NATURE|Vol 453|15 May 2008|doi:10.1038/nature07042

A chemical approach to stem-cell biology and regenerative medicine

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An improved understanding of stem-cell and regenerative biology, as well as a better control of stem-cell fate, is likely to produce treatments for many devastating diseases and injuries. Chemical approaches are starting to have an increasingly important role in this young field. Attention has focused on chemical approaches that allow the precise manipulation of cells *in vitro* to obtain homogeneous cell types for cell-based therapies. Another promising approach is the development of conventional chemical and biological therapeutics to stimulate endogenous cells to regenerate. Such therapeutics can act on target cells or their niches *in vivo* to promote cell survival, proliferation, differentiation, reprogramming and homing.

Recent breakthroughs in stem-cell biology have generated enthusiasm for therapeutic developments towards tissue and organ repair and/or regeneration. These advances include insights into the intrinsic mechanisms[1–4] and the niche interactions[5] that regulate the fate of embryonic stem (ES) cells and adult stem cells. Other recent advances are the ability to manipulate and control stem-cell fate and function in a more precise and defined manner[6–9] and the ability to reprogramme lineage-committed cells to revert to more-primitive multipotent[10] states (in which the cells can form many cell types) or even pluripotent states[11] (in which the cells can form all the cell types of the body). Not only are various types of stem cell excellent model systems for studying the fundamental biology of human development and diseases, but they also provide superior vehicles compared with conventional cell lines for drug discovery (from disease modelling, target validation and high-throughput screening to development of clinical candidates). Furthermore, stem cells can be used to replace cells that have been damaged as a result of disease and injury. They can also be endogenous targets for conventional small-molecule or biological therapeutics that stimulate a body's own cells to regenerate *in vivo*.

Chemical approaches (Fig. 1) and small molecules have provided the key to many biological discoveries. Understanding of a biological phenomenon often begins by observing it and then discovering or designing ways to perturb it. Although genetic methods have been widely used for this purpose, a chemical approach offers distinct advantages. For example, small molecules typically provide a high degree of temporal control over protein function, inducing rapid inhibition or activation, and their effects are often reversible. Their effects can also be finely tuned by varying the concentrations of the small molecule. Furthermore, a single small molecule can simultaneously modulate multiple specific targets within a protein family or across different protein families. This allows the production of a desirable phenotype in a synergistically favourable manner. It also has considerable advantages when viewed from a primary screening and clinical-development perspective.

Examples of small molecules that modulate single or multiple targets[12] are retinoic acid, cytidine analogues, histone-deacetylase inhibitors and protein kinase inhibitors (Fig. 2). These chemicals have been used to modulate and dissect stem-cell phenotypes and may well have a role in treatment of stem- and progenitor-cell-based diseases such as some types of cancer.

In this review, we discuss chemical approaches to stem-cell and regenerative biology, including the identification of new chemical entities or chemically defined conditions for studying cell-fate regulation *in vitro* and *in vivo*. We also discuss ways in which such chemical approaches enhance cell-replacement therapy and the use of therapeutic molecules to control endogenous cell fate *in vivo* for the treatment of degenerative diseases, injuries, ageing and cancers.

Stem-cell modulation *in vitro*

The ability to isolate stem cells and progenitor cells from tissues and manipulate them *in vitro* gives rise to several attractive opportunities. It can provide model systems for understanding the intrinsic regulation of these cells and their interactions with exogenous signals, including those underlying development and disease. Moreover, controlling self-renewal expansion and differentiation will be necessary to generate sufficient homogeneous functional cell types for cell-based therapies. And such homogeneous and physiologically relevant cell populations would also be useful for drug discovery.

Self-renewal

Pluripotent stem cells give rise to all cell types in a body. These typically include the following: conventional ES cells derived from the inner cell mass of pre-implantation embryos[13]; germline stem cells and derivatives[14–16]; epiblast stem cells derived from post-implantation epiblast-stage embryos[17,18]; and induced pluripotent stem (iPS) cells derived from somatic cells[11]. Because ES cells are an excellent model for pluripotent stem cells, we focus on the regulation of ES cells by chemical approaches.

