [PubMed: 20842192]

Wang J, Gallagher D, DeVito Loren M, Cancino Gonzalo I, Tsui D, He L, Keller Gordon M, Frankland Paul W, Kaplan David R, Miller Freda D. Metformin Activates an Atypical PKC-CBP Pathway to Promote Neurogenesis and Enhance Spatial Memory Formation. Cell stem cell. 2012; 11:23–35. [PubMed: 22770240]

Wang L, Yang L, Debidda M, Witte D, Zheng Y. Cdc42 GTPase-activating protein deficiency promotes genomic instability and premature aging-like phenotypes. Proceedings of the National Academy of Sciences. 2007; 104:1248–1253.

Wang T, Chen K, Zeng X, Yang J, Wu Y, Shi X, Qin B, Zeng L, Esteban Miguel A, Pan G, et al. The Histone Demethylases Jhdm1a/1b Enhance Somatic Cell Reprogramming in a Vitamin-C-Dependent Manner. Cell stem cell. 2011a; 9:575–587. [PubMed: 22100412]

Wang W, Yang J, Liu H, Lu D, Chen X, Zenonos Z, Campos LS, Rad R, Guo G, Zhang S, et al. Rapid and efficient reprogramming of somatic cells to induced pluripotent stem cells by retinoic acid receptor gamma and liver receptor homolog 1. Proceedings of the National Academy of Sciences. 2011b

Wang Y, Adjaye J. A Cyclic AMP Analog, 8-Br-cAMP, Enhances the Induction of Pluripotency in Human Fibroblast Cells. Stem Cell Rev and Rep. 2011; 7:331–341.

Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T, Takahashi JB, Nishikawa S, Nishikawa S, Muguruma K, et al. A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol. 2007; 25:681–686. [PubMed: 17529971]

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

Xu Y, Shi Y, Ding S. A chemical approach to stem-cell biology and regenerative medicine. Nature. 2008; 453:338–344. [PubMed: 18480815]

Xu Y, Zhu X, Hahm HS, Wei W, Hao E, Hayek A, Ding S. Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proceedings of the National Academy of Sciences. 2010; 107:8129–8134.

Yanes O, Clark J, Wong DM, Patti GJ, Sánchez-Ruiz A, Benton HP, Trauger SA, Desponts C, Ding S, Siuzdak G. Metabolic oxidation regulates embryonic stem cell differentiation. Nat Chem Biol. 2010; 6:411–417. [PubMed: 20436487]

Yang W, Wei W, Shi C, Zhu J, Ying W, Shen Y, Ye X, Fang L, Duo S, Che J, et al. Pluripotin Combined with Leukemia Inhibitory Factor Greatly Promotes the Derivation of Embryonic Stem Cell Lines from Refractory Strains. Stem Cells. 2009; 27:383–389. [PubMed: 19056907]

Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, Cohen P, Smith A. The ground state of embryonic stem cell self-renewal. Nature. 2008; 453:519–523. [PubMed: 18497825]

Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW, Crabtree GR. MicroRNA-mediated conversion of human fibroblasts to neurons. Nature. 2011; 476:228–231. [PubMed: 21753754]

Yuan X, Wan H, Zhao X, Zhu S, Zhou Q, Ding S. Brief report: combined chemical treatment enables Oct4-induced reprogramming from mouse embryonic fibroblasts. Stem cells (Dayton, Ohio). 2011; 29:549–553.

Zaruba M-M, Theiss HD, Vallaster M, Mehl U, Brunner S, David R, Fischer R, Krieg L, Hirsch E, Huber B, et al. Synergy between CD26/DPP-IV Inhibition and G-CSF Improves Cardiac Function after Acute Myocardial Infarction. Cell stem cell. 2009; 4:313–323. [PubMed: 19341621]

Zhang J, Nuebel E, Daley George Q, Koehler Carla M, Teitell Michael A. Metabolic Regulation in Pluripotent Stem Cells during Reprogramming and Self-Renewal. Cell stem cell. 2012; 11:589–595. [PubMed: 23122286]

Zhou H, Li W, Zhu S, Joo JY, Do JT, Xiong W, Kim JB, Zhang K, Scholer HR, Ding S. Conversion of mouse epiblast stem cells to an earlier pluripotency state by small molecules. Journal of Biological Chemistry. 2010

Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell. 2009; 4:381–384. [PubMed: 19398399]

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature. 2008; 455:627–632. [PubMed: 18754011]

Zhu S, Li W, Zhou H, Wei W, Ambasudhan R, Lin T, Kim J, Zhang K, Ding S. Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell stem cell. 2010; 7:651–655. [PubMed: 21112560]

