Review

Direct cell-fate conversion of somatic cells: Toward regenerative medicine and industries

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Abstract: Cells of multicellular organisms have diverse characteristics despite having the same genetic identity. The distinctive phenotype of each cell is determined by molecular mechanisms such as epigenetic changes that occur throughout the lifetime of an individual. Recently, technologies that enable modification of the fate of somatic cells have been developed, and the number of studies using these technologies has increased drastically in the last decade. Various cell types, including neuronal cells, cardiomyocytes, and hepatocytes, have been generated using these technologies. Although most direct reprogramming methods employ forced transduction of a defined sets of transcription factors to reprogram cells in a manner similar to induced pluripotent cell technology, many other strategies, such as methods utilizing chemical compounds and microRNAs to change the fate of somatic cells, have also been developed. In this review, we summarize transcription factor-based reprogramming and various other reprogramming methods. Additionally, we describe the various industrial applications of direct reprogramming technologies.

Keywords: direct reprogramming, cell-fate conversion, regenerative medicine, cell transplantation therapy, transcription factor, industrial application

1. Introduction

All cell types in the bodies of higher multicellular animals have extremely diverse phenotypes despite their genetic identity. A single zygote can divide and differentiate into a considerable number and wide variety of cells with unique genome sequences during development. The differences in the phenotypic characteristics among cell types result in different transcriptome and epigenome variations. As Waddington predicted in the 1950s,1) the differentiation and identity of the cells are governed by a non-genetic system for the regulation of gene expression termed “epigenetics”. Although it has long been thought that terminally differentiated somatic cells cannot cross over the “epigenetic barrier” and trans-differentiate into other cell types, some somatic cells have plasticity for de- or re-differentiation, e.g., cardiomyocytes in zebrafish hearts2,3) and pigmented epithelial cells in newt eyes.4) These examples suggest that terminally differentiated animal cells can convert into other cell types.

In 2006, Takahashi and Yamanaka initialized the cell fate of fibroblasts from adult mammals and re-differentiated them into various cell lineages through the forced transduction of a defined set of transcription factors.5) Their study provided information regarding the nature of cell differentiation as well as the possibility of induced pluripotent stem cell (iPSC)-based regenerative therapies. Indeed, many basic and applied studies have been performed for the realization of iPSC-based regenerative medicine. The successful application of this type of medicine has been reported, including for clinical trials of cell transplantation therapy for age-related macular degeneration,6) Parkinson’s disease,7) and corneal epithelial stem cell deficiency.8) At present, clinical studies of other diseases, such as spinal cord injury, severe heart failure, and stroke, are being undertaken with regards to iPSC-based regenerative medicine. Furthermore, several drug discovery studies employ-
ing patient-derived iPSCs have identified a number of hopeful pharmaceutical candidates for treating fibrodysplasia ossificans progressiva,9) Pendred syndrome,10) Parkinson’s disease,11) Alzheimer’s disease,12) amyotrophic lateral sclerosis,13) and muscular dystrophy.14) In addition, the in vitro reconstruction of complicated and functional tissues and organs using iPSCs has also been reported, including in the brain,15) intestines,16) liver,17) and kidneys.18)

Although iPSCs are a promising technology for future medicine, alternative methods for cell fate conversion, termed “direct reprogramming”, have also been developed in the last decade. Direct reprogramming involves the trans-differentiation of somatic cells directly into other cell types without complete initialization. Before the first study regarding iPSCs, only a few types of direct reprogramming had been reported, including the reprogramming of fibroblasts into myoblast and pancreatic β cells. In recent studies, various cell types have been directly converted from other somatic cell types. Most of the direct reprogramming methods applied the forced transduction of defined sets of transcription factors to convert terminally differentiated cells into specific target cells artificially. Initially, only single-transcription factor-based direct reprogramming methods were reported.19)–24) However, most recent studies for direct reprogramming employ a combinatorial transduction strategy similar to that of the iPSC, especially in the conversion between embryologically divided cell types. Although the combinatorial expression strategy of transcription factors with viral vectors is a powerful and stable technique for direct reprogramming, alternative methods are increasing because genomic integration of the transgenes causes genomic damage, and might induce unexpected functional attenuation, abnormal proliferation, and transformation. As explained above, the major applications of the direct reprogramming technologies are bedside clinical uses, such as cell transplantation and regenerative medicine. Meanwhile, other medical, e.g., drug discovery and disease study, and non-medical, e.g., livestock and pet industry, applications of the direct reprogramming technology have been used increasingly in recent years. Therefore, direct reprogramming technology will play a crucial role in both medical and non-medical industry.