Conventionally, ES cells are maintained and expanded in the presence of feeder cells, serum and additional exogenous factors[13] such as leukaemia inhibitory factor (LIF) for mouse ES cells and basic fibroblast growth factor (bFGF; also known as FGF2) for human ES cells. The use of such conventional culture conditions has presented a number of problems. First, such conditions are highly variable both in the way feeder cells are used and in the composition of serum products. Thus, maintaining large-scale, consistent and robust long-term ES-cell cultures has been a challenge. Second, unknown factors from feeder cells and/or serum, as well as certain defined exogenous factors, may bias ES cells towards having specific lineage differentiation properties. They

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do this by modulating additional gene expression that coexists with the basic pluripotency network. This adds to the existing complexity of ES cell lines, especially in humans. Different ES cell lines show different self-renewal properties, as well as showing different propensities to differentiate into different lineages (or different subtypes of the same lineage) owing to their own unique genetic and epigenetic background[19]. Third, unknown factors may operate in conjunction with a specific treatment to change the outcome of a particular cellular process.

To address these problems, we carried out a high-throughput screen of 50,000 synthetic small molecules using a transgenic reporter mouse ES cell line expressing green fluorescent protein (GFP) under control of the *Oct4* gene (a gene specifically expressed in pluripotent stem cells) regulatory elements. The screen was conducted in the absence of feeder cells, serum and LIF, to search for small molecules that can maintain self-renewal of ES cells in chemically defined conditions. OCT4–GFP expression and the characteristic compact domed colony morphology of mouse ES cells were used as criteria to select primary hits in the screen. Secondary confirmation assays and structure–activity-relationship studies led to the identification of a novel compound named pluripotin (also known as SC1)[6]. Pluripotin sustains homogeneous self-renewal of mouse ES cells long-term in chemically defined medium conditions in the absence of feeder cells, serum, LIF and bone morphogenetic proteins (BMPs). The cells remain pluripotent in serially passaged culture *in vitro* without losing their germline transmission ability *in vivo*[6]. Furthermore, pluripotin functions independently of exogenous activation of LIF–STAT3, BMP–SMAD-ID and WNT–β-catenin pathways. Through affinity pull-down experiments using a pluripotin-immobilized matrix, the molecular targets of pluripotin were identified as RasGAP and extracellular-signal-regulated kinase 1 (ERK1), two endogenously expressed proteins with differentiation-inducing activity. Additional biochemical, genetic and pharmacological studies have shown that simultaneous inhibition of ERK1 and RasGAP by pluripotin or other independent methods is sufficient for long-term self-renewal of mouse ES cells[6].

From a small-molecule and drug-discovery perspective, this study demonstrated that controlling a complex phenotype (or curing a disease) can require modulation of more than one target. A single small molecule with the desirable, specific polypharmacological activity can be selected out through a rationally designed phenotypic screen. From