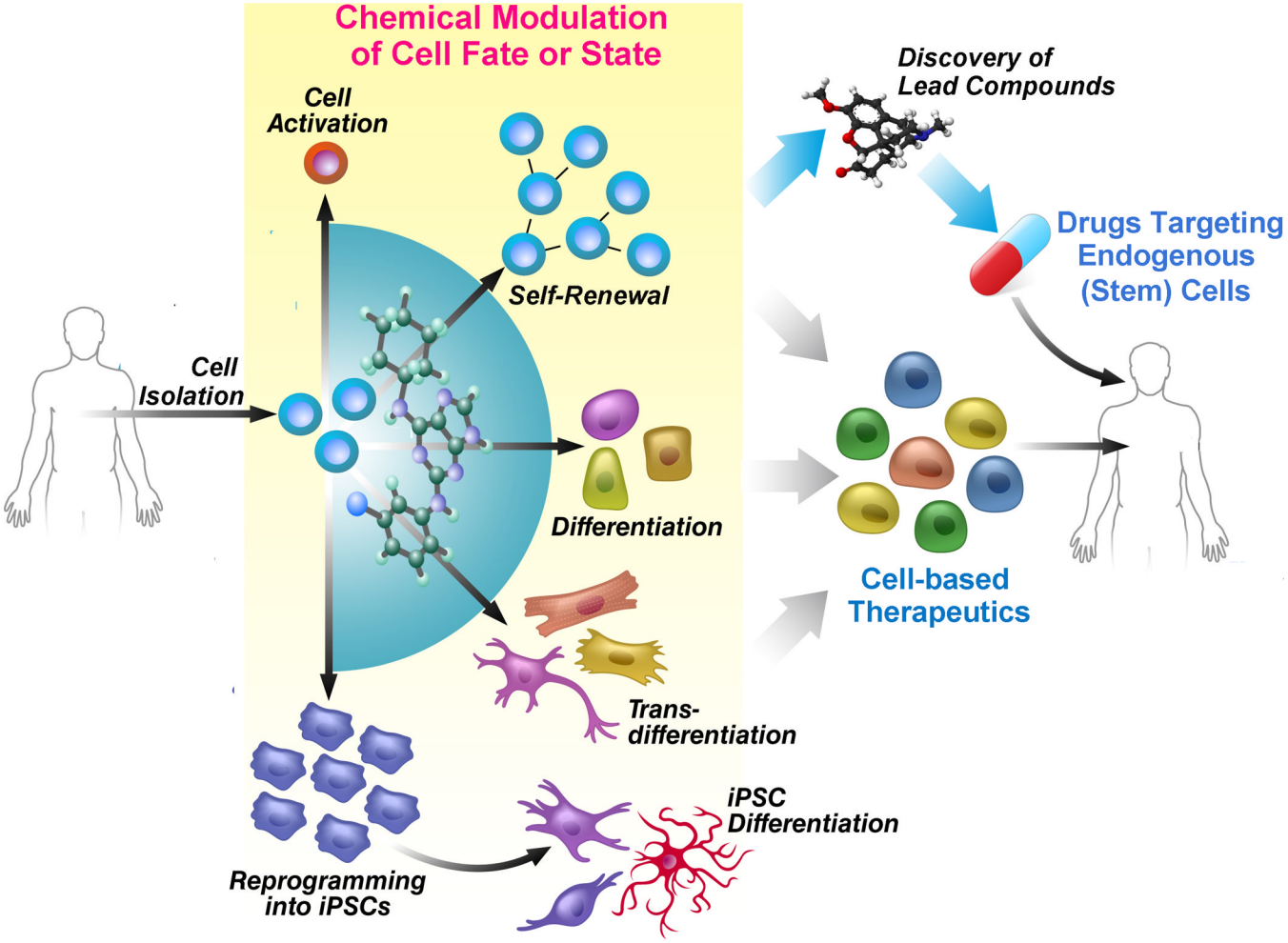

Figure 1. Chemical approaches to stem cell biology and therapeutics

Chemical approaches are applied *in vitro* and *in vivo* to manipulate cell fate toward desired therapeutic applications, which include cell activation, expansion, differentiation, somatic lineage-specific trans-differentiation and iPSC reprogramming. The functional cells generated through chemical approaches could be used as the cell source for cell-based therapy. In addition, the chemical compounds that can regulate appropriate cell fate or function could be further developed as small molecule therapeutics for modulating endogenous cells underlying disease or injury conditions.