2. Transcription factor-based direct reprogramming technologies

Similar to iPSC technology, most direct reprogramming methods employ forced expression of the defined set of transcription factors for cell-fate conversion (Table 1). Many are tissue-specific or developmental process-related genes; thus, it is considered that these transcription factors act as a “kickstarter” to drive the cell type-specific transcription networks. Furthermore, almost all of the

| Source cells | Target cells | Transgenes and/or Treatment | Species | Cell transplantation | Published year | PMID |
|--------------|--------------|-----------------------------|---------|---------------------|----------------|------|
| Astrocytes   | Glutamatergic neurons | Pax6, Mash1, or Gln2 | Mouse | YES | 2007 | 17687043(25) |
| Embryonic fibroblasts and Hepatocytes | Neuronal cells | Brn2, Ascl1, and Myt1l | Mouse | 2010, 2011 | 20107439(26); 21962018(27) |
| Astrocytes   | GABAergic neurons | Dlx2; Dlx2 and Ascl1 | Mouse | 2010 | 20502524(23) |
| Embryonic fibroblasts and Adult skin fibroblasts | Dopaminergic neurons | Ascl1, Lmx1a, and Nur1 | Mouse & Human | YES | 2011 | 21725324(27) |
| Fetal fibroblasts and Postnatal foreskin fibroblasts | Neuronal cells | BRN2, ASCL1, MYT1L, and NEUROD1 | Human | 2011 | 21617644(28) |
| Embryonic fibroblasts and Postnatal fibroblasts | Neuronal cells | ASCL1, BRN2, MYT1L, LMX1A, and FOXA2 | Human | 2011 | 21646515(29) |
| Embryonic fibroblasts | Neural stem cells | Brn4/Pou3f4, Sox2, Klf4, c-Myc, and E47/Tcf3 | Mouse | YES | 2012 | 22445517(30) |

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| Cell Type                        | Source Cells                      | Target Cells            | Pluripotency |
|---------------------------------|-----------------------------------|-------------------------|--------------|
| Embryonic fibroblasts and Fetal foreskin fibroblasts | Neural stem cells, Sox2, Pax6, Ngn2, Hes1, Id1, Ascl1, Brn2, c-Myc, and Ki67 | Mouse & Human, YES, 2012 | 22683203[31] |
| Sertoli cells                   | Neural stem cells                 | Mouse                   | YES, 2012    | 2206470[32] |
| Fibroblasts (IMR90 cells)       | Dopaminergic neurons, MASH1, NGN2, SOX2, NURR1, and PTFX3 + A dominant-negative P53 | Human                   | YES, 2014    | 25129808[214] |
| Non-sensory cochlear epithelial cells | Neuronal cells, Ascl1, Ascl1 and Neurod | Mouse                   | 2014         | 2492835[30] |
| Astrocytes                      | Neuronal cells, Brn4              | Mouse                   | 2014         | 24887289[185] |
| Skin fibroblasts                | Dopaminergic precursors, Brn2, Sox2, and Foxa2 | Mouse, YES              | 2015         | 26224135[215] |
| Adult skin fibroblasts          | Motor neurons, NEUROG2, SOX11, ISL1, and LHX3 | Human                   | 2016         | 26725112[40] |
| Fibroblasts (3T6 cells)         | Neuronal cells, Ascl1, Brn4, and Tc3 | Mouse                   | YES, 2017    | 29137640[216] |
| Umbilical cord blood cells      | Neural stem cells, SOX2 and HMGA2 | Human                   | 2017         | 28844127[43] |
| Fibroblasts (3T6 cells)         | Neuronal cells, Ascl1, Brn2, and Foxa1 | Mouse                   | 2017         | 28855426[139] |
| Resident glial cells            | Neuronal cells, Ascl1, Lmx1a, and Nurr1 | Mouse                   | 2017         | 28844658[41] |
| Fibroblast-like cells from retinal tissues | Neuronal cells, ASCL1 and PAX6 | Human                   | 2017         | 28697461[217] |
| Pharyngeal mesenchymal cells     | Neuronal cells, Brn1, Ascl1, Myt1l, and Neurod | Mouse & Human, 2017     | 28327614[218] |
| Fibroblasts                      | Motor neurons, ASCL1, ISL1, NEUROD1, BRN2, HB9, LHX3, MYT1L, and NGN2 | Human                   | 2017         | 28099929[219] |
| Fibroblasts                      | Neuronal cells, SOX2, GATA3, and NEUROD1 | Human                   | 2017         | 2858733[62] |
| Dermal fibroblasts               | Neural precursor cells, SOX2 and PAX6 | Human                   | 2018         | 30450440[44] |
| Embryonic fibroblasts and Newborn foreskin fibroblasts | Neural stem cells, Ptf1a | Mouse & Human, YES, 2018 | 30030434[35] |
| Adult fibroblasts                | Neural precursor cells, SOX2, SOX2 and PAX6; SOX2, LMX1A, FOXA2 | Human                   | 2018         | 3001847[36] |
| Spiral ganglion non-neuronal cells | Neuronal cells, Ascl1 and Neurod | Mouse                   | 2018         | 29492404[220] |
| Umbilical cord mesenchymal stem cells | Neuronal cells, SOX2, ASCL1, and NEUROG2 | Human                   | 2018         | 29937717[221] |
| Pericytes                        | Neuronal cells, ASCL1 and SOX2    | Human                   | 2018         | 29915193[183] |
| Cord blood CD133(+) cells        | Neuronal cells, FOXM1, SOX2, MYC, SALL4, and STAT6 | Human                   | 2018         | 29653196[38] |

