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Small molecule-induced cellular fate reprogramming: promising road leading to Rome

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Cellular fate reprogramming holds great promise to generate functional cell types for replenishing new cells and restoring functional loss. Inspired by transcription factor-induced reprogramming, the field of cellular reprogramming has greatly advanced and developed into divergent streams of reprogramming approaches. Remarkably, increasing studies have shown the power and advantages of small molecule-based approaches for cellular fate reprogramming, which could overcome the limitations of conventional transgenic-based reprogramming. In this concise review, we discuss these findings and highlight the future potentiality with particular focus on this new trend of chemical reprogramming.

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Introduction

In mammals, most terminal differentiated cells show limited regenerative ability under acute damage or diseased conditions. The major goal of regenerative medicine is to generate functional cells to replace lost or injured cells, which could be achieved by differentiation of stem/progenitor cells or reprogramming from somatic cells. Importantly, the advent of induced pluripotent stem cells (iPSCs) took a ground-breaking leap to prove the principle of reprogramming cell fates by using combinations of transcription factors [1]. This principle has been adapted to induced cell fate conversion between different lineages and across the germ layers [2•]. Although these advances have opened promising ways to obtain functional cells, the transgenic approach in these studies raises the safety concern for its future applications, which demands for developing novel strategies. Remarkably, increasing studies have shown the feasibility and advantages of using small molecules to induce cell fate conversion. In this review, we discuss these findings and prospect the promising future of the small-molecule based approach for cellular reprogramming.

Chemical reprogramming: potential game changer

Utilizing strategies from the iPSC generation [1], combined transcription factor-based approach has demonstrated its power in reprogramming and has made remarkable progress [2•]. However, the risks of genomic insertion of exogenous DNA sequences and reactivation of exogenous genetic factors, as well as unpredictable side effects associated with ectopic gene expression, stirred up safety concerns [2•,3]. Although integration-free methods were reported by transient delivery or using special particles for delivery, the relatively high cost, low efficiency, and technical challenges (e.g. complicated experimental setup and required repeated transfections) remain as hurdles for desirable outcomes [4]. As a result, there is great demand of developing novel strategies that can circumvent these problems.

In recent years, small molecules have shown their power in reprogramming [3]. Small molecule-based reprogramming has distinct advantages over transgenic and other approaches, including cell permeability, reversibility, structural and functional versatility, fine-tuned-ability, and low cost [3,5]. In addition, small molecules could be used to target signalling pathways and targets that are critical for regulating cellular fate and functionality [3,4]. Because chemical compounds can be easily manufactured, scalable throughput chemical screening is feasible to be performed for a specific reprogramming purpose. The identified chemical modulators can be further used as chemical probes to understand intrinsic molecular networks during reprogramming [3]. Inspiringly, a growing number of studies have shown the feasibility of reprogramming cellular fates by pure chemical compounds, for generating different cell types across lineages and germ layers.

Landmarks of chemical reprogramming: CiPSCs, CiNs, CiNSCs, CiCs and CiHs

Our group is the first to show that chemical compounds can replace all the transcription factors for inducing iPSCs, which proves that small molecules alone are capable of reprogramming somatic cell fates [[8••]]. Notably, the chimeric mice generated from these chemically induced iPSCs (CiPSCs) displayed an improved viability over Yamanaka factor-induced iPSCs [[8••]], suggesting a potential advantageous property of CiPSCs in application. Moreover, one recent study revealed that mouse CiPSCs have closer epigenetic features to mouse embryonic stem cells than OSKM-integrated iPSCs [7], thus providing a strong evidence that chemical reprogramming might be a better strategy than transcription factor-integrated reprogramming. In 2015, our group further developed an improved 3-step protocol to induce CiPSCs from fibroblasts and identified that CiPSC generation goes through a transition from an extra-embryonic endoderm (XEN)-like state to a pluripotent state [8••]. It demonstrated that the reprogramming route of CiPSCs is substantially different from that of Yamanaka transcription factors-induced iPSCs [[8••],8••]. In addition, we further demonstrated that CiPSCs can be induced from cells of different tissue origins, suggesting that chemical reprogramming can be extended to more initial types [9]. Importantly, our protocol of generating CiPSCs have been independently reproduced by different groups. Basing on our original protocol, Long *et al.* found that Bromodeoxyuridine could further enhance the efficiency of CiPSC induction [10]. More recently, Cao *et al.* modified our 3-step chemical reprogramming protocol and integrated Bromodeoxyuridine to develop a 2-step protocol to generate CiPSCs, which further confirms the importance of XEN-like state to CiPSC generation [11].