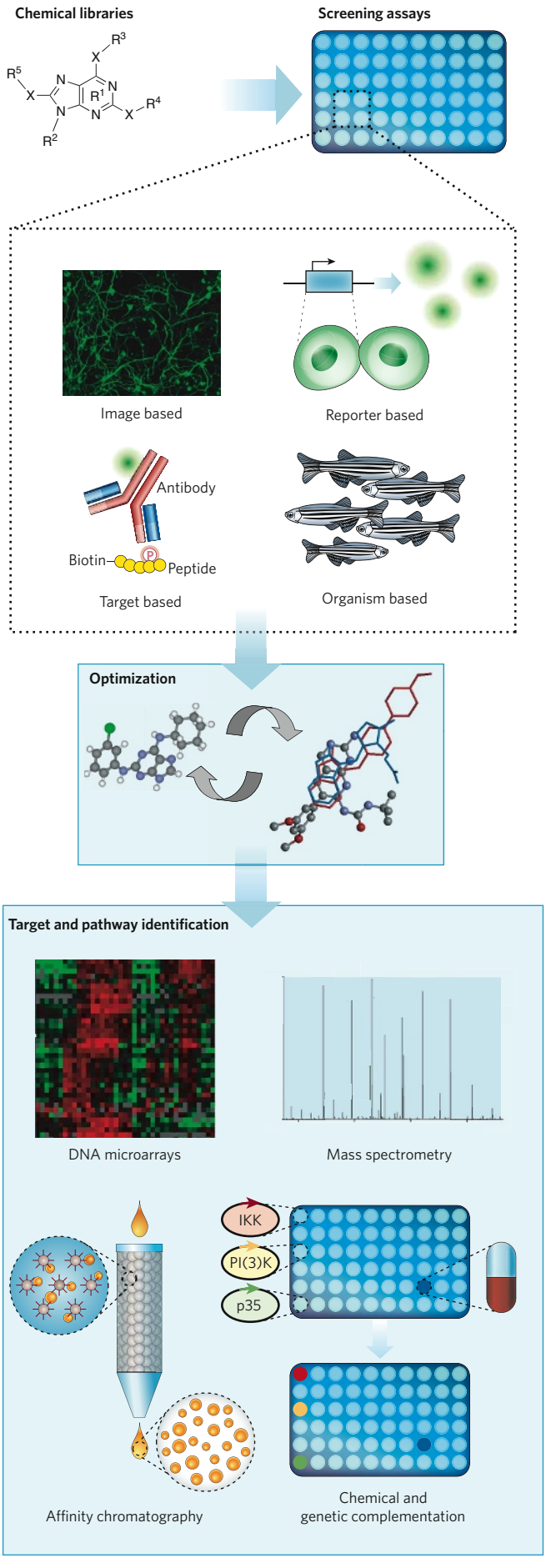

Figure 1 | A screening approach. Chemical libraries for biological screens can be assembled from synthetic compounds and natural products through chemical synthesis and/or from commercial sources on the basis of their biological fitness and molecular diversity. Screening against a defined molecular target usually exploits a protein's function (for example, enzymatic activity or signal transduction) or molecular interactions with its partners. Phenotypic screens are powerful, in that prior knowledge of defined targets and mechanisms for the given desirable phenotype is not required. Such screens can be carried out in cells or whole organisms by examining multiple markers and functional changes (for example, cell morphology and behaviour) using automated high-content imaging technologies in a high-throughput manner (see page 345). After hit compounds are identified from a primary screen, they are typically confirmed by using a series of secondary assays, which are more functional, as well as being analysed by informatics tools. Before a compound enters mechanistic studies, it is normally optimized through structure–activity-relationship studies to improve its properties, such as increasing its potency, specificity and enhancing its pharmacokinetic properties. To identify the molecular targets and pathways of an unknown compound, affinity chromatography with labelled compounds in conjunction with mass spectrometry is most commonly used. Target proteins are typically validated by gain- and loss-of-function studies (for example, through microarray analysis and RNA interference) to recapitulate the compound's activity, as well as by using other relevant biochemical and cellular assays (shown here as chemical and genetic complementation). Unbiased expression analysis and genetic epistasis may also be used to provide mechanistic insights. R^1 denotes a heterocycle; X denotes O, N, S or C. IKK, inhibitor of nuclear factor-κB (IκB) kinase; PI(3)K, phosphatidyl-inositol-3-OH kinase.

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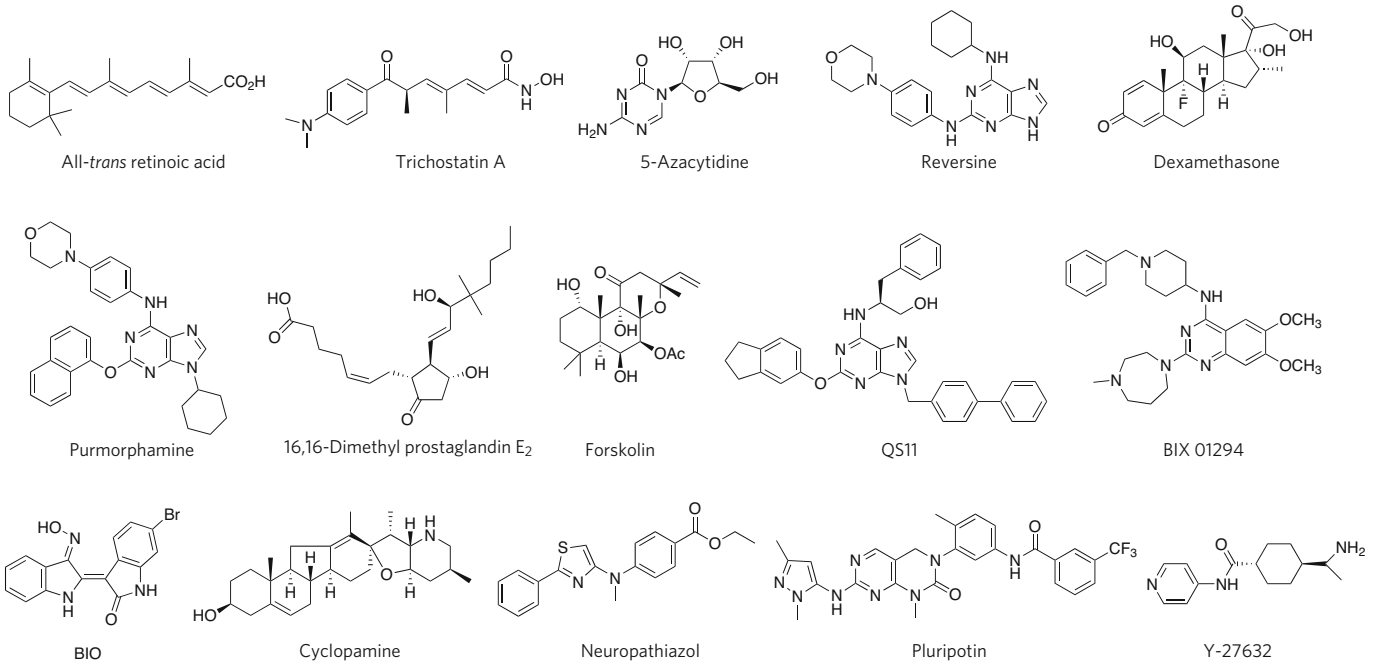