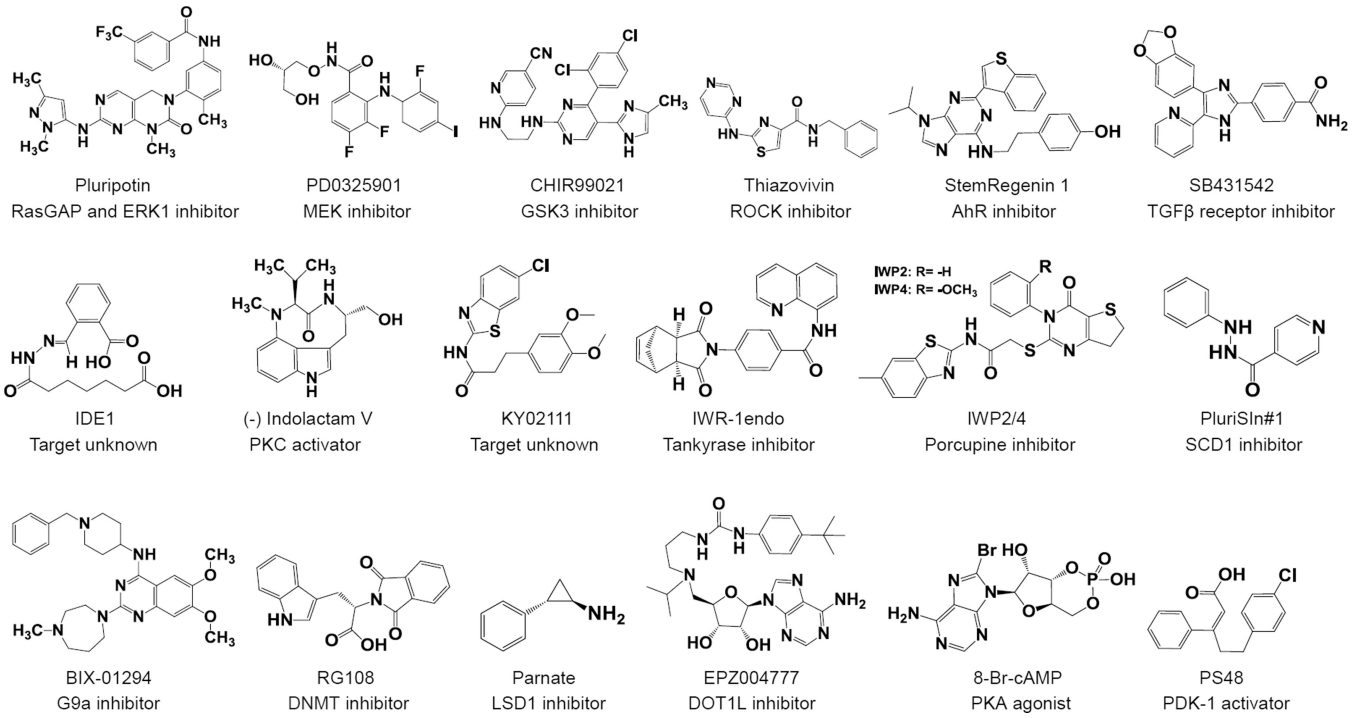

Figure 2. The structures and mechanisms of representative chemicals for cell fate modulation

Pluripotin or the combination of **PD0325901** and **CHIR99021** can support mESC self-renewal by inhibiting the differentiation-inducing signaling. **Thiazovivin** is a ROCK inhibitor that enhances hESC survival after dissociation. **StemRegenin 1** enhances HSC self-renewal by inhibition of AhR. **SB431542** (a TGFβ receptor inhibitor) or other small molecules inhibiting TGFβ signaling sustain primitive NSC self-renewal when combined with CHIR99021 and LIF; promote neural induction from PSC; or enhance reprogramming. **IDE1** promotes differentiation of PSCs into definitive endoderm (DE). **Indolactam V**, a PKC activator, promotes differentiation of PSC-derived DE cells toward pancreatic lineage. **KY02111, IWR-1endo** and **IWP2/4** can inhibit Wnt signaling through distinct mechanisms and facilitate cardiac specification of PSC-derived cardiac precursor cells. **PluriSIn#1** is toxic specifically to pluripotent cells by inhibiting SCD1. Modulation of various epigenetic proteins, including HDACs, DNMTs, HMTs, HDMs, and signaling pathways, including Wnt, cAMP and PDK-1, can induce and enhance reprogramming.

RasGAP: Ras GTPase activating protein; ERK1: extracellular signal-regulated kinase-1; MEK: Mitogen-activated protein/ERK kinase; GSK3: glycogen synthase kinase-3; ROCK: Rho-associated Kinase; AhR: aryl hydrocarbon receptor; TGFβ: transforming growth factor β; PKC: protein kinase C; SCD1: stearoyl-CoA desaturase-1; G9a: G9a histone methyltransferase; DNA MTase: DNA methyltransferase; LSD1: lysine-specific demethylase 1; DOT1L: DOT1-like, histone H3 methyltransferase; PKA: protein kinase A; PDK-1: 3´-phosphoinositide-dependent kinase-1;.

