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Chemically induced cell fate reprogramming and the acquisition of plasticity in somatic cells

Yang Zhao

The nature of somatic cell fate has always been considered relatively unchangeable. Only in rare cases, in response to highly specific environmental cues, do differentiated mammalian somatic cells transform into other cell types. However, the fact that cell fate reprogramming can be accomplished by utilizing chemical cocktails, in the absence of any genetic alterations, suggests that the fate determination of somatic cells is much more malleable than previously believed. The use of chemical cocktails to directly alter cell fate sheds light on an important, yet less explored approach to regenerative medicine: the use of chemicals to restore functions to injured, aging or diseased tissues. Here, we review and discuss the recent developments, inspirations, and challenges encountered when modulating cell fate reprogramming with chemicals, and investigate how chemical biology impacts the future of cell fate reprogramming and regenerative medicine.

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Cell fate reprogramming with chemical compounds

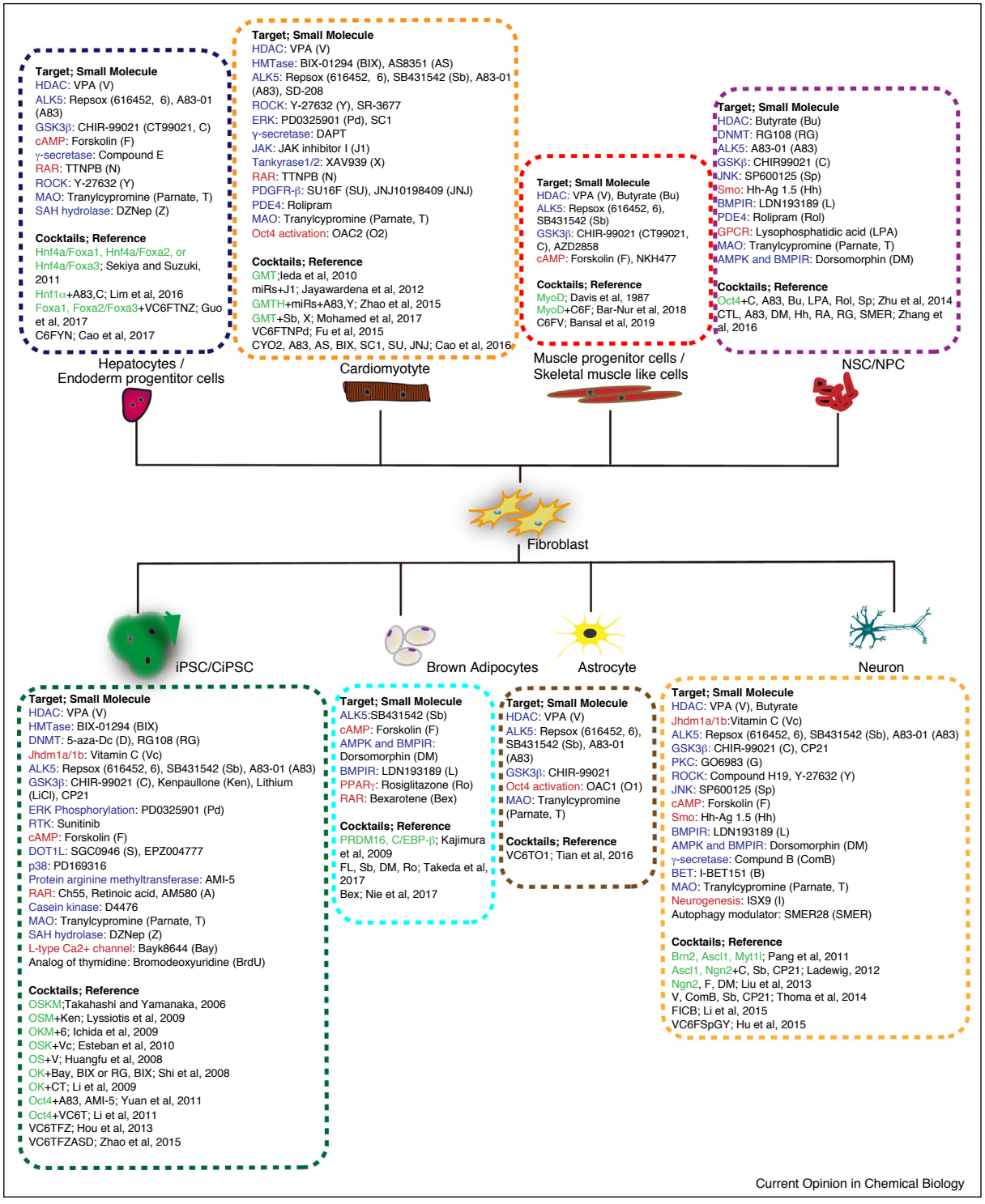
The earliest reported cell fate reprogramming by chemicals in mammalian traces back to the conversion of myocytes from fibroblasts by DNA analogue 5-azacytidine in 1979 [1•], almost a decade before the first transgene-mediated reprogramming in 1987 [2•]. The study of chemicals in directing cell fate conversion intensified after the discovery of induced pluripotent stem cells (iPSCs) and transgene-mediated reprogramming across different cell type lineages [3••] (Figure 1). Large single and combinatorial screens of hundreds of thousands chemicals identified dozens with the capacity to enhance overall reprogramming efficiency, to replace some of the reprogramming genes during induction, or to benefit the overall quality of iPSCs produced [4–11] (Figure 1).

