Review Article

Small Molecule Epigenetic Modulators in Pure Chemical Cell Fate Conversion

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Although innovative technologies for somatic cell reprogramming and transdifferentiation provide new strategies for the research of translational medicine, including disease modeling, drug screening, artificial organ development, and cell therapy, recipient safety remains a concern due to the use of exogenous transcription factors during induction. To resolve this problem, new induction approaches containing clinically applicable small molecules have been explored. Small molecule epigenetic modulators such as DNA methylation writer inhibitors, histone methylation writer inhibitors, histone acylation reader inhibitors, and histone acetylation eraser inhibitors could overcome epigenetic barriers during cell fate conversion. In the past few years, significant progress has been made in reprogramming and transdifferentiation of somatic cells with small molecule approaches. In the present review, we systematically discuss recent achievements of pure chemical reprogramming and transdifferentiation.

1. Introduction

In 1958, Gurdon et al. first reported unknown factors in the oocyte cytoplasm could reprogram differentiated cells to a pluripotent state [1]. The breakthrough suggested that somatic cells are flexible and could be converted to other cell types. In 1987, Davis et al. discovered that a single transcription factor, MyoD, was able to induce fibroblasts directly into myoblasts, which indicated only a few transcription factors could make cell fate decisions [2]. Nearly 20 years later, Yamanaka’s team found that pluripotent stem cells (iPSCs) could be obtained from somatic cells using four key transcription factors (Oct4, Sox2, Klf4, and c-Myc, termed OSKM) [3]. One year later, two research groups independently succeeded in creating human iPSCs using a similar method [4, 5]. With this new iPSC technology, the molecular mechanisms of cell fate transition could be investigated and diverse applications, including drug screening, disease modeling, and cell therapy, could be developed [6].

Although the medical applications of iPSCs are promising, transgenic approaches raise safety concerns because of the use of oncogenes and the potential for the integration of exogenous factors. Therefore, several new methods have been developed to resolve these issues, including nonintegrating vectors, nonviral gene delivery methods, miRNAs, cell membrane permeable proteins, and small molecule compounds [7–11]. Compared to other approaches, chemical compounds similar to those employed to treat human diseases for decades have several unique advantages. For example, their structural versatility permits modulation of induction time and concentration [12]. In this review, omitting differentiation, we will focus on pure small molecule inductions for reprogramming or transdifferentiation (Figure 1). The dramatic progress in small molecule induction of cell fate
molecules (AM580 and EPZ004777) to induce mouse fibroblasts into a stage named “XEN-like cell transition,” while three small molecules (5-aza-dC, EPZ004777, and SGC0946) were sufficient to convert these transitional cells to CiPSCs. Compared with the original protocol, the induction efficiency for CiPSCs was raised by 1000-fold via fine-tuning of the factors during these two stages. At the same time, Xie’s team discovered that a chemical cocktail including bromodeoxyuridine (BrdU), CHIR99021, RepSox, and Forskolin was able to induce mouse fibroblasts into CiPSCs [19].

In 2016, Deng’s team also reported that CiPSCs were reprogrammed from neural stem cells and intestinal epithelial cells [21]. A similar chemical cocktail (VPA, CHIR99021, RepSox, Parnate, Forskolin, AM580, and DZNep) was applied to the reprogramming of MEFs and intestinal epithelial cells. Two extra small molecules Ch55 and EPZ004777 were used in the reprogramming of neural stem cells. In 2018, Pei’s team found that three types of mouse cell lineages could be induced to CiPSCs through an epithelial colony stage [22]. A chemical combination containing Vitamin C (VC), bFGF, CHIR99021, BrdU, RepSox, FSK, VPA, AM580, EPZ5676, DZNep, SGC0946, and BMP4 was applied for the induction of epithelial colonies, and then, 2iL (CHIR99021, PD0325901, and LIF) were used to induce full pluripotency in the second stage. In contrast to Deng’s and Xie’s methods, the induction efficiency and time were dramatically improved in Pei’s protocol.