Figure 2 | Selected chemical compounds that regulate cell fate. Shown are synthetic small molecules and natural products that bind to nuclear receptors (all-*trans* retinoic acid and dexamethasone), histone-modifying enzymes and DNA-modifying enzymes (trichostatin A, BIX 01294 and 5-azacytidine), and protein kinases and signalling molecules (reversine, purmorphamine, 16,16-dimethyl prostaglandin E_2, forskolin, QS11, BIO, cyclopamine, neuropathiazol, pluripotin and Y-27632).

a stem-cell perspective, it suggests that self-renewal of ES cells, and perhaps other types of stem cell as well, may be largely driven by intrinsic regulators in the cell and does not require activation of additional pathways by exogenous factors, such as LIF or BMPs[20].

Maintenance of self-renewal in stem cells can be simply viewed as involving multiple processes of continued proliferation, as well as inhibiting differentiation and cell death. This requires a fine balance and cross-regulation between positive and negative regulators. Stem cells might autonomously express almost all gene products that are essential for self-renewal. However, endogenous expression of some differentiation-inducing genes at a certain level in the undifferentiated ES cells could cause differentiation in culture conditions that do not inhibit their negative effects. Therefore, the key to achieving self-renewal in ES cells may be to inhibit the negative effects of endogenously expressed, pluripotency inhibitory proteins (for example, proteins involved in differentiation or cell death).

A balanced self-renewal state can also be achieved through a specific combination of multiple pathway activation by exogenous factors (for example, LIF, BMPs and WNT proteins for mouse ES cells[20], or bFGF, activin and WNT proteins for human ES cells[21,22]). Although these exogenous factors at the appropriate concentrations inhibit each other's differentiation activity, they can also mediate lineage-specific gene expression, which coexists with the pluripotency gene networks, or induce specific lineage differentiation. This idea is also supported by an independent study in which a combination of three specific chemical inhibitors of the protein kinases FGF receptor (FGFR), mitogen-activated protein kinase kinase (MEK) and glycogen-synthase kinase 3 (GSK3) supports derivation and long-term self-renewal of mouse ES cells in the absence of exogenous cytokines[68]. Not only are these chemicals useful for defining cell-culture conditions and regulatory mechanisms (replacing exogenous factors and providing a platform for supporting a more reliable and robust cell culture), but they also present an opportunity for derivation of new cell lines from strains or species for which this has been difficult. For example, a combination of the three inhibitors of FGFR, MEK and GSK3 was used to derive ES cells from the mouse strain CBA and *Stat3*$^{-/-}$ mice.

Small molecules can function intracellularly to inhibit key differentiation-inducing proteins, thereby bypassing the need to express receptors for exogenous factors that inhibit differentiation. Positive results have been obtained using chemical approaches to derive pluripotent stem cells from other species (for example, rat and human) that resemble more closely the conventional inner-cell-mass-stage mouse ES cells. This is also in line with the recent finding[17,18] that the conventional human ES cells represent a later epiblast stage of pluripotent cells and have defining features in common with rodent epiblast stem cells.

Various exogenous growth factors, including bFGF[7,8,22], TGF-β and activin[21,23], WNT proteins[7,24] and insulin-like growth factor[25], support self-renewal of human ES cells in chemically defined conditions. Identification of small molecules that function similarly to pluripotin in mouse ES cells may lead to improved control of human ES-cell fate. For example, the extremely poor survival of dissociated single human ES cells had been a problem for routine large-scale culture and clonal selection of human ES cells[26]. Testing of caspase and protein-kinase inhibitors identified an inhibitor of the protein kinase ROCK, known as Y-27632, which allows survival of dissociated human ES cells. Recently, we carried out high-throughput screens of large-scale combinatorial chemical libraries in human ES cells and identified distinct small molecules that can replace bFGF in maintaining long-term self-renewal of human ES cells in a feeder-free and serum-free chemically defined condition or can potently promote single-cell survival of human ES cells. Further characterization of the mechanisms involved in the action of these small molecules will provide a better understanding of human ES-cell biology.