The discovery of chemical replacers for transgenes and chemical boosters of cell fate reprogramming elicit an intriguing question as to whether reprogramming transgenes can be replaced entirely with chemical compounds. By several rounds of intensive chemical screen, a cocktail of a minimum four chemical compounds, Forskolin, CHIR99021, 616452 and DZNep, has been developed that can reprogram mouse somatic cells into chemically induced pluripotent stem cells (CiPSCs) [12••]. In comparison with transgenic methods, chemical approach could be chromosome non-integrative, functionally reversible, cost-efficient, and easier to control and standardize, making it the more pragmatic choice for future applications. Chimeric mice generated from CiPSCs have higher survival rates than mice generated from iPSCs using c-Myc, suggesting that CiPSCs are overall much safer [12••]. Besides, CiPSCs bear more epigenetic resemblances to embryonic stem cells than do iPSCs induced with transgenes [13].

Several boosters for chemical reprogramming were further identified, such as TTNPB, Rolipram, UNC0638, and BrdU [12••,14]. Chemically induced reprogramming can be fine-tuned through changes in concentration, duration, cocktail composition, and slight changes in structure, allowing the induction efficiency of CiPSCs to be increased [15]. This is evidenced by the dramatically enhanced chemical reprogramming system using an RA agonist, AM580, Dolt1l inhibitors, EPZ004777 and SGC0946, and a Dnmt inhibitor, 5-aza-dC, in a stepwise manner. Moreover, an extraembryonic endoderm (XEN)-like intermediate state was found to mediate chemical reprogramming for somatic cells to CiPSCs [15••], even without serum or serum replacement [16]. Chemical reprogramming efficiency can be further increased if the reprogramming steps are divided temporally and controlled more precisely [17].

Not long after the discovery of chemically induced pluripotent stem cells, the chemical reprogramming approach was extended into direct cell lineage reprogramming. Many functional cells such as neurons, neural stem cells, astrocytes, brown adipocytes, skeletal muscle

Figure 1



Representative chemicals or cocktails used in cell fate reprogramming from fibroblasts.
Potential targets of small molecules used in cell fate reprogramming are listed in the doted box. Red, positive regulation; Blue, negative regulation. Black, downstream effects or potential functions of small molecules. Alias and abbreviations for small molecules are listed in brackets. Shown are the representative chemicals and cocktails used in reprogramming.

progenitors, and cardiomyocytes were reported to be induced from fibroblasts through the use of chemical cocktails without a pluripotent state [18–21,22••,23••,24–26] (Figure 1). Neural stem cells, gastric epithelial cells, and astrocytes are also used as the initial cell types for chemical reprogramming, either into pluripotent stem cells or directly into differentiated cell types [21,27,28]. Overall, chemical cocktails show their power in generating various ultimate cell types and altering cell fates previously considered stable.