Although mouse CiPSCs have advanced in the last several years, generation of human CiPSCs have remained elusive. Based on different pluripotent signaling pathways in mice and humans [23, 24], a large-scale screening of small molecules may be necessary. Currently, the small molecules involved in induction are classified into three categories, including epigenetics, signaling pathways, and metabolism (Table 1 and Figure 2). As for different starting cells and targeted cells, some clues could be obtained to select small molecules for reprogramming or transdifferentiation from this review.

2.2. Extended Pluripotent Stem Cells (EPSs). In 2017, Deng’s team found that ESCs or iPSCs could be reprogrammed into extended pluripotent stem cells (EPSs) that could differentiate into four lineages including trophoderm, ectoderm, endoderm, and mesoderm via a chemical cocktail consist of LIF, CHIR99021, (S)-(+) Dimethindene maleate, and Minocycline hydrochloride [25]. After half a year, Liu’s team also obtained EPSs using a different small molecule combination containing hLIF, CHIR99021, PD0325901, JNK inhibitor VIII, SB203580, A-419259, and XAV939 [26]. As for a means to create new animal models, EPS cell lines could be applied to explore fundamental questions such as the development of the placenta, yolk sac, and embryo proper.

2.3. Chemical-Induced Neural Stem Cells (CiNSCs). In 2012, we first found a pure small molecule combination (VPA, RG108, VC, BIX01294, A83-01, CHIR99021, and PD032591) was able to induce mouse embryonic and adult tail-tip fibroblasts into neural stem cells [27, 28]. CiNSCs are similar to neural stem cells in morphology, gene
| Name of the compounds | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|-----------------------|-------------------------|-----------------------------------------------|------------|
| **Signaling pathways** |                         |                                               |            |
| **TGF-β signaling pathways** |                         |                                               |            |
| A83-01                | TGF-beta RI (ALK4/5/7) inhibitor | CiNSCs, CiNs, CiBCs, CiPSCs, CiBLPCs, CiCMs, CiEPCs | [16, 27, 28, 30–34, 46, 55–57, 62, 64] |
| RepSox (E-616452)     | TGF-beta RI (ALK5) inhibitor | CiPSCs, CiNs, CiCMs, GPCs, CiSMCs, CiCCs       | [14, 17–19, 21, 22, 40, 41, 45, 52, 61, 69, 71, 79] |
| SB431542              | Inhibitor of TGF-βRI, ALK4, and ALK7 | CiEPCs, CiNs, CiCMs, CiLCs                     | [35, 43, 55–57, 59, 75] |
| IDE 1                 | Activator of TGF-β signaling pathway | CiBCs                                          | [64] |
| DMH1                  | Inhibitor of ALK2         | CiNs                                           | [41] |
| **BMP signaling pathways** |                         |                                               |            |
| Dorsomorphin           | BMP receptor inhibitor    | CiNs                                           | [42] |
| LDN193189             | BMP type I receptor (ALK2/3) inhibitor | CiNSCs, CiNs                                  | [30, 42, 43] |
| **Wnt signaling pathway** |                         |                                               |            |
| CHIR99021             | GSK3 inhibitor           | CiPSCs, EPSs, CiNSCs, CiBLPCs, CiCMs, CiPCs, CiSMCs | [15, 17–19, 21, 22, 25–28, 30–34, 39–43, 45, 47, 52, 59, 61, 62, 69, 78, 79] |
| LiCl                  | GSK3 inhibitor           | CiBCs                                          | [64] |
| XAV939                | Wnt/beta-catenin inhibitor | EPs                                            | [26] |
| IWR1                  | Wnt/beta-catenin inhibitor | CiPCs                                          | [52] |
| CHIR-98014            | GSK3 inhibitor           |                                               |            |
| TWS119                | GSK3 inhibitor           |                                               |            |
| Tidelogusib           | GSK3 inhibitor           |                                               |            |
| BIO                   | GSK3 inhibitor           |                                               |            |
| AZD2858               | GSK3 inhibitor           |                                               |            |
| TDZD-8                | GSK3 inhibitor           |                                               |            |
| Indirubin             | GSK3 inhibitor           |                                               |            |
| PNU-74654             | Wnt/beta-catenin inhibitor |                                               |            |
| IWP-2                 | Wnt/beta-catenin inhibitor |                                               |            |
| **MAPK/ERK signaling pathway** |                         |                                               |            |
| PD0325901             | Inhibitor of MEK1/2      | CiPSCs, CiCMs, CiNs, EPSs                     | [22, 26, 42, 61] |
| SC1                   | ERK1 and RasGAP inhibitor | CiCMs                                          | [62] |
| **Rho signaling pathway** |                         |                                               |            |
| Thiazovivin           | ROCK inhibitor           | CiNs                                           | [43] |
| Y-27632               | ROCK inhibitor           | CiNs, CiBLPCs, CiCMs                          | [31–33, 40, 42, 46, 47, 58, 62, 78] |
| **Notch signaling pathway** |                         |                                               |            |
| DAPT                  | Gamma-secretase inhibitor | CiNs, CiBCs                                    | [42, 43, 64] |
| **SHH signaling pathway** |                         |                                               |            |
| Cyclophamine-KAAD     | Hedgehog/smoothened inhibitor | CiBCs                                         | [64] |
| Hh-Ag 1.5             | Smoothened agonist       | CiNSCs                                         | [30] |
| Purmorphamine         | Smoothened agonist       | CiNs                                           | [42, 43, 46] |
| **Other signaling pathways** |                         |                                               |            |
| A-419259              | An inhibitor of Src family kinases (SFK) | EPS                                           | [26] |
Table 1: Continued.