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| Cell Type                          | Associated Cells                                                                 | Species | Year | PubMed ID |
|------------------------------------|-----------------------------------------------------------------------------------|---------|------|-----------|
| Hepatocytes                        | Neuronal cells, Suz12, EzH2, Meis1, Sry, Smarca4, Esr1, Pparγ, and Stat3         | Mouse   | 2018 | 29653196(38) |
| Peripheral CD34(+) cells           | Neuronal cells, AR, SOX2, SMAD3, MYC, JUN, WT1, TAL1, SPI1, and RUNX1            | Human   | 2018 | 29653196(38) |
| Urine epithelial-like cells        | Neural stem cells, POU3F2, SOX2, BACH1, AR, PBX1, and NANOG                      | Human   | 2018 | 29653196(38) |
| Muller glia cells                  | Neural stem cells, Bmi1, Spi1, Lmo2, and Cebpα                                    | Mouse   | 2018 | 29653196(38) |
| Astrocytes and Foreskin fibroblasts| Noradrenergic neurons, Ascl1, Phox2b, Ap-2α, Gata3, Hand2, Nurr1, and Phox2a    | Mouse & Human | YES | 2019 | 31315047(222) |
| Bone marrow-derived cells, Fibroblasts, and Keratinoocytes | Neural precursor cells, MSI1, NGN2, and MBD2 | Human | YES | 2019 | 31196173(37) |
| Microglial cells                   | Neuronal cells, Neurod1                                                           | Mouse   | 2019 | 30638745(44) |

**Cardiomyocytes**

| Cell Type                          | Cardiomyocytes                                                                 | Species | Year | PubMed ID |
|------------------------------------|--------------------------------------------------------------------------------|---------|------|-----------|
| Cardiac fibroblasts                | Gata4, Mef2c, and Tbx5                                                          | Mouse   | YES  | 20691899(49) |
| Cardiac fibroblasts                | Gata4, Mef2c, Tbx5, and Hand2                                                    | Mouse   | 2012 | 22660318(50) |
| Cardiac fibroblasts and Embryonic fibroblasts | Mef2c and Tbx5 + Myocd or Gata4                                             | Mouse   | 2012 | 22575762(51) |
| Cardiac fibroblasts                | GATA4, MEF2C, TBX5, MESP1, and MYOCD                                            | Human   | 2013 | 23861494(52) |
| Embryonic stem cells-derived fibroblasts | GATA4, MEF2C, TBX5, ESRRG, MESP1, ZFPM2, and MYOCD   | Human   | YES  | 24319666(53) |
| Adult tail-tip fibroblasts and Cardiac fibroblasts | Me2c and Tbx5 + Bmi1 knockdown                                    | Mouse   | 2016 | 26942853(175) |
| Adult fibroblasts                  | Gata4, Hand2, Mef2c, Tbx5, and Znf281                                          | Mouse   | 2017 | 28082760(167) |

**Hepatocytes**

| Cell Type                          | Hepatocytes, Hnf4a and Foxa1, Foxa2, or Foxa3                                 | Species | YES   | 21716291(55) |
|------------------------------------|--------------------------------------------------------------------------------|---------|-------|-------------|
| Caudal fibroblasts                 | Gata4, Hnf1a, and Foxa3 + p19knockdown                                        | Mouse   | YES   | 21562492(56) |
| Embryonic fibroblasts              | HNF1A, HNF4A, HNF6, ATF5, PROX1, CEBPA, and MYC + TP53 knockdown              | Human   | YES   | 24582926(57) |
| Fetal and adult fibroblasts and Adipose tissue-derived mesenchymal stem cells | FOXA3, HNF1A, and HNF4A + SV40 large T antigen                               | Human   | YES   | 24582927(58) |

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| Cell Type                                      | Conversion Type | Factors                                      | Species | Year       | Reference |
|------------------------------------------------|-----------------|----------------------------------------------|---------|------------|-----------|
| Fibroblasts (BJ and MRC-5 cells) Hepatocytes   | Direct          | HNF1A and Any two of the three factors: FOXA1, FOXA3, and HNF4A | Human   | 2014       | 24963715(59) |
| Liver cells in mouse models of chronic liver disease Hepatocytes | Direct          | Foxa3, Gata4, Hnf1a, and Hnf4a | Mouse   | 2016       | 26923201(60) |
| Fetal lung fibroblasts Hepatocytes            | Direct          | ATF5, PROX1, FOXA2, FOXA3, and HNF4A         | Human   | 2017       | 29192290(61) |
| Fibroblasts Hepatocytes                       | Direct          | OCT4, FOXA2, HNF1A, and GATA3               | Human   | 2017       | 28587331(62) |
| Embryonic fibroblasts Hepatocytes             | Direct          | Foxa3, Hnf1a, and Gata4                     | Mouse   | YES        | 29959867(63) |
| Embryonic fibroblasts Hepatocytes             | Direct          | Hnf4a, Foxa3, Klf4, and c-Myc                | Mouse   | YES        | 30635054(64) |