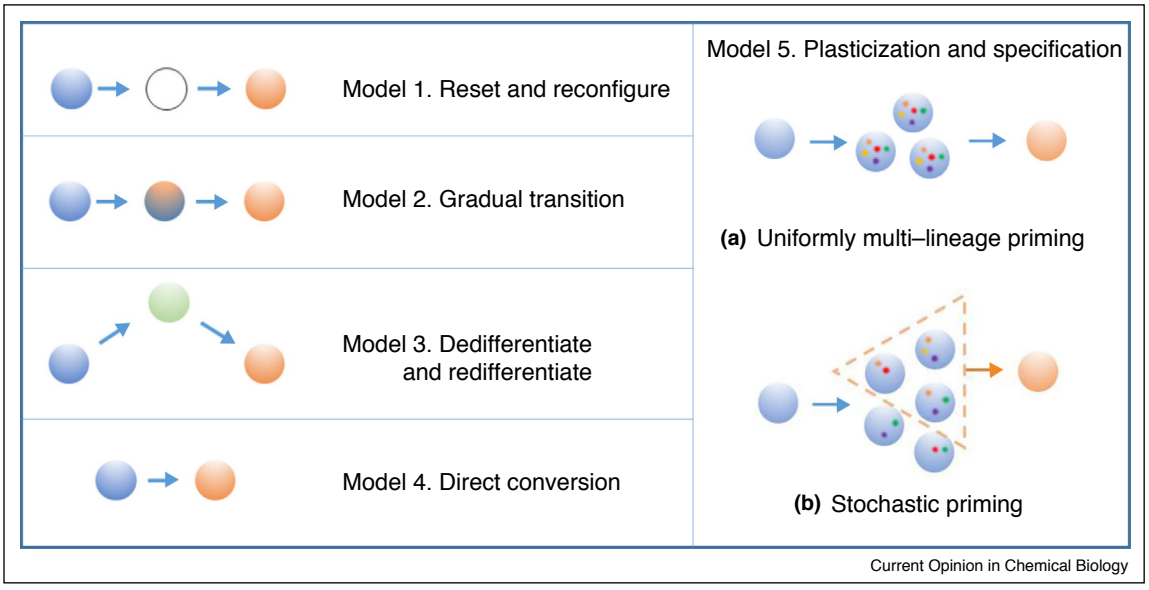
Molecular dynamics during chemical reprogramming

The discovery of the iPSC induction method came as the direct result of the intention to test 24 pluripotency-associated genes. And Yamanaka factors, the final four, are all pluripotency-associated factors that highly expressed in embryonic stem cells and known to be important regulators of pluripotency [29••]. Direct cell lineage reprogramming factor cocktails were also developed based on the idea of inducing the target cell fates by the delivery of target cell type-associated master transcription factors. The master transcription factors may act as pioneer factors to directly shape the cell type favorable chromatin accessibility profiles and activates the cell type-specific gene expression program [29–31]. In contrast, the chemical compounds used in chemical reprogramming are not obviously associated with specific cell fates, and directly target proteins involving signaling transduction pathways or epigenetic factors that play roles in multiple biological processes (Figure 1). This raises the question of how exactly the target cell type is determined by each chemical cocktail, and what exactly happens during chemical reprogramming that determines cell fate.

Study of the cellular dynamics indicated that unlike transgenic reprogramming, chemically induced reprogramming undergo a unique roadmap to achieve pluripotency mediated by an XEN-like state [15••]. For XEN specification, the core chemicals in chemical reprogramming, Forskolin, CHIR99021 and 616452, first activate the expression of *Sox17* in an initial plasticity acquisition stage, and further support the endogenous activation of other essential XEN cell-specific transcription factors, such as *Gata4*, *Sall4* and *Foxa2*, thereby established the entire network of XEN cells (Yang *et al.*, unpublished) (Figure 2).