| Name of the compounds | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|-----------------------|--------------------------|-----------------------------------------------------|-------------|
| dbcAMP                | Activates cAMP-dependent protein kinases              | GIPSCs, CiNs, CiPCs, CiSMCs, CiLCs                   | [78]        |
| Forskolin             | Adenylyl cyclase activator                             | CiPSCs, CiNs, CiPCs, CiSMCs, CiLCs                   | [18, 19, 22, 39–42, 45–47, 52, 59, 61, 69, 75, 78, 79] |
| Gö6983                | Inhibitor of protein kinase C (PKC)                   | CiNs                                                | [40]        |
| Indolactam V          | Activator of protein kinase C (PKC)                   | CiBCs                                               | [64]        |
| JNJ10198409           | PDGFR-a and PDGFR-b inhibitor, PDGFR tyrosine kinase inhibitor IV | GiCMs                                               | [62]        |
| SB203580              | P38 MAPK inhibitor                                    | EPSs, CiBCs                                         | [26, 64]    |
| SP600125              | JNK inhibitor                                          | CiNs                                                | [40, 41]    |
| SU16F                 | PDGFR-b inhibitor                                     | CiCMs                                               | [62]        |
| Celecoxib             | COX inhibitor                                          | CiCCs                                               | [71]        |

Epigenetic modifications

DNA methylation inhibitor

| Name | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|------|--------------------------|-----------------------------------------------------|-------------|
| 5-Aza-dC | DNMT inhibitor | GIPSCs | [20] |
| BrdU  | Analog of thymidine     | GIPSCs | [19, 22] |
| DZNep | SAH hydrolase inhibitor | GIPSCs | [18, 21, 22] |
| RG108 | DNA methyltransferase inhibitor | GINSCs, GIEPCs, CiNs | [27, 28, 30, 35, 42] |
| AMI-5 | Protein methyltransferase inhibitor | GIPSCs | [16] |
| PF-6405761 | BET inhibitor | GIPSCs | [27, 28, 30, 35, 42] |

Histone deacetylation inhibitor

| Name | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|------|--------------------------|-----------------------------------------------------|-------------|
| NaB  | HDAC inhibitor           | CiN, CiCMs                                          | [47, 60]    |
| VPA  | HDAC inhibitor           | GIPSCs, GINSCs, CiNs, CiCMs, CiPCs, CiSMCs, CiCCs    | [13, 17, 18, 21, 22, 27, 28, 40, 43, 45, 47, 52, 61, 69, 71, 79] |
| I-BET-762 | BET inhibitor | GIPSCs, GINSCs, CiNs, CiCMs, CiPCs, CiSMCs, CiCCs | [13, 17, 18, 21, 22, 27, 28, 40, 43, 45, 47, 52, 61, 69, 71, 79] |