**Islet-related cells**

| Cell Type                                      | Conversion Type | Factors                                      | Species | Year       | Reference |
|------------------------------------------------|-----------------|----------------------------------------------|---------|------------|-----------|
| Liver cells in vivo β cells Pdx1               | Direct          |                                              | Mouse   | 2000, 2003 | 10802714(20); 12775714(21) |
| Pancreatic exocrine cells in vivo β cells Ngn3, Pdx1, and Maf | Direct          |                                              | Mouse   | 2008       | 18754011(65) |
| Hepatocytes Islet cells Ngn3                   | Direct          |                                              | Mouse   | 2009       | 19793886(24) |
| Liver cells β cells PDX1, PAX4, and MAFA       | Direct          |                                              | Human   | 2014       | 24504462(66) |
| Cultured adult pancreatic duct cells β cells Pdx1, Ngn3, and Maf | Direct          |                                              | Mouse   | 2015       | 25836667(67) |
| Gallbladder cells β cells Pdx1, Ngn, Maf, and Pax6 | Direct          |                                              | Mouse   | YES        | 27833043(68) |

**Hematopoietic system-related cells**

| Cell Type                                      | Conversion Type | Factors                                      | Species | Year       | Reference |
|------------------------------------------------|-----------------|----------------------------------------------|---------|------------|-----------|
| T precursor cells Macrophages Cebpa or Cebpb   | Direct          |                                              | Mouse   | 2006       | 17088084(22) |
| T precursor cells Dendritic cells Pu.1         | Direct          |                                              | Mouse   | 2006       | 17088084(22) |
| B cells T cells Pax5 knockout                   | Direct          |                                              | Mouse   | YES        | 17851532(73) |
| Fibroblasts (3T3 cells), Embryonic fibroblasts, and Adult skin fibroblasts Macrophage-like cells Pu.1 and Cebpa or Cebpb | Direct          |                                              | Mouse   | 2008       | 18424555(74) |
| B cells Erythroid cells Gata1, Scl, and Cebpa  | Direct          |                                              | Mouse   | 2012       | 22968040(223) |
| Fibroblasts (3T3 cells) and Adult dermal fibroblasts Megakaryocyte Nfe2, Mafg, and Mafk | Direct          |                                              | Mouse & Human | YES | 22855609(75) |
| Skin fibroblasts Monocytes Spl1, Cebpa, Mnda, and Irf8 | Direct          |                                              | Mouse   | 2012       | 22428058(76) |
| Embryonic fibroblasts and Adult ear skin fibroblasts Hematopoietic progenitor cells Erg, Gata2, Lmo2, Runx1c, and Scl | Direct          |                                              | Mouse   | YES        | 25466247(77) |
| Fibroblasts Antigen-presenting dendritic cells Pu.1, Irf8, and Baf3 | Direct          |                                              | Mouse & Human | YES | 30530727(78) |

**Bone-related cells**

| Cell Type                                      | Conversion Type | Factors                                      | Species | Year       | Reference |
|------------------------------------------------|-----------------|----------------------------------------------|---------|------------|-----------|
| Neonatal foreskin fibroblasts Chondrogenic cells c-MYC, KLF4, and SOX9 | Direct          |                                              | Human   | YES        | 24146084(224) |

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| Cells/Progenitor Cells | Matched Cells | Matched Factors | Species | Year | PubMed ID |
|------------------------|---------------|----------------|---------|------|-----------|
| Dermal fibroblasts     | Osteoblasts   | OCT3/4 and OCT6 or OCT9 + L-MYC, c-MYC, or N-MYC | Human | 2015 | 26499074<sup>79</sup> |
| Fibroblasts            | Osteoblasts   | RUNX2, OCT4, OSTERIX, and L-MYC | Human | YES 2015 | 25918395<sup>80</sup> |
| Gingival fibroblasts and Adult dermal fibroblasts | Osteoblasts | OCT4, OSTERIX, and L-MYC | Human | YES 2016 | 26990860<sup>81</sup> |
| Embryonic fibroblasts  | Osteoblasts   | c-Myc, Oct4, and hLMP3 | Mouse | YES 2019 | 30453092<sup>82</sup> |

**Skeletal muscle-related cells**

| Fibroblasts (C3H10T1/2 cells) | Myoblasts | Myod | Mouse | 1987 | 3690668<sup>19</sup> |
| Dermal fibroblasts | Myoblasts | MYOD1 and MYCL | Human | YES 2017 | 28501623<sup>83</sup> |
| Embryonic fibroblasts | Skeletal muscle progenitor cells | Mef2b and Pitx1 + Pax3 or Pax7 | Mouse | YES 2018 | 28808339<sup>84</sup> |
| Adult fibroblasts | Skeletal muscle progenitor cells | Pax7, Mef2b, and Myod | Mouse | YES 2018 | 28808339<sup>85</sup> |