In contrast with the progress made with ES cells, maintaining long-term self-renewal of multipotent tissue-specific stem cells (Fig. 3Aa), especially at the most primitive stage, remains a challenge. For example, long-term haematopoietic stem cells (HSCs) quickly lose their ability to serially repopulate the entire haematopoietic system during *in vitro* culture, despite the use of multiple exogenous protein factors. Similarly, conditions with growth factors (for example, bFGF and/or EGF) can only expand primitive neural stem cells for a limited number of generations *in vitro*. In such conditions, they typically become more glia-restricted and lose the ability to be patterned to subtype-specific neuronal types[27]. Finding small molecules that can inhibit their differentiation would be useful.

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To this end, a recent study[28] used such a chemical approach to study multipotent islet1 (Isl1)$^+$ cardiovascular progenitor cells (MICPs), which reside in both embryonic and adult hearts. MICPs can generate the three main heart cell types: cardiac muscle, smooth muscle and endothelial cells[29]. A high-throughput screen was carried out to identify small molecules capable of inducing expansion of MICPs; Isl1–LacZ was used as a marker. Several compounds were identified that significantly increased MICP expansion, with the potent GSK3 inhibitor BIO being one of the strongest. This discovery led to a series of *in vitro* and *in vivo* studies of the role of the WNT–β-catenin signal (as secreted from the cardiac mesenchymal niche cells), which functions in a sequential manner first to block the specification of the MICPs from mesodermal precursor cells and then to promote their self-renewal by inhibiting their further differentiation into cardiomyocyte and smooth muscle cells. Interestingly, BIO treatment also induced expansion of human Isl1$^+$ cardiovascular progenitor cells, suggesting a conserved role of WNT-mediated signalling in self-renewal of MICPs.

Differentiation

Conventional differentiation of ES cells has been an inefficient and nonspecific process typically involving co-culture with feeder cells[30,31] or growing them in suspension to form embryoid bodies in the presence of complex serum and additional factors. The cell type of interest is then selected from a heterogeneous cell population by the expression of markers. The development of small molecules and/or chemically defined conditions for more effective stem-cell differentiation has attracted significant efforts, for practical reasons and for improving the understanding of the mechanisms regulating differentiation.

Advances in developmental biology have guided the design of directed, stepwise differentiation of stem cells in ways that recapitulate the progression of embryonic development. This was achieved by sequentially treating cells with combinations of embryonic signalling molecules. An early elegant example was the generation of functional motor neurons from mouse ES cells by first neuralizing and caudalizing (differentiating and patterning) cells with retinoic acid. Subsequently, the cells were ventralized (positioned) by a specific small-molecule agonist of the hedgehog-mediated signalling pathway[32]. Conceptually similar, but technically more sophisticated, methods have since been developed for directing human ES cells to differentiate along the following paths: neural precursor cells → subtype-specific neuronal progenitor cells → mature functional neurons[33,34]; mesendoderm → mesoderm → cardiovascular precursor cells → immature cardiac cell types → mature cardiomyocytes[35,36]; and mesendoderm → definitive endoderm → primitive gut tube → posterior foregut → pancreatic endoderm → endocrine precursor cells → hormone-producing endocrine cells[9,37].

To substantially increase the homogeneity, functionality and yield of the intermediate progenitor cells or terminally differentiated cell types, a more precise knowledge of lineage specification will be needed. Thus it would be highly desirable to identify additional small molecules (Fig. 3Ab) that can function synergistically with factors that have already been defined, such as FGF and WNT proteins. The ultimate goal is the development of specific, efficient and completely chemically defined medium conditions. Towards this goal, we have used high-content imaging-based analysis of immunostained cells with a neuron-specific marker to identify a novel synthetic small molecule named neuropathiazol from a large chemical library. This molecule can specifically induce robust differentiation of primary multipotent hippocampal neural progenitor cells into neurons, even in gliogenic conditions[38]. More recently, screening of libraries of pharmacologically active compounds using neurospheres and simple homogeneous assays has identified drugs and endogenous metabolites for inhibiting neurosphere proliferation[39], and/or promoting neuronal differentiation and/or survival[40]. Some of those small molecules — as well as others such as histone-deacetylase inhibitors[41] and serotonin-uptake blockers[42] — promote neurogenesis in cell culture, as well as *in vivo*, with specific behaviour modulation when administered directly. These studies have demonstrated the great utility of chemical approaches in regulating cell fate for *in vitro* applications. Even more importantly, they hold promise that relevant cell-fate modulation *in vitro* by small molecules will provide models and leads for therapeutic development towards *in vivo* regeneration.