Histone methylation modulator

| Name | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|------|--------------------------|-----------------------------------------------------|-------------|
| AS8351 | Inhibitor of histone demethylase | CiCMs | [62] |
| Bx01294 | Histone methyltransferase inhibitor | CiCMs, GIEPCs, CiNSCs | [27, 28, 35, 62] |
| BRD 7552 | Increases acetylation of histone H3 and trimethylation of H3K4 and H3K9 | CiBCs | [64] |
| EPZ5676 | DOT1 inhibitor | GIPSCs | [22] |
| EPZ004777 | DOT1L inhibitor | GIPSCs | [20, 22] |
| SGC0946 | DOT1L inhibitor | GIPSCs | [20, 22] |
| CPI-0610 | BET inhibitor | GIPSCs | [20, 22] |
| GS-5829 | BET inhibitor | GIPSCs | [20, 22] |

Histone acetylation modulator

| Name | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|------|--------------------------|-----------------------------------------------------|-------------|
| I-BET151 | Inhibitor of epigenetic reader | CiNs | [39, 45, 78] |
| INCB057643 | BET inhibitor | CiNs | [39, 45, 78] |

Metabolic processes

| Name | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|------|--------------------------|-----------------------------------------------------|-------------|
| AMS80 | RAR agonist             | GIPSCs | [20–22] |
| Bexarotene | RAR agonist | GIPSCs | [20–22] |
| Ch55  | RAR agonist             | GIPSCs | [21] |
| Retinoic acid | RAR ligand | GINSCs, CiNs | [30, 46] |
| TTNPB  | RAR ligand              | GIPSCs, CiNs, CiSMCs, CiCCs | [18, 43, 47, 61, 69, 71] |
induce mouse hepatocytes with dielines obtained human bipotent liver progenitor cells from Y-27632, CHIR99021, and Wnt3a [32]. Later, two research progenitor cells using four small molecules A83-01, Y-Hui in vitro three small molecules (Y-27632, A83-01, and CHIR99021) into bipotent liver progenitor cells with (CiEPCs).

In 2016, Pei’s team revealed that human gastric

## 2.5. Chemical-Induced Endodermal Progenitor Cells (CiEPCs)

In 2016, Pei’s team revealed that human gastric epithelial cells could be reprogrammed to endodermal progenitors with a small molecule cocktail (Bay-K-8644, Bix01294, RG108, and SB431542) used to treat tissue-specific mesenchymal feeders [35]. The resulting chemical-induced endodermal progenitors were able to be amplified in culture and differentiated to hepatocytes, pancreatic endocrine cells, and intestinal epithelial cells without generation of teratomas in vivo.

Although the mechanism of small molecule induction remains elusive, some clues can be obtained from the current literature. Taken together, to complete reprogramming, the epigenetic barrier has to be overcome and the starting cell identity should be gradually removed, while the target cell identity should be built up. In pure small molecule reprogramming, epigenetic modulators such as DNA methylation writer inhibitors (5-aza-dC, BrdU, DZNep, and RG108), histone methylation writer inhibitors (Bix01294, EPZ004777, EPZ5676, and SGC0946), and histone acetylation eraser inhibitors (VPA) were involved in this process (Table 1 and Figure 2). If fibroblasts are the starting cells, the TGF-β signaling pathway needed to be shut down by chemicals (SB431542, A83-01, and RepSox), which indicates this pathway is essential to keep the identity of fibroblasts. To create target cell identity, the Wnt signaling pathway needed be activated to reverse the induced cells back to an earlier developmental stage during reprogramming with an activator (CHIR99021). Due to cell death caused by oxidative stress and an epigenetically unstable state during the reprogramming process, metabolic regulators ([(S)-(+)Dimethindene maleate, Vitamin C, Parnate, Ch55, SMER28, AM580, and TTNPB])

