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Chemical reprogramming and transdifferentiation

Xin Xie[1,2], Yanbin Fu[2] and Jian Liu[1]

The revolutionizing somatic cell reprogramming/transdifferentiation technologies provide a new path for cell replacement therapies and drug screening. The original method for reprogramming involves the delivery of exogenous transcription factors, leading to the safety concerns about the possible genome integration. Many efforts have been taken to avoid genetic alteration in somatic cell reprogramming/transdifferentiation by using non-integrating gene delivery approaches, cell membrane permeable proteins, and small molecule compounds. Compared to other methods, small-molecule compounds have several unique advantages, such as structural versatility and being easy to control in a time-dependent and concentration-dependent way. More importantly, small molecules have been used as drugs to treat human diseases for thousands of years. So the small molecule approach to reprogramming might be more acceptable in clinical-related uses. In the past few years, small molecule approaches have made significant progresses in inducing pluripotent or functional differentiated cells from somatic cells. Here we review the recent achievements of chemical reprogramming/transdifferentiation and discuss the advantages and challenges facing this strategy in future applications.

Addresses
[1] CAS Key Laboratory of Receptor Research, The National Center for Drug Screening, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
[2] Shanghai Key Laboratory of Signaling and Disease Research, Laboratory of Receptor-based Bio-medicine, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China

Corresponding author: Xie, Xin (xxie@simm.ac.cn)

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Induced pluripotent stem cells (iPSCs)

Not too long ago, people still believed that animal development is an epigenetically programmed process and is irreversible in mammals. In 2006, Yamanaka *et al.* demonstrated that ectopic expression of defined transcription factors (*Oct4* (O), *Sox2* (S), *Klf4* (K), *c-Myc* (M)) could reprogram murine somatic cells into induced pluripotent stem cells (iPSC) [1]. Human iPSCs were generated shortly after that with a similar strategy [2,3]. These iPSCs resemble embryonic stem cells (ESCs) in term of gene expression and chromatin signatures, and some mouse iPSCs can develop into individuals after tetraploid complementation, indicating the full developmental potential of these cells [4].

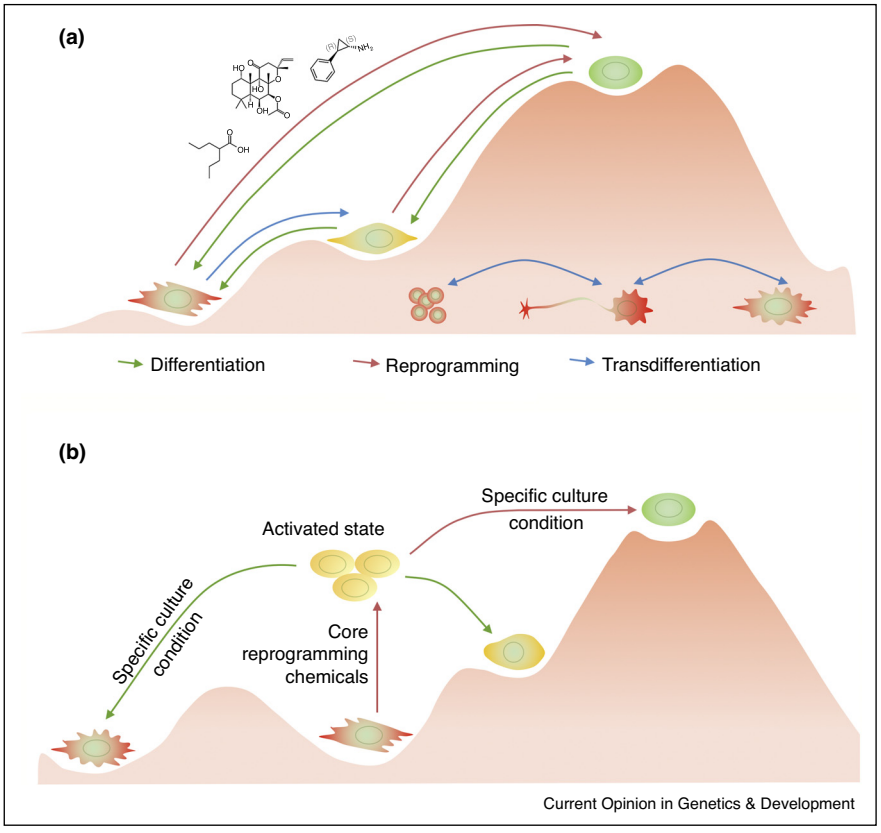
After the publication of the first iPSC paper, scientists have been searching for other factors that can replace the Yamanaka factors OSKM. The original idea was to search among the pluripotency-associated or maternal genes, which led to the identification of factors like *Nanog*, *Prdm14*, *Sall4*, *Esrrb*, *Utf1*, *Tet2*, *Glis1*, *etc.* [5,6]. The most interesting finding is that many lineage specifiers could replace pluripotency factors *Oct4* and *Sox2* and lead to the generation of iPSCs [7], indicating a balance established with pluripotency factors and/or counteracting lineage specifiers facilitates reprogramming. A number of factors involved in chromatin remodeling have also been identified. Depleting *Mbd3*, a key component in the nucleosome remodeling and deacetylation complex, boosted the efficiency of OSKM-mediated reprogramming to almost 100% [8].