Reprogramming

Mammalian tissue-specific stem cells and progenitor cells typically have restricted developmental potential *in vivo*. Such cells can only differentiate towards cell types with a more restricted potential within the same lineage boundary. However, in certain conditions, mammalian

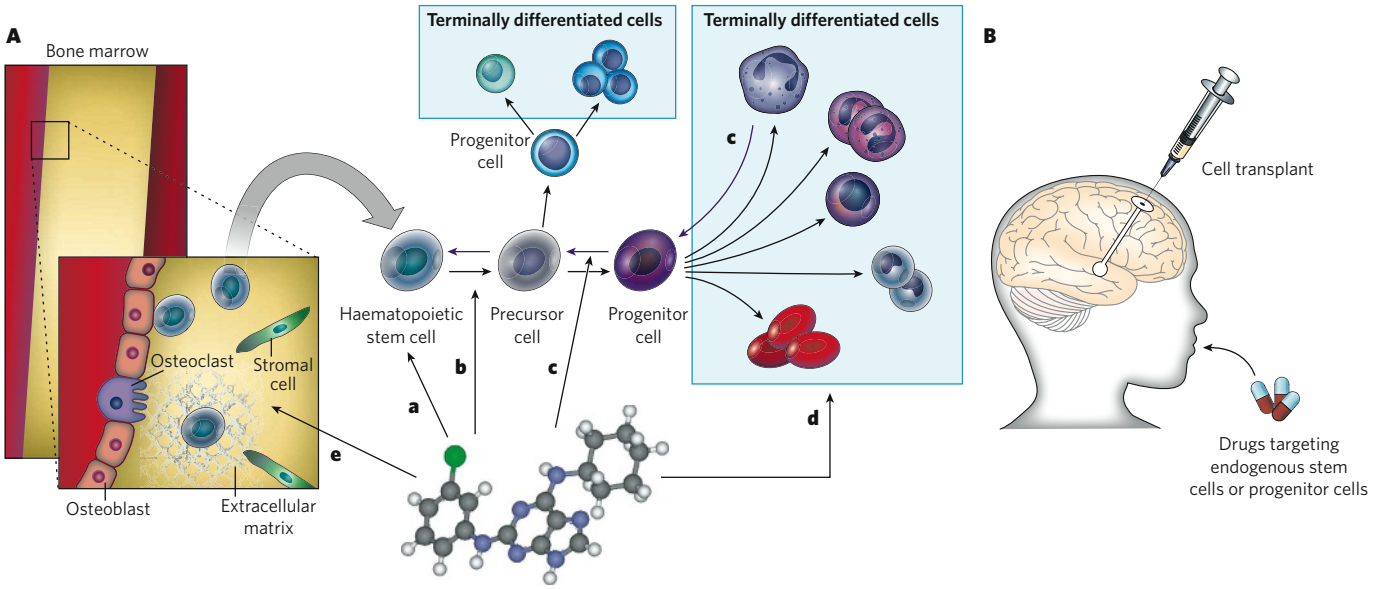

Figure 3 | Therapeutic strategies for regenerative medicine. A, Small molecules can target stem cells or progenitor cells for self-renewal (**a**) or differentiation (**b**). Small molecules can also target lineage-restricted cells for the generation of more-primitive cells or other tissue cell types (**c**), or they can regulate the survival, proliferation, homing or reprogramming of terminally differentiated cells (**d**). They can also control the cell fate and/or function of target cells by regulating the appropriate cellular niches (**e**). **B**, Homogeneous and functional cell types generated in chemically defined conditions can be used for cell-based therapy; and alternatively, conventional chemical and biological therapeutics can be developed to target patients' own cells or their niches to stimulate regeneration *in vivo*.

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cells can be reprogrammed to adopt alternative cell fates across lineage boundaries or to return to more-primitive states *in vitro*, as well as *in vivo*, through various mechanisms. Such techniques include somatic cell nuclear transfer (to generate totipotent cells, which are sufficient to form an entire organism)[43,44], cell (or cell extract) fusion[45–47], genetic alterations[11,48,49] or defined exogenous molecules[10,50,51]. At the molecular level, these studies suggest that a stably balanced epigenetic state can be shifted by specific treatments. Recent successes in generating ES cells by somatic cell nuclear transfer into mitotic zygotes from mice[52] and into oocytes from non-human primates[53] suggest that it might be possible to generate human ES cells by using a similar approach, which might lessen ethical concerns.