### Table 1: Continued.

| Name of the compounds | Main mechanism of action | Application in reprogramming or transdifferentiation | References |
|-----------------------|--------------------------|-----------------------------------------------------|------------|
| Bay-K-8644            | Ca2+ channel activator   | GiEPCs                                             | [35]       |
| ISX9                  | Neurogenesis inducer     | GiNs                                               | [39, 42, 78]|
| LPA                   | A ligand activator for EDG-2, EDG-4, and EDG-7 | GiBLPCs                                            | [33]       |
| Minocycline hydrochloride | Bind to the bacterial 30S ribosomal subunit and inhibiting protein synthesis | EPsS                                               | [25]       |
| OAC2                  | Activator of octamer-binding transcription factor 4 (Oct4) | GiCMs                                              | [62]       |
| P7C3                  | Monoamine oxidase inhibitor, LSD1 inhibitor | GiPSCs, GiNSCs, GiCMs, GiSMCs                      | [15, 17, 18, 21, 30, 59, 61, 69, 79] |
| Rolipram              | Targets NAMPT enzyme     | GiNs                                               | [42]       |
| SMER28                | Autoagy modulator        | GiNSCs                                             | [30]       |
| (-)-Dimethindene maleate | Antagonist of muscarinic M2 and histamine H1 receptors | EPsS                                               | [25]       |
| Vitamin C             | A strong antioxidant     | GiPSCs, GiNSCs, GiBCs                              | [22, 27, 28, 64] |

GPsAs: chemical-induced adipocytes; GPsCs: chemical-induced beta cells; GiBLPCs: chemical-induced bipotent liver progenitor cells; GPsCs: chemical-induced cartilaginous cells; GiCMs: chemical-induced cardiomyocytes; GPsCs: chemical-induced epithelial colonies; GiEPCs: chemical-induced endodermal progenitor cells; GiLs: chemical-induced Leydig cells; GiNs: chemical-induced neurons; GiNPCs: chemical-induced neuroprogenitor cells; GiNSCs: chemical-induced neural stem cells; GiPCs: chemical-induced photoreceptor cells; GiPSCs: chemical-induced pluripotent stem cells; GiSMCs: chemical-induced skeletal muscle cells; EPsS: extended pluripotent stem cells.
have been applied to enhance cell survival during the conversion.

3. Transdifferentiation In Vitro

Pluripotent stem cells (ESCs and iPSCs) should be converted into functional target cells before injection for cell therapy because they could generate teratomas in vivo [36]. The technology of transdifferentiation (i.e., the transition from one functional cell type to another without a requirement of a pluripotent state) represents a shortcut to achieve sufficiently functional cells for cell therapy [37]. At present, several types of functional cells including neurons, photoreceptor cells, cardiomyocytes, beta cells, adipocytes, skeletal muscle cells, cartilaginous cells, and Leydig cells have been successfully obtained using small molecule-mediated transdifferentiation methods in vitro.

3.1. Chemical-Induced Neurons (CiNs). As life expectancy is increasing, the number of people suffering from neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease is on the rise [38]. Thus, it is urgent to obtain adequate quantities of patient-tailored neural cells for cell therapy and drug screening. Nowadays, scientists have made great progress in small molecule-based direct induction for neurons. In 2015, Deng’s team used a combination of four small molecule compounds (Forskolin, ISX9, CHIR99021, and I-BET151) to transdifferentiate mouse fibroblasts into neurons [39]. The authors suggested that I-BET151 (a BET family bromodomain inhibitor) disrupted the fibroblast-specific program, while ISX9 (a neurogenesis inducer) activated neuronal-specific genes. At the same time, Pei’s work revealed that human lung fibroblasts could be...
converted into neurons using a similar small molecule combination, including VPA, CHIR99021, DMH1, RepSox, Forskolin, Y-27632, and SP600125 [41].