**Other cells**

| Embryonic fibroblasts and Newborn foreskin fibroblasts | Brown fat cells | Prdm16 and Cebp | Mouse & Human | YES 2009 | 19641492<sup>95</sup> |
| Embryonic fibroblasts | Sertoli cells | Nr5a1, Wt1, Dmrt1, Gata4, and Sox9 | Mouse | YES 2012 | 22958931<sup>96</sup> |
| Iris-derived cells | Photoreceptor cells | CRX, RAX, and NEUROD | Human | 2012 | 22558175<sup>85</sup> |
| Embryonic fibroblasts and Adult tail-tip dermal fibroblasts | Melanocytes | Mitf, Sox10, and Pax3 | Mouse & Human | YES 2014 | 25510211<sup>97</sup> |
| Adipose tissue-derived stromal cells | Endothelial cells | SOX18 | Human | 2014 | 25290189<sup>91</sup> |
| Dermal fibroblasts | Photoreceptor cells | CRX, RAX, OTX2, and NEUROD | Human | 2014 | 24456169<sup>96</sup> |
| Embryonic fibroblasts | Thymic epithelial cells | Foxn1 | Mouse | YES 2014 | 25150981<sup>98</sup> |
| Fibroblasts | Sweat gland cells | NF-κB and LEF-1 | Human | YES 2015 | 26566868<sup>91</sup> |
| Amniotic fluid stem cells | Pluripotent stem cells | OCT4 | Human | 2016 | 27019637<sup>225</sup> |
| Cardiac mesenchymal progenitors | Adipocytes | Klf4 and c-Myc | Mouse | 2016 | 27077806<sup>7</sup> |
| Embryonic fibroblasts, Adult tail-tip dermal fibroblasts, Postnatal foreskin fibroblasts, and Fetal dermal fibroblasts | Renal tubular epithelial cells | Emx2, Hnf1b, Hnf4a, and Pax8 | Mouse & Human | YES 2016 | 27820600<sup>99</sup> |
| Endothelial progenitor cells | Smooth muscle cells | MYOCD | Human | 2016 | 26874281<sup>80</sup> |

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reprogramming factor gene sets contain at least one pioneer factor, which is a transcription factor capable of opening closed chromatin sections. These factors may play a pivotal role in the machinery of direct reprogramming. In this section, examples and applications for various types of transcription factor-based direct reprogramming are introduced.

### 2.1. Neuronal cells.

By the end of 2018, the most reported target cells for direct reprogramming were neuronal lineage cells, including neural stem/progenitor cells (Fig. 1). The first example of the direct lineage conversion to neuronal cells was the reprogramming of astrocytes toward glutamatergic neurons with the transduction of single transcription factors, as shown in Table 1.

![Fig. 1. The number of publications relating to direct reprogramming studies.](image)

**Table 1. Examples of direct reprogramming studies.**

| Cell Type & Source | Factors | Target Cells | Species | Year | PMCID |
|------------------|---------|--------------|---------|------|--------|
| Embryonic stem cells & Trophoblast stem cells | Cdx2, Arid3a, and Gata3 | Mouse | 2017 | 28973471 |
| Embryonic fibroblasts & Adult tail-tip dermal fibroblasts & Leydig cells | Dmrt1, Gata4, and Nr5a1 | Mouse | 2017 | 28017657 |
| Postnatal dermal fibroblasts & Endothelial cells | ER71/ETV2 (ETS variant 2) | Human | 2017 | 28003219 |
| Dermal fibroblasts & Adipocytes | PPARG2 | Human | 2017 | 28982679 |
| Embryonic fibroblasts & Intestine progenitor cells | Hafla, Foxo, Gata6, and Cdx2 | Mouse & Human | 2017 | 28943092 |
| Embryonic fibroblasts & Adult dermal fibroblasts & Smooth muscle cells | Myocd, Gata6, and Mef2c | Mouse & Human | 2018 | 30026272 |
| Epidermal cells & Sweat gland cells | Foxc1 | Mouse | 2019 | 30894517 |
| Renal proximal tubular epithelial (HK2) cells | SNAI2, EYA1, and SIX1 | Human | 2019 | 30827514 |