After XEN-like cells are generated by chemical cocktails, DZNep, a SAH hydrolase inhibitor, can initiate the expression of Oct4, probably by suppressing histone and DNA methylation, following the activation of upstream factors Sall4 and Sox2 (or Gata4) in the first stage of reprogramming [12••,15••]. Pluripotency factors, such as Oct4, form positive feedback regulatory networks with master genes of embryonic 2-cell (2C) state, bolstering a 2C-like state from XEN-like cells that further

Figure 2



Potential models for cell fate reprogramming.
The blue ball represents initial cell type, and orange ball represents the ultimate cell type during cell fate reprogramming. Different kinds of intermediated cells are proposed as shown. Cell fate reprogramming in model 1 was mediated by an intermediate cell without the feature of initial or ultimate cells. In model 2, reprogramming is mediated by a hybrid cell of initial and ultimate cell type. Model 3 reprogramming is mediated by a stem cell, which is developmentally more naive than initial and ultimate cell type (green ball). Model 4 reprogramming have no intermediate cell type. In model 5, reprogramming was mediated by a priming state, in which multi-lineage factors (colorful dots) are activated for further specification into ultimate cell types. 5a indicates a uniformly primed cell state, while 5b indicates stochastic priming of chemical reprogramming, in which the multi-lineage gene activation is stochastic and dispersed in different cells.

promotes CiPSC induction [17]. In summary, a hierarchical regulation circuitry linking the sequential activation of master transcription factors was revealed throughout chemical reprogramming process (Figure 3).