In 2019, Dai’s research group found a rapid and efficient method to convert human fibroblasts into neurons with twelve small molecules (CHIR99021, LDN193189, Dorso-morphin, ISX9, RG108, PD0325901, Purmorphamine, DAPT, Forskolin, ISX9, Y-27632, and P7C3) [42].

In 2015, Chen’s team identified a combination of nine small molecules (LDN193189, SB431542, TTNPB, Thiazovivin, CHIR99021, VPA, DAPT, Smoothed agonist, and Purmorphamine) for reprogramming human astrocytes into neurons [43]. These induced neurons could survive for more than 5 months in culture and generated functional synaptic networks in vitro, and they were able to survive for over 1 month in mouse brains and merge with local circuits. Later, they also implied that six signaling pathways including SHH, Notch, Wnt, TGF-β, and JA/STAT played a pivotal role during the transdifferentiation [44]. Similar work was reported by Pei’s lab with a different small molecule combination (VPA, Chir99021, RepSox, Forskolin, I-Bet151, and ISX-9) two years later [45].

Furthermore, subtype neurons also have been obtained. In 2018, human and mouse motor neurons were created by a chemical combination containing Kenpaullone, Forskolin, Y-27632, Purmorphamine, and retinoic acid [46]. One year later, Li’s team reported that a chemical cocktail (CHIR99021, A83-01, Y-27632, VPA, TTNPB, Forskolin, and NaB) induced human urine-derived cells into neurons, while the majority of induced cells were glutamatergic neurons [47].

3.2. Chemical-Induced Photoreceptor Cells (CiPCs). Vision loss resulting from retinal neuron damage causes retinopathies, including age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa [48, 49]. As a favorable method, stem cell therapy could substitute for the loss of retinal neurons [50, 51]. Recently, Chavala’s team reported five small molecules (VPA, CHIR99021, RepSox, Forskolin, and IWR1) were able to transdifferentiate fibroblasts into photoreceptor-like cells [52]. The authors also confirmed that CiPCs could mend pupil reflex and vision when transplanted into the subretinal space of mice with retinal degeneration. Additionally, they implied that the AXIN2–NF-κB–ASCL1 pathway enhanced retinal lineage commitment and mitochondria were the signaling hub during transdifferentiation.

3.3. Chemical-Induced Cardiomyocytes (CiCMs). It is widely known that the regeneration of the adult mammalian heart after injury is limited [53]. Therefore, heart failure resulting from cardiomyocyte loss is a major cause of mortality around the world [54]. As the most common cell type in the heart, cardiac fibroblasts are considered promising for cardiac reprogramming.

Small molecules are also able to replace transcription factors and provide an alternative means of cardiac reprogramming. It was reported that TGF-β inhibitors (SB431542 or A83-01) could improve the efficiency of cardiomyocyte induction [55–57]. The small molecule Y-27632 also enhanced cardiac reprogramming [58]. Furthermore, Ding’s group reported that a small molecule combination (CHIR99021, SB431542, Parnate, and Forskolin) was sufficient to complete the conversion of cardiomyocytes from mouse fibroblasts with Oct4 alone [59]. It was also reported that small molecules (NaB, RA, and ICG-001) were able to improve rat and human cardiac cell generation induced by transcription factors (Gata4, Mef2C, and Tbx5) [60]. In 2015, Xie’s team transdifferentiated mouse fibroblasts into cardiomyocytes by passing a cardiac progenitor stage with six small molecules (CHIR99021, RepSox, Forskolin, VPA, Parnate, and TTNPB), while the induced cardiomyocytes were cultured in cardiomyocyte maintenance medium containing CHIR99021, PD0325901, LIF, and insulin [61]. One year later, Ding’s lab reported that human functional cardiomyocytes were induced by a combination of nine small molecules (CHIR99021, A83-01, BIX01294, AS8351, SCI, Y-27632, OAC2, SU16F, and JNJ1019409) [62]. Furthermore, the induced human fibroblasts were able to be efficiently converted into cardiomyocyte-like cells in infarcted mouse hearts.