Another breakthrough in reprogramming is the identification of an easy genetic approach to convert somatic cells back to iPS cells[11,54–59] (see page 322). The simplicity of this genetic approach has opened up tremendous opportunities to generate patient-specific cells for various applications (for example, cell-based therapy or drug discovery) without the controversies associated with the conventional human ES cells. In addition, it facilitates studies of the intriguing epigenetic reversal process on a fully defined basis.

Practical clinical applications of the iPS-cell approach will largely depend on the resolution of two crucial issues. The first is elimination of the risks associated with exogenous genetic manipulations, as well as possible endogenous genetic alterations, during the slow and inefficient reprogramming process. The second is the generation of homogeneous populations of lineage-specific cell types from iPS cells. One strategy is to replace the genetic method with chemically defined approaches, such as protein or RNA transduction and/or growth factor or small-molecule treatment. We have explored two approaches for replacing viral transduction of transcription factors. The first approach was to test methods that induce pluripotency in different cell types on the basis of the idea that certain accessible cell types may endogenously express or have less silenced loci of some of the required genes for inducing pluripotency. Thus the cells can be more efficiently reprogrammed with fewer genetic manipulations. The second approach was to identify defined exogenous factors and small molecules that can activate a pluripotency network, inhibit negative regulators of pluripotency or directly regulate chromatin modifications. Both approaches independently or in conjunction have generated positive results that may ultimately lead to development of fully chemically defined conditions for the generation of iPS cells.

Given the difficulties with differentiating pluripotent stem cells for specific applications, an attractive approach to reprogramming is to generate intermediate lineage-specific stem cells or progenitor cells or other types of differentiated functional cell through lineage reversal or transdifferentiation (Fig. 3Ac). Although problems with genetic manipulations (if used), and efficiency and heterogeneity, would still need to be resolved, these reprogrammed cells might be advantageous for differentiation and/or have a lower cancer risk because they are lineage restricted and do not form teratomas *in vivo*.

Reprogramming of somatic cells to express previously silenced genes or to become another cell type has been extensively studied, providing the conceptual and technical basis for epigenetic reprogramming biology. Examples of such *in vitro* studies include heterokaryon (a fusion of two different cell types) formation-induced reprogramming, which is independent of cell division[60], phenotypic conversion of certain pancreatic cells to hepatocytes[51] by dexamethasone treatment or by overexpression of the transcription factor C/EBP-β, conversion of hepatocytes to a pancreatic phenotype by overexpression[61] of PDX1, reprogramming of B cells through the transcription factor *Pax5* deletion[48,49], reprogramming of normally glia-restricted oligodendrocyte precursor cells to multipotent neural precursor cells by sequential treatment with BMPs and bFGF[50], and mesenchymal-cell reprogramming by controlling matrix elasticity[62].

To screen rationally for small molecules that would reprogramme lineage-committed cells to become more-primitive precursor cells, we carried out a cell-based functional screen based on the idea that lineage-reversed cells would regain multipotency. Lineage-committed myoblasts were screened using a two-stage screening protocol, in which cells were initially treated with libraries of compounds to induce dedifferentiation. They were then assayed for their ability to differentiate into otherwise non-permitted osteoblasts in osteogenic conditions. A synthetic small molecule named reversine was identified and shown to reprogramme multiple lineage-restricted cell types to a more-primitive multipotent state at the clonal level[10,63]. The cellular targets of reversine were identified through affinity chromatography as MEK1 and non-muscle myosin II heavy chain. Mechanistic studies suggested that inhibition of both target proteins' activities is required, which entails cell-cycle G2–M phase staging, cytoskeletal reorganization and cell signalling modulation.

Targets for therapeutic interventions

Cell-based therapy using tissue-specific cells either isolated from donors or derived from pluripotent stem cells holds promise for treatment of many devastating diseases and injuries. Clinical successes have included several cell-replacement therapies, such as HSC transplantation for blood-related disorders and pancreatic islet or β-cell transplantation for type 1 diabetes. However, challenging issues remain. It is difficult to obtain donor cells, and there are issues with immunological compatibility and the precise control of cell fate in defined conditions *ex vivo*. As highlighted above, chemical approaches would support cell-based therapy by generating and/or enhancing transplantable cells through better *ex vivo* control of cell survival, growth, differentiation and reprogramming.