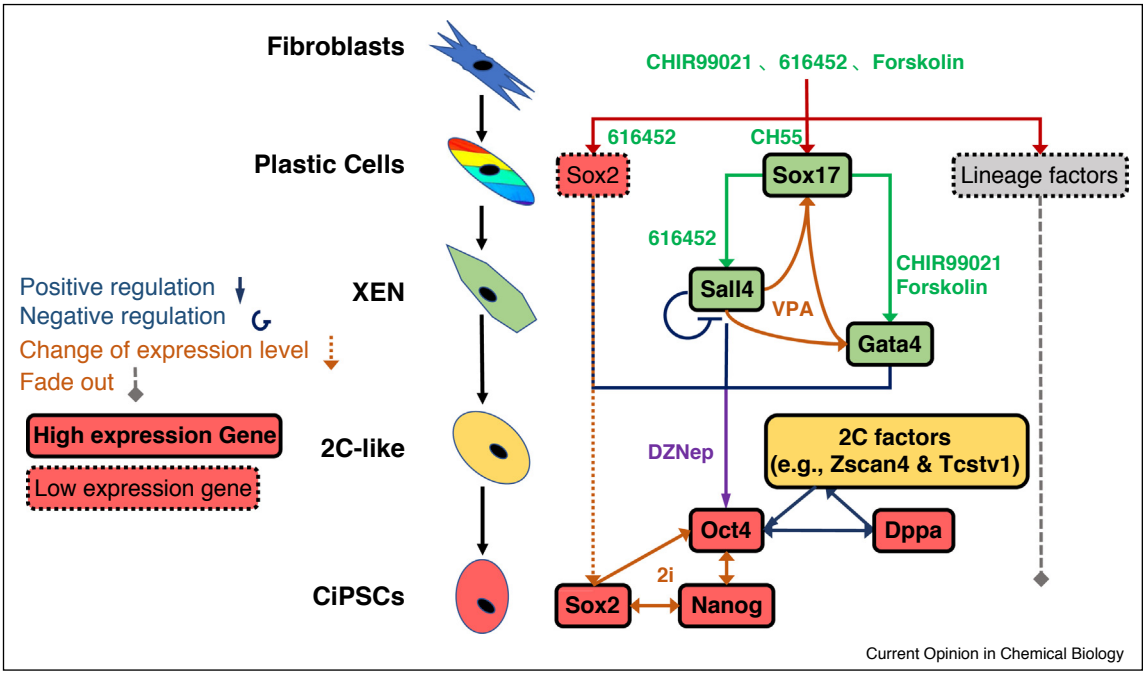
Cell plasticity induced with chemicals

The term cell plasticity has traditionally been used to describe either the potential of cells to change their fates or the ability of cancer cells to transition between epithelial and mesenchymal states. The acquisition of plasticity in a somatic cell is necessary in somatic reprogramming; this may counteract the machinery for the safeguarding of cell identity. Histone chaperons, CAF1 and FACT, and Cbp9-mediated sumoylation modification which stabilize the epigenetic state of a cell identity are barriers in somatic reprogramming [32–34]. The p53-mediated tumor suppressor pathway was reported as a general barrier in different reprogramming systems, which may involve in cell fate stabilization [35,36•,37–39].

Some chemicals used in reprogramming to obtain different cell types may act in the acquisition of cell plasticity, rather than the choice of ultimate cell fate. For instance, a Brd4 inhibitor, I-BET-151, was initially found to disrupt the cell identity of fibroblasts during reprogramming toward functional neurons [23••]. This was then found to be useful for directing cellular reprogramming into iPSCs [40]. HDAC inhibitors were also found to be beneficial in a lot of reprogramming systems (Figure 1). Interestingly, the core chemical cocktail that initially discovered in CiPSC generation, CHIR99021, 616452 and Forskolin, are also widely used in various reprogramming systems into different lineages, suggesting their broad roles in inducing cell plasticity rather than determine the cell fate directions (Figure 1).

Detailed investigation of the chemically induced cell fate transition process from fibroblasts to XEN cells uncovered two major steps, plasticity acquisition and cell fate specification (Yang *et al.*, unpublished). In the plasticity acquisition step, a chemically induced multi-lineage priming (CiMP) state initiates, in which the endogenous expression of a wide range of transcription factors not-specific to a certain cell fate is activated. Expression of these transcription factors is stochastically dispersed in different cells, and have overall low correlations with each other, which could be a unique model in cell fate reprogramming (model 5b in Figure 2). Cells in a CiMP state are plastic and can be rewired into different other cell types by fine-tuning of the cocktails and mediums of the subsequent stage, providing a shortcut for obtaining functional desirable cell types in regenerative medicine, bypassing long-term culture in iPSC inductions and directed differentiation mimicking developmental process (Yang *et al.*, unpublished). As these chemicals are all

Figure 3



Transcriptional activation circuitry in chemical reprogramming.
Schematic diagram for transcriptional activation circuitry in chemical reprogramming process. Transcription factors are hierarchically regulated and stimulated through a XEN-like state and 2C-like state. The chemicals function at different stages and play different roles on endogenous gene activation. Multi-lineage factors are activated as the side effects in the initial stage of reprogramming, which can be rewired into other cell fates (Yang *et al.*, unpublished).

commonly known as cell signaling modulators, the question remains as to how these chemicals function to induce such a plastic state on a molecular level.

Challenges and new thoughts in developing chemical cocktails for reprogramming

Since the traditional strategy to develop chemical compounds such as phenotypic screening is labor intensive and time-consuming, an effective strategy to develop chemical reprogramming cocktails is still needed. In accordance with the fact that, during chemical-induced reprogramming, cells pass through multiple discrete stages, a more effective way to discover chemicals for reprogramming could involve adjusting the small molecule cocktails during cell specification process after chemically inducing cell plasticity. In addition, since the chemical reprogramming process may be mediated by hierarchical activation of transcription factors, it could be valuable to screen for potential chemical candidates by activating the endogenous expression of one or more master transcription factors, based on each specific cell type. In addition, the reprogramming barriers identified in mechanism study on somatic reprogramming could also be potential targets for use of target-based chemical screening. However, because chemical reprogramming always requires a combo of more than 2–3 chemicals, it is still a challenge to systematically 'design' specific cocktails but not a single candidate compound for cell fate reprogramming.