3.4. Chemical-Induced Beta Cells (CiBCs). Diabetes mellitus, which results from pancreatic β cell damage, is an international health epidemic and influences more than 300 million people in the world [63]. Therefore, producing plenty of functional pancreatic β cells for studying diabetes and treating patients is an urgent task. In 2015, we successfully induced human urine cells to insulin-secreting beta cells by passing through three stages with pure small molecules [64]. Firstly, urine cells were induced into an endodermal lineage using a chemical cocktail (IDE 1, LiCl, and VC) for 6 days. The induced cells were then differentiated into pancreatic precursors in two steps. The first step induction medium contained cyclopamine–KAAD, Indolactam V, RA, VC, A83-01, and BRD 7552 for 1 day, while the secondary step induction used chemicals, including cyclopamine–KAAD, Indolactam V, VC, A83-01, and BRD 7552, for 6 days. Insulin-secreting beta cells were obtained in the tertiary induction medium (SB203580, VC, and DAPT) for 9 days. Furthermore, the induced beta cells could reduce glucose levels and enhance survival rates in diabetic mice.

3.5. Chemical-Induced Adipocytes (CiAs). As a promising therapy for obesity and metabolic diseases, brown adipose tissue (BAT) has been intensively studied [65, 66]. The energy balance in the body is balanced with white adipose tissue collecting energy, while BAT expends energy and produces heat [67]. In 2017, Ding’s research group converted mouse myoblasts into brown adipocyte-like cells with a retinoid X receptor (RXR) agonist, bexarotene. They implied that Rxra/γ activation is required for the induction of BAT [68].

3.6. Chemical-Induced Skeletal Muscle Cells (CiSMCs). Muscle-related maladies including muscle wasting and muscular dystrophy have yet-to-be adequately treated using traditional medicine. The cell therapy technique brings a promising
approach to resolve this issue. Recently, it was reported that mouse fibroblasts could be converted to skeletal muscle cells by a combination of six small molecules (VPA, Chir99021, RepSox, Forskolin, Parnate, and TTNPB) [69]. The authors implied that three signaling pathways Wnt, TGF-β, and cAMP were crucial for the transdifferentiation.

3.7. Chemical-Induced Cartilaginous Cells (CiCCs). Cartilage defects cause joint pain and diminish quality of life. Recently, autologous chondrocyte therapy was proposed as a means of cartilage healing [70]. Ouyang’s team revealed that mouse embryonic fibroblasts could be converted to functional cartilaginous cells by a chemical cocktail (VPA, CHIR98014, Forskolin, TTNPB, and Celecoxib) [71]. These CiCCs could enhance defective healing and restore 63.4% of mechanical function damage in vivo.

3.8. Chemical-Induced Leydig Cells (CiLCs). Affecting about 30% of men aged 40–79 years, late-onset hypogonadism (LOH) with a serum testosterone deficiency could result in sexual dysfunction, central adiposity, mood disturbance, osteoporosis, amyotrophy, and other abnormalities [72–74]. Leydig cells produce testosterone, so Leydig cell transplanta- tion could be an ideal tool to heal LOH. Recently, Huang’s team reported that functional mouse Leydig cells could be transdifferentiated from fibroblasts using a small molecule combination (Forskolin, 20ɑ-hydroxycholesterol, luteinizing hormone, and SB431542) [75]. Moreover, these CiLCs could survive in the testes and produce testosterone in a circadian rhythm. As for the mechanism of small molecule transdifferentiation, collectively, in contrast to reprogramming, transdifferentiation is an easier process because it does not need more energy to pull the starting cells to a less differentiated level for cell conversion. Compared to reprogramming, epigenetic modulators, the histone methylation writer inhibitor was replaced with the histone acylation reader inhibitor (I-Bet151) in transdifferentiation, which implies less epigenetic barrier is required to be overcome during transdifferentiation. Furthermore, more metabolic modulators are involved in the confirmation of the new cell identity, such as OAC2 for cardiomyocytes, ISX9 for neurons, and beaxarotene for brown adipose tissue.