**Fig. 1.** The number of publications relating to direct reprogramming studies. The publications relating to direct reprogramming studies of different target cell types are shown as a bar chart. Each different color refers to an individual target cell type. In order to survey the direct reprogramming studies published previously, original articles were searched on PubMed with the following search formula: "direct reprogramming" [All Fields].
factors in vitro: Pax6, Mash1, or Ngn2.25) However, the presynaptic output function of the astrocyte-derived neurons was insufficient, and the conversion was only achieved between closely related cells. In 2010, more successfully reprogrammed neuronal cells, termed induced neuronal (iN) cells, were reported. Combinatorial transduction of a set of transcription factors, Brn2, Ascl1, and Myt1l, induced functional neuron-like cells from mouse embryonic fibroblasts (MEFs) and hepatocytes in vitro.26) One year later, some studies generated iN cells from human fibroblasts.27–29) Although the combination and member of the transcription factors in each study were different, only one transcription factor, Ascl1, was employed in almost every iN cell conversion method. This indicated that Ascl1 may be a vital regulator of neuronal reprogramming but can also be compensated for by other related factors. In addition to terminally differentiated neuronal cells, the direct induction of neural stem/progenitor cells, which are expandable and differentiate to a wide variety of neuronal lineage cells, was also reported.30) The combinations of transcription factors for the induction of neural stem/progenitor cells were significantly different from those of the differentiated iN cells; thus, this might indicate that the transcription networks in the differentiated neuronal cells and the neural stem/progenitor cells are significantly different. In recent years, studies on the generation of multipotent neural stem/progenitor cells have increased because these cells can proliferate in vitro for use in cell transplantation therapies.30–38) On the other hand, in vivo direct reprogramming studies for terminally differentiated iN cells are also increasing. In these studies, endogenous cells in the brain, such as glial cells, are converted into functional neuron-like cells in situ with plasmid transfection or viral vector infection.39–44) Furthermore, iN cells have been applied for non-therapeutic applications; for example, direct neuronal reprogramming of cells from patients with severe nerve diseases, such as Huntington’s disease, amyotrophic lateral sclerosis, and myoclonus epilepsy associated with ragged red fibers, have been developed for pathological analysis and drug discovery studies.45–48)

2.2. Cardiomyocytes. Cardiomyocytes, striated muscle cells in the heart, are the second most frequently reported target cells in the field of direct reprogramming (Fig. 1). Cardiomyocytes are one of the most desirable cells for establishment in regenerative medicine because they are not expandable in vivo or in vitro. The first study of mouse-induced cardiomyocyte-like (iCM) cells was published in 2010,49) which was followed by other studies using different combinations of transcription factors.50,51) Human fibroblast-derived iCM cells were established in 2013.52,53) As well as iN cells, the combination of transcription factors for iCM cells shows wide variation, which might indicate redundancy of the transcription network of cardiomyocytes. However, only Tbx5 was included in all of the combinations of transcription factors for iCM cells. Therefore, it is considered that Tbx5 plays a central role in the transcription network of cardiomyocytes, similar to Ascl1 in neuronal cells. In previous studies, fibroblasts have frequently been employed as source cells for iCM cells because cardiac fibroblasts, which exist abundantly in the heart, are considered a potential candidate for source cells of in situ direct reprogramming. Some studies have achieved successful in vivo direct reprogramming of iCM cells in mouse hearts by using lentivirus vectors.50,54) This technology may benefit patients with heart injury, such as myocardial infarction.

2.3. Hepatocytes. Hepatocytes are parenchymal cells of the liver and have vital roles in this organ, i.e., lipid metabolism, glycogen accumulation, detoxification, and endocrine activities. As well as neuronal cells and cardiomyocytes, hepatocytes are also expected to be induced artificially for therapeutic uses because the specific functions of liver-derived hepatocytes cannot be maintained in canonical in vitro culture conditions and are unsuitable for cell transplantation therapy. Although iPSC-derived hepatocytes have been actively studied, the direct reprogramming of hepatocytes is also considered a promising alternative for regenerative treatments of the liver. The first studies of mouse-induced hepatocyte-like (iHep) cells were published by two independent groups simultaneously.55,56) Although these studies used different protocols to induce iHep cells from fibroblasts, the resulting iHep cells showed similar phenotypes to those of endogenous hepatocytes, i.e., secretion of albumin, uptake of low-density lipoprotein, and xenobiotic metabolism. Furthermore, studies were also conducted using human and mouse iHep cells in a wide variety of reprogramming factor combinations.55–64) The transcription network defining the cell fate of hepatocytes appears complex and may have redundancy. Among the transcription factors for iHep cell conversion, the Foxa family of transcription factors, i.e., Foxa1, Foxa2, and Foxa3, are considered primary factors for hepatic reprogramming because almost all iHep cell
induction methods employ this family proteins as well as Ascl1 and Tbx5 in other direct reprogramming methods. iHep cells are expected to have potential in cell transplantation therapies because the induced cells are expandable and maintain their hepatic functions, unlike the liver-derived hepatocytes. Nevertheless, the human iHep cells required unfavorable factors, such as a virus-derived gene, to be expandable cells; therefore, further improvements are necessary for the medical use of iHep cells. In vivo reprogramming of the iHep cells with an adeno-associated virus (AAV) vector is also studied to treat liver disorders, such as cirrhosis, because the fibrous liver contains a massive number of fibroblasts as source cells for the direct reprogramming and exclusion target cells.

2.4. Islet-related cells. Among the five types of endocrine cells in pancreatic islets, β cells are the most critical targets for regenerative medicine because they are the unique source of insulin and are destroyed in type 1 diabetes. Of note, all of the cells reported to be reprogrammed into β cells were endoderm-derived cells, e.g., hepatocytes, bile duct cells, pancreatic exocrine cells, and gallbladder cells, without exception. The fact that only cells of closely related lineages have been converted into β cells might indicate the peculiarity of β cells from other lineages and plasticity among endoderm-derived cells. Relatively large numbers of studies of the in vivo direct reprogramming of these cells have been reported, because the generation of insulin-secreting cells in digestive organs is an easy and effective solution for type 1 diabetes.