Although effective in inducing reprogramming, the transgenic approaches have also raised safety concerns involving the use of oncogenes and possible genetic integration of exogenous factors. Many efforts have been put forward to optimize the reprogramming system using non-integrating vectors, non-viral gene delivery methods, miRNAs, cell membrane permeable proteins, and small molecule compounds (Figure 1a) [9].

Small molecules improving reprogramming efficiency

Compared to other methods, small molecule compounds have several unique advantages, such as structural versatility, relatively cheap to prepare in large quantities and easy to control in a time-dependent and concentration-dependent way. Histone deacetylase inhibitors and DNA methyltransferase inhibitors are the earliest discovered compounds that facilitate the generation of iPSCs [10]. After that, several dozens of compounds have been reported to facilitate factor-mediated somatic cell reprogramming, or replace one or several reprogramming factors (reviewed in [11–13]). As early as 2010, the combination of chemical compounds and a single transcription factor *Oct4* was found to be sufficient to reprogram somatic cells into iPSCs [14,15]. But replacing *Oct4* and

Figure 1



Model of somatic cell reprogramming/transdifferentiation. **(a)** A schematic definition of differentiation, reprogramming and transdifferentiation. During development, pluripotent stem cells roll down the hill (differentiation) to generate tissue specific stem cells with lower potency or terminally differentiated cells. These differentiated cells can be pushed uphill towards pluripotent state (reprogramming) by external forces, such as transcription factors and chemical cocktails. Transdifferentiation is reprogramming process between functional somatic cells, which is also aided by forces like transgenes and small molecules. **(b)** Possible existence of a core reprogramming chemicals which might induce the generation of an activated cell population (or mixed progenitors, unstable intermediates) from the relatively static somatic cells. With favorable culture conditions, these activated cells might be induced to pluripotent stage or various functional cells.

generating full chemical-induced pluripotent cells have been proven to be extremely difficult.

Full chemical induction of iPSCs (CiPSCs)

In 2013, Hou *et al.* demonstrated that iPSCs could be induced from mouse fibroblasts by using a cocktail of seven small molecules (VPA, CHIR99021, E-616452, Tranylcypromine, Forskolin, DZNep and TTNPB; Figure 2a) [16••]. The discovery that Forskolin could replace *Oct4* and induce iPSCs with three transcription factors SKM was believed to be critical in this study. The scientists also reported that C6FZ (CHIR99021, E-616452, Forskolin, DZNep) among the 7 compounds were the core compounds in inducing reprogramming, they were able to generate CiPSCs, although the efficiency was lower than the 7-compound combination. Long *et al.* also discovered that a commonly used biological reagent, bromodeoxyuridine (BrdU), was able to replace *Oct4* in reprogramming and enable CiPSC generation from mouse fibroblasts with several chemical cocktails (Figure 2b), and the minimal combination was BrdU, CHIR99021, Repsox, and Forskolin [17••]. The CiPSCs reported by these studies resemble ESCs in terms of their gene expression, epigenetic status, *in vivo* differentiation and chimera generation.

The mechanisms underlying chemical reprogramming are largely elusive. Current understandings mainly come from the known target or pathway regulated by the compounds used in the induction (Table 1). Recently, Zhao *et al.* discovered the formation of an extra-embryonic endoderm (XEN)-like state during CiPSC induction, a route fundamentally differs from that of the transcription factor-induced reprogramming (Figure 2c) [18••]. In a step-wise manner, the scientists identified that an RAR agonist, AM580, and a DOT1L inhibitor, EPZ004777 were able to enhance the fibroblast to XEN-like cell transition (Stage 1), and 5-aza-dC, EPZ004777 and