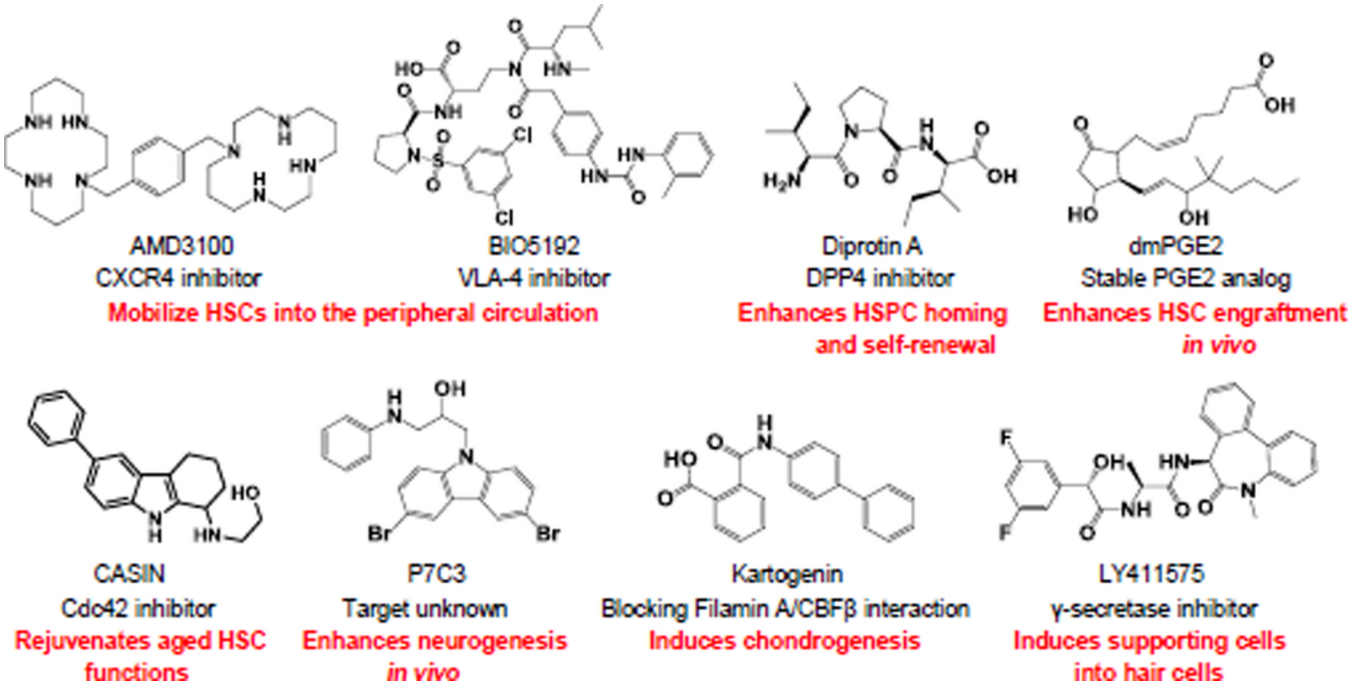

Figure 3. The structures and mechanisms of representative chemicals for modulating endogenous stem cells and tissue regeneration

CXCR4: C-X-C chemokine receptor type 4; VLA-4: very late antigen 4; DPP4: dipeptidylpeptidase IV; PGE2: prostaglandin E2; Cdc42: cell division control protein-42; CBFβ: core-binding factor β subunit;.

Table 1

The comparison of different strategies for regenerative medicine

	Cell therapy	**Enhanced cell therapy via *ex vivo* cell modulation**	**Chemical or biologic therapeutics targeting cells *in vivo***
Therapeutic Mechanisms	Replace the lost and damaged cells by healthy ones or stimulate regeneration via paracrine mechanisms	Replace the lost and damaged cells by healthy ones with enhanced capacity of homing, survival, expansion, differentiation, and engraftment etc.	Enhance the endogenous cells to repair and regenerate to restore tissues' normal functions
Immune Rejection	Possible	Can be ameliorated	Typically not applicable
Safety	Potential side effects could be due to aberrant cell function, cell-borne contamination, immune reaction, or cell carcinogenesis potential.	Potential side effects may be similar to conventional cell-based therapy with improved safety and efficacy.	Potential side effects could be due to therapeutic molecule's on-target mechanism, off-target effects, and systematic exposure.
Feasibility of Development and Use	Overall with relatively limited experiences	May have advantages over conventional cell-based therapy	Overall with extensive experiences
Cost Burdens for Patients	Relatively high	High to low	Relatively low