2.5. Hematopoietic system-related cells. Several terminally differentiated blood cells, e.g., dendritic cells, macrophages, and T cells, were easily converted from other blood cells by transducing with single transcription factors. Therefore, these studies were reported earlier the other direct reprogramming studies. On the other hand, the direct reprogramming of blood cells from fibroblasts requires much more complex sets of transcription factors. The long-distance lineage conversion appears to require a larger number of transcription factors than the short-distance lineage conversion to drastically change their transcription network. Toward practical use, the in vitro conversion of various types of blood cells has been studied vigorously to prepare cells for blood component transfusion.

2.6. Other types of cells. Since the first report of the direct reprogramming of somatic cells into myoblasts in 1987, a vast number of different direct reprogramming techniques have been reported; for example, osteoblasts, myoblasts, skeletal muscle progenitor cells, photoreceptor cells, adipocytes, smooth muscle cells, sweat gland cells, and endothelial cells have been induced with several combinations of transcription factors. In addition, the direct conversion of various types of cells, such as brown fat cells, Sertoli cells, melanocytes, thymic epithelial cells, renal tubular epithelial cells, intestine progenitor cells, Leydig cells, trophoblast stem cells, and nephron progenitor cells has been achieved successfully, and this method is expected to be applied in medical treatment, drug discovery, and pathological studies. Indeed, the researchers of some studies employed the directly reprogrammed cells from patient-derived somatic cells for their disease research.

2.7. Prediction of the combinations of transcription factors. Although most direct reprogramming studies select transcription factor combinations from factor lists developed in previous studies in genetics, genomics, and developmental biology, this type of screening is unreliable and inefficient. Therefore, several tools for the prediction of reprogramming factors have been developed. For these studies, a large amount of comprehensive biological data, e.g., transcriptome and topologically associating domains, with computer algorithms, e.g., machine learning and network analysis, were employed to develop the prediction model. These tools made the selection of transcription factor candidates and combinations easier. Therefore, the number and variation of direct reprogramming techniques using transcription factors will continue to increase.

3. Methods for direct cell fate conversion

Although the majority of direct reprogramming has been achieved through the forced transduction of transcription factors with viral vectors, these methods are expected to be replaced with alternative methods (Table 2). This is because frequently used viral vectors, retroviruses, and lentivirus vectors integrate the transgenes into the host genome and increase the risk of unexpected cell proliferation, transformation, and oncogenesis. Furthermore, although integration-free transduction methods, e.g., adenovirus vectors, AAV vectors, and plasmid vectors, are employed, the possibility of accidental genome integration of foreign DNA remains. Therefore, integration-free, DNA-free, and transgene-free methods are desired for medical applications. In this
| Source cells                              | Target cells                                      | Transgenes and/or Treatment                                                                 | Species               | Cell transplantation | Published year | PMID           |
|-------------------------------------------|--------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------|---------------------|-----------------|----------------|
| Fibroblasts (BJ and MRC-5 cells)          | Hepatocytes                                      | HNF1A and Any two of the three factors: FOXA1, FOXA3, and HNF4A (mRNA transfection)        | Human                 |                     | 2014            | 24963715(50)   |
| Non-sensory cochlear epithelial cells     | Neuronal cells                                   | Ascl1; Ascl1 and Neurod (plasmid electroporation)                                          | Mouse                 |                     | 2014            | 24928351(39)   |
| Cardiac fibroblasts                      | Cardiomyocytes                                   | Gata4, Me2c, and Tbx5 (peptide-enhanced mRNA transfection)                                 | Mouse                 |                     | 2015            | 25834424(114)  |
| Astrocytes                               | Neural stem cells                                | Sox2 (Leu3p-αIPM system)                                                                  | Mouse                 |                     | 2016            | 27148066(121)  |
| Fetal and embryonic fibroblasts and Brain cells in vivo | Neuronal cells                              | Ascl1, Brn2, and Myt11 (mRNA transfection with a GO-PEI-based reagent in vitro and in vivo) | Mouse & Human         |                     | 2016            | 28145631(40)   |
| Peripheral blood mononuclear cells       | Photoreceptor cells                              | CRX, RAX1, and NEUROD1 (SeV infection)                                                    | Human                 |                     | 2016            | 27170256(112)  |
| Gingival fibroblasts and Adult dermal fibroblasts | Osteoblasts                               | OCT4, OSTERIX, and L-MYC (plasmid transfection)                                            | Human YES             |                     | 2016            | 26990868(113)  |
| Fibroblasts                              | Hepatocytes                                      | OCT4, FOXA2, HNF1A, and GATA3 (protein transduction with HVJ-E)                           | Human                 |                     | 2017            | 28587331(42)   |
| Fibroblasts                              | Neurocytes                                       | SOX2, GATA3, and NEUROD1 (protein transduction with HVJ-E)                               | Human                 |                     | 2017            | 28587331(42)   |
| Fibroblasts (3T6 cells)                  | Neuronal cells                                   | Ascl1, Brn2, and Foxa1 (plasmid transfection with a polysaccharide-based reagent)         | Mouse                 |                     | 2017            | 28855426(119)  |
| Mesenchymal stem cells                   | Hepatocytes                                      | Hnf4a and Foxa3 (PiggyBac transposon)                                                     | Mouse                 |                     | 2017            | 28295042(114)  |
| Dermal fibroblasts                       | Endothelial progenitor cells                     | ETV2 (mRNA transfection)                                                                  | Human YES             |                     | 2017            | 27778229(117)  |
| Fibroblasts (3T6 cells)                  | Neuronal cells                                   | Ascl1, Brn4, and Tcf3 (plasmid transfection with a polysaccharide-based reagent)         | Mouse                 |                     | 2017            | 29137640(120)  |
| Mesenchymal stem cells and Dermal fibroblasts | Neural stem cells                  | SOX2 (mRNA transfection)                                                                 | Human                 |                     | 2018            | 29909688(118)  |
| Adult fibroblasts                        | Neural precursor cells                           | SOX2; SOX2 and PAX6; SOX2 and LMX1A; SOX2, LMX1A, and FOXA2 (plasmid transfection)      | Human                 |                     | 2018            | 30018471(36)   |
| Dermal fibroblasts                       | Neural precursor cells                           | SOX2 and PAX6 (chemically modified mRNA or plasmid transfection)                          | Human                 |                     | 2018            | 30450440(14)   |
| Embryonic fibroblasts                    | Hepatocytes                                      | Hnf4a and Foxa3 (modified mRNA transfection)                                              | Mouse YES             |                     | 2018            | 30327781(115)  |