Opportunities and challenges of chemical regeneration *in situ*

One of the ultimate goals of chemical reprogramming is to develop drugs that enable direct reprogramming and regeneration *in vivo* to repair damaged tissues. In recent years, evidence of *in vivo* reprogramming has been shown in mouse models through both genetic manipulation [41•,42–46] and chemical compounds [47•,48]. The microenvironments *in vivo* may provide specific opportunities for cell fate reprogramming. For instances, *in vivo* iPSC generation was found to be promoted by IL-6 secretion from senescent cells in the niche [49]. Injury signaling may play an essential role to direct reprogramming from Muller glia cells to retinal neurons [50]. Direct reprogramming toward pancreatic beta cells, T cells, and hematopoietic stem cells was achieved only *in vivo*, and has not been achieved *in vitro* [41•,51,52]. Moreover, *in vivo* activated astrocyte exhibits more similarities with neural stem cells after injury, and thereby may be more amenable for reprogramming [53]. In addition, cell types *in vivo* may be more heterogeneous, in contrast to cloned cells *in vitro*, which could have more chances to cell fate reprogramming in response to stimuli or chemicals. *In vivo* cell fate adaptive reprogramming naturally occurs in mammals in response to injury [54–56,57], the mechanism of which may be manipulated by chemicals. These are opportunities to induce *in situ* reprogramming.

However, there are also several challenges to develop chemicals for *in situ* reprogramming. For example, chemical cocktails developed *in vitro* may not work *in vivo* because the *in vitro* cultured cell types may not have exact counterparts *in vivo*. Chemicals may lead to unintended off-target effects in certain cell types in the heterogeneous *in vivo* environment. It could be ideal if chemical cocktails can be developed to have activities only in excessively deposited cell types, such as myofibroblasts in the injured region or activated astrocytes, since different cell types may have different responses with use of the same chemicals. Otherwise, targeted deliver methods of chemicals might be required to reduce the risks in clinical applications. Moreover, the partially reprogrammed cells and unintended cell products may be induced *in vivo*, which could be harmful for tissue repair. Further efforts and new strategies are necessary to improve *in vivo* reprogramming efficiency, to efficiently deliver compounds locally for drug sustained release and even to reduce the number of compounds, before *in situ* chemical reprogramming can be widely used in regenerative medicine.