Two years after the CiPSCs, our group developed a chemical approach to convert mouse fibroblasts into neurons [12••]. *Ngn2* and *NeuroD1* were identified as the major intrinsic players for establishing neuronal cell fate from fibroblasts [12••]. Another group showed the feasibility of reprogramming human fibroblasts, including fibroblasts from familial Alzheimer's disease patients, into neurons [13••]. These chemically-induced neurons (CiNs) produced by the two groups are electrophysiologically active and possess functional hallmarks of neurons. In the same year, Zhang *et al.* showed the success of inducing functional neurons from human astrocytes [14•]. In accordance with our study, the neuronal reprogramming requires the silencing of original fibroblast/glial genes and concomitant activation of *Ngn2* and *NeuroD1* for neurogenesis [12••,14•]. Very recently, Gao *et al.* established an improved cocktail with small molecules used in our study, to efficiently reprogram human adult astrocytes into functional neurons [15]. These CiNs together demonstrate the feasibility of chemically-induced lineage reprogramming to generate functional cells, even across germ layers.

Multipotent neural stem cells/progenitor cells (NSCs/NPSCs) have been identified as a life-long source of neurons and glial cells in the Central Nervous System (CNS). Two studies have developed chemical cocktails to induce NSCs/NPSCs from mouse fibroblasts and human urinary cells [16,17•]. The neurons derived from these chemically induced NSCs (CiNSCs) generated action potentials. Other neural cell types including astrocytes and oligodendrocytes were also obtained from the CiNSCs. In particular, a physiological hypoxic condition is shown to facilitate CiNSC induction [16]. These studies demonstrate the feasibility of chemically-induced lineage reprogramming to generate progenitor cells, even across germ layers.

In 2016, an important study demonstrated generating chemically-induced functional cardiomyocytes (CiCs) from human fibroblasts [18•]. This group set up a stepwise protocol and develop a chemical cocktail to robustly generate functional cardiomyocytes with mature electrophysiological properties [18•]. Another group developed a chemical cocktail to obtain spontaneously beating cardiac cells from mouse fibroblasts, which resembles atrial and ventricular mouse adult cardiomyocytes [19]. Interestingly, some compounds used in this study were also used for iPSC reprogramming [[18•],19].

Recently, our group revealed that the intermediated XEN-like state during CiPSCs induction is plastic that have different lineage potentials [20••]. We identified the principle of specifying this XEN-like state into functional cells, inducing neurons and hepatocytes [20••]. The route of lineage reprogramming via the XEN-like state is potential for generating other cell types. Later, another group showed the feasibility of inducing hepatocytes from fibroblasts via an intermediated progenitor cell state (ciEPCs) [21]. Interestingly, this group used similar chemical cocktail used for XEN-like state induction [20••], and identified *Sox17*, a master gene of the XEN-like state, as the key inducer for the ciEPCs [21]. Both of the intermediated states induced by the two groups are highly expandable, which enables generating sufficient functional cells for applications [20••,21]. The two studies indicate the potential of stepping stone from an intermediate cell state to derive diverse functional cell types, without reaching the pluripotent stage.

Collectively, these studies have fully demonstrated the feasibility of small molecule-induced cellular fate reprogramming (Figure 1). This strategy is improving and evolving rapidly for potential therapeutic applications.

Lessons of chemical reprogramming: strategy, toolbox, commonalities, and implications

Studies of chemical reprogramming guide us to new insights for cellular fate plasticity and shed light on