Given that many adult tissues and organs have endogenous stem cells and progenitor cells that participate in normal tissue homeostasis and regenerative processes in response to injuries, it is conceivable that the body's own cells can be targeted *in vivo* to enhance regeneration. As the scientific understanding of adult stem-cell biology increases, this approach will be further strengthened by the decades of experience in development of conventional drugs. Consequently, endogenous stem cells and progenitor cells and their cellular niches are targets for therapeutic development.

To identify new mechanisms and small molecules that can influence endogenous stem-cell behaviour, phenotypic functional screens at the cellular or organismal level have been particularly useful. A recent phenotypic screen of known drug collections in zebrafish embryos identified small-molecule regulators of prostaglandin E_2 (PGE_2) synthesis that modulate HSC numbers *in vivo*[64]. This discovery was further extended to show that a stabilized PGE_2 analogue, 16,16-dimethyl PGE_2, improved kidney-marrow recovery after irradiation injury in the adult zebrafish. *Ex vivo* treatment of mouse bone-marrow cells with 16,16-dimethyl PGE_2 increased the frequency of long-term HSCs present in mouse bone marrow after limiting-dilution competitive transplantation[64]. This suggests that PGE_2 functions as a potent regulator of vertebrate HSC homeostasis, and its pathway modulation by small molecules may be useful for treating patients undergoing bone-marrow transplantation.

Another strategy to develop small molecules for regeneration is to focus on defined molecular targets or pathways, such as WNT- and hedgehog-mediated signalling, which are implicated in specific regenerative processes. One potential concern about activating a regenerative pathway is the risk of causing cancer, as certain genetic alterations or abnormal gene expressions that lead to the activation of some of those pathways have been associated with cancer. Strategies such as temporal and/or synergistic activation may provide a viable solution. To identify novel compounds and pathways that interact with the canonical WNT–β-catenin signalling pathway, we recently carried out a reporter-based high-throughput screen for molecules that synergistically activate the reporter in the presence of WNT3A. A 2,6,9-trisubstituted purine compound, QS11, was found to synergize with canonical WNT proteins both *in vitro* and *in vivo*[65]. Affinity chromatography identified ARF-GAP as a target of QS11. Additional biochemical, genetic and functional studies have established that QS11 inhibits ARF-GAP, and as a consequence modulates ARF activity and β-catenin localization so that it crosstalks with WNT-mediated signalling.

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Significant advances in defining adult stem-cell niches and understanding how they regulate stem-cell function *in vivo* have provided new strategies for controlling cell fate by pharmacologically manipulating the niches[5] (Fig. 3Ae). Such approaches alone or in combination with approaches to target stem cells directly may provide broader selection of molecular targets or elicit more robust response in specific settings. A proof-of-concept demonstration in mice showed that daily treatment with a parathyroid hormone that augments osteoblasts, a key component of the HSC niche, resulted in expansion and protection of HSCs with therapeutic benefit in three clinically relevant models of stem-cell-based therapy[66]. In another example, regeneration of damaged axons in the adult central nervous system is restricted by both their intrinsic incompetence and microenvironment, consisting of myelin and the glial scar. A small-molecule screen for compounds that can neutralize inhibitory activity, such as that associated with central-nervous-system myelin, identified EGFR inhibitors as potent promoters of neurite outgrowth on cerebellar granule neurons on an immobilized myelin substrate[67]. More importantly, local administration of EGFR inhibitors led to significant regeneration of injured optic nerve fibres in mice, suggesting that targeting the inhibitory microenvironment may provide a therapeutic avenue for enhancing axon regeneration after central-nervous-system injury.

Perspectives

Stem cells present enormous opportunities for basic research, drug discovery and therapies. Conventional small-molecule or biological therapeutics will probably become a more convenient form of regenerative medicine, working to unleash the body's own regenerative capacities by promoting survival, migration, proliferation, differentiation or reprogramming of endogenous cells.

Continued development and application of chemical approaches in stem cells will undoubtedly lead to identification of additional small molecules and more precisely defined and 'individualized' conditions for controlling cell fate *in vitro* and *in vivo*. ■

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Acknowledgements We thank members of the Ding laboratory for stimulating work and discussions. S.D. is supported by funding from the Scripps Research Institute, the National Institutes of Health (grant numbers MH074404, HD053759, HL084295 and HD058110), the Juvenile Diabetes Research Foundation and the California Institute of Regenerative Medicine.

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