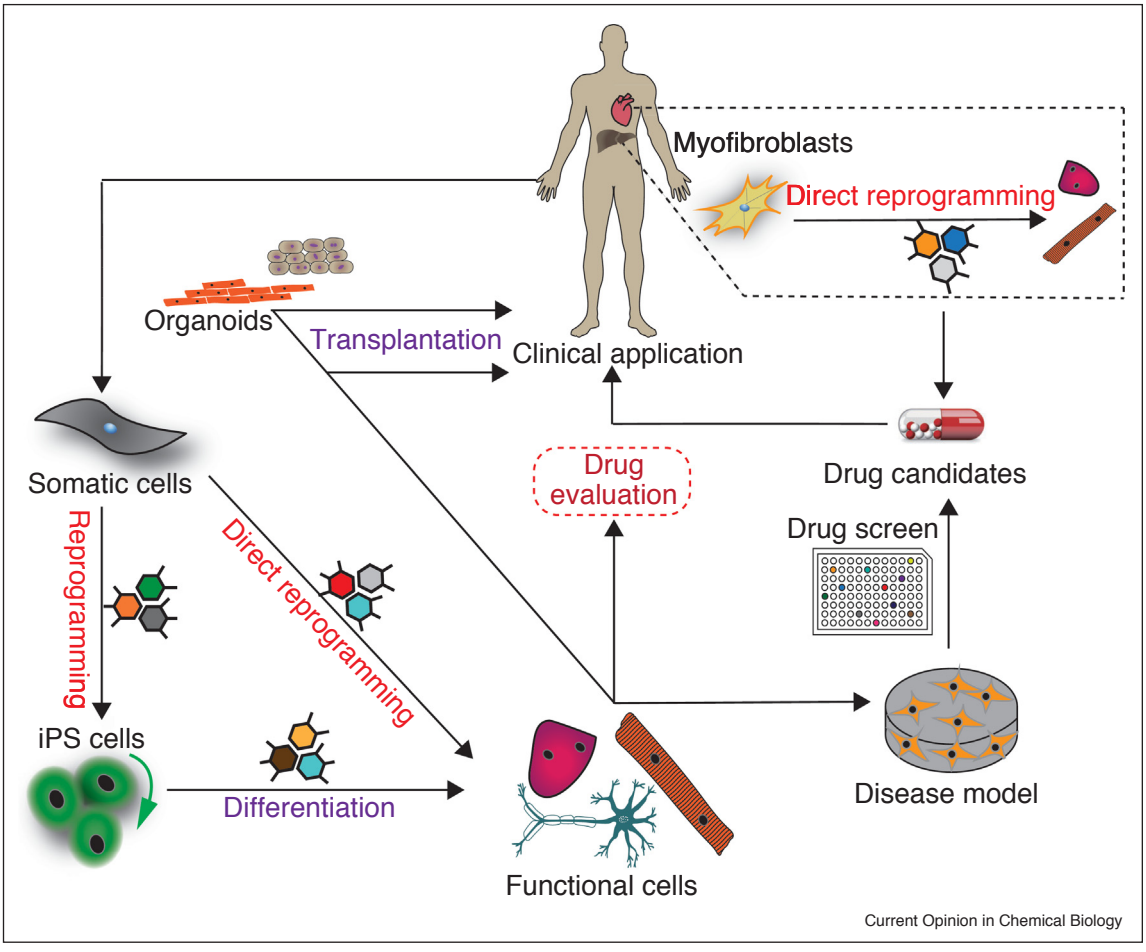
Conclusions and perspective

Even before the discovery of chemically induced cell reprogramming, small molecules have been used to regulate natural biological processes, such as in stem cell self-renewal and differentiation, even *in vivo*. However, it is only due to the establishment of a method to chemically direct the reprogramming of a cell fate that we now have the means to produce functional cells from more available cell sources both *in vitro* and *in vivo*, even without a relying on the use of genetic modifications. Hopefully this will pave the way for more efficient use of cell fate manipulation and cell fate engineering in furthering future research in drug screens, drug evaluation and in regenerative medicine, by cell transplantation or pharmacological reprogramming (Figure 4).

Moreover, the recent developments on chemical reprogramming make it possible to ask new questions and to further our understanding of cell fate determination and reprogramming. For example, is there any chemical cocktail that functions as a general eraser of a cell fate by breaking down the cell fate keepers? Could chemical cocktails reprogram cell aging, without changing cell type? Could chemical reprogramming be further extended into cancer therapy by altering the fate of cancer cells into a more differentiated one or more normal one, just like reported very recently [58,59,60••,61]? In addition, some traditional concepts may also need to be reconsidered. Because chemically treated somatic cells exhibit plasticity similar to that of stem cells, how can we distinguish the differences between reprogrammable somatic cells and adult stem cells that can be activated to change their fates? [62]

Overall, the use of chemicals to induce cell fate reprogramming has revolutionized our concepts of cell

Figure 4



Cell fate reprogramming and chemical biology.
Schematic diagram for chemical biology in the field of somatic reprogramming. Small molecules (triple hexagon) can be used in somatic reprogramming, stem cell differentiation and direct reprogramming. The functional cell types obtained by somatic reprogramming can be used in drug evaluation, disease model for drug screening, and cell transplantation therapy. Moreover, chemicals that discovered in direct *in vivo* reprogramming of myofibroblasts can be developed into drug candidates for fibrosis alleviation and regenerative medicine.

plasticity and cell fate determination, and provides probes to study the molecular mechanisms that participate in cell fate determination. The complete bypass of the risks and concerns commonly associated with transgenes suggests a promising for future in clinical applications. In addition, the chemicals that are used for direct reprogramming *in situ* may also be translated into potential drug candidates for the amelioration of tissue fibrosis, through altering the cell fate of myofibroblasts that are excessively accumulated in tissues as the result of injury. One can anticipate the future of chemical reprogramming to play more in exploring the mechanisms of cell fate regulation and in developing new methods to restore tissue functions, rejuvenate cell aging and even eliminate cancers cells.

Conflict of interest statement

Nothing declared.

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