4. Transdifferentiation In Vivo

Although functional cells could be obtained by differentiation from pluripotent stem cells or transdifferentiation from somatic cells, induction efficiency, ultimate maturation of cells, and survival rates after cell transplantation are still the three biggest obstacles to cell therapy [76]. Due to safety and technical difficulties of cell transplantation therapy, in vivo reprogramming may become the next generation of regenerative medicine with therapeutic potential [77].

4.1. Neurons. In 2018, Deng’s team released their data about in vivo transdifferentiation of neurons from mouse astrocytes with a cocktail combination consist of dbcAMP, Forskolin, ISX9, CHIR99021, I-BET151, and Y-27632 [78]. The combination of chemicals was injected into mouse brains at a stable rate for two weeks with an osmotic minipump. The induced cells not only formed endogenous neurons with similar neuron-specific marker expression and electrophysiological properties but also merged with local circuits in vivo.

4.2. Cardiomyocytes. In 2018, Xie’s team reported that a small molecule combination of CRFVPTM (CHIR99021, RepSox, Forskolin, VPA, Parnate, TTNPB, and Rolipram) mediated transdifferentiation of cardiac fibroblasts into cardiomyocytes in normal adult mice with a low efficiency of 1% [79]. CRFTM were administrated orally and VP were intraperito- neally injected once for 6 weeks. The transdifferentiation only happened in the heart, which suggests the local niche also plays a critical role in small molecule-mediated cardiac induction. Furthermore, the induced cardiomyocytes dramatically repressed the scar formation and promoted cardiac function in mice with myocardial infarction.

To explore the mechanism of small molecule transdifferentiation in vivo and in vitro, additional small molecules were applied to activate the cAMP signaling pathway (dbcAMP for neurons and Rolipram for cardiomyocytes), which suggested targets downstream of the PKA signaling pathway are important to overcome the disturbance from in vivo environment during transdifferentiation.

In summary, although the mechanism of full small molecule induction is unknown, some implications can be observed. By examining signaling pathways, it is apparent that certain pathways are preferred for transdifferentiation (Figure 2), such as inhibiting BMP for ectodermal induction, activation of LIF-STAT3 for creating pluripotent stem cells, and inhibition of Notch, SHH, and Rho for the induction of ectodermal or endodermal lineages. On the other hand, some signaling pathways are preferred for induction (e.g., activation of Wnt and inhibition of TGF-β and MAPK/ERK). As for the induction process, it seems that there is an intermedi- ate state by which various target cells could be achieved in certain culture conditions.

5. Perspective

Despite the exciting progress that has been achieved in the field of pure small molecule-induced cells, there are still some key problems such as apoptosis due to oxidative stress, death from an epigenetically unstable state, genomic integrity, gen- toxicity, scaling production for large animals’ safety and efficacy trials, and producing a safe delivery system as well as induction methods [77]. Moreover, the majority of pure small molecule cocktails for human cells still remain to be determined.

Without cell transplantation, direct in vivo reprogram- ming for local in situ conversion of cells is emerging as a new way to produce cells for regenerative medicine. Although in situ chemical induction will be a focus for the next decade, how these small molecules could be precisely delivered to the desired tissues or organs to produce fully integrated functional cells is a primary challenge. Biomate- rials that can deliver small molecules to targeted organs, for example, nanoparticles containing specific signals for
recognizing specific cell types, can assist in vivo reprogramming studies and future clinical applications (Figure 3). On the other hand, small molecule-induced cells could be constructed for organs such as the heart, liver, or brains using 3D printers in vitro (Figure 3). In addition, recent scientific tools such as single-cell sequencing [80] and CRISPR-based genome-wide screening [81] will help exploring new chemical cocktails and illustrate the induction mechanisms.

Conflicts of Interest

The authors declare no competing interests.

Authors’ Contributions

Z.-D.Y. and W.-N.Z. prepared the manuscript. Z.-D.Y. and K.-Z.L. drew graphics. Y.-C.H. wrote the manuscript. Z.-P.H. reviewed and edited the manuscript. Z.-P.H drafted the final version of the manuscript. All authors read and approved the final manuscript. Zhao-Di Yuan and Wei-Ning Zhu contributed equally.

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