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### Direct cell-fate conversion of somatic cells

| Somatic cells | Neuronal lineages | Transcription factors or compounds | Species | Year | PubMed ID |
|---------------|-------------------|------------------------------------|---------|------|-----------|
| Foreskin fibroblasts | Neuronal cells | ASCL1 + miR-124 + P53 knock-down (episomal plasmid transfection) | Human | 2019 | 30539819 |
| Bone marrow-derived cells, Fibroblasts, and Keratinocytes | Neural precursor cells | MS1, NGN2, and MBD2 (plasmid transfection) | Human | YES | 31196173 |
| Renal proximal tubular epithelial (HK2) cells | Nephron progenitor cells | SNAI2, EYA1, and SIX1 (PiggyBac transposon) | Human | 2019 | 30827514 |
| Chemical compounds (with or without transcription factors) |
| Fibroblasts (IMR90 or MRC5 cells) | Cholinergic neurons | NGN2 + Forskolin and dorsomorphin treatment | Human | 2013 | 23873306 |
| Embryonic fibroblasts and Neonatal tail-tip fibroblasts | Cardiomyocytes | CHIR99021, RepSox, Forskolin, Valproic acid, Parnate, and TTNPB treatment | Mouse | 2015 | 26292833 |
| Embryonic fibroblasts and Adult tail-tip fibroblasts | Neural stem cells | Valproic acid, Bix01294, RG108, PD0325901, CHIR99021, Vitamin C, and A83-01 treatment | Mouse | YES | 26788068 |
| Fibroblasts | Neuronal cells | Valproic acid, CHIR99021, RepSox, Forskolin, SP600125, GO6983, and Y-27632 treatment | Human | 2015 | 26253202 |
| Fibroblasts | Neuronal cells | ISX9, SB431542, Forskolin, CHIR99021, and I-BET151 treatment | Mouse | 2015 | 26253201 |
| Embryonic fibroblasts | Neural stem cells | A83-01, Purmorphamine, Valproic acid, and Thiazovivin treatment | Mouse | 2017 | 27207831 |
| Urine-derived cells | Hepatocytes | FOXA3 and HNF1A or HNF4A + CHIR99021, RepSox, Valproic acid, Parnate, TTNPB, and Dznep treatment | Human | 2018 | 36315254 |
| Embryonic and adult fibroblasts | Skeletal muscle progenitor cells | Myod + CHIR99021, RepSox, and Forskolin treatment | Mouse | YES | 29742392 |
| Adult dermal fibroblasts, Fetal pancreas fibroblasts, and Peripheral blood mononuclear cells | Neural plate border stem cells | BRN2, KLF4, SOX2, and ZIC3 + CHIR99021, ALK5 inhibitor, Purmorphamine, Tranexycyprosine treatment | Human | YES | 30581079 |
| Striatal neurons | Dopaminergic neurons | Sox2, Nur1, Lmx1a, and Foxa2 + Valproic acid treatment (in vivo infection) | Mouse | 2018 | 30318292 |
| Glioblastoma cells (U87MG cells) | Neuronal cells | Forskolin, ISX9, CHIR99021, I-BET151, and DAPT treatment | Human | 2018 | 30091580 |
| Dermal fibroblasts | Neuronal cells | NGN2 and ASCL1 + Pyr integrin, ZM336372, AZ960, and KC7F2 treatment | Human | 2019 | 31099332 |

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| Culture condition and soluble factors (with transcription factors) |
|---------------------------------------------------------------|
| **Liver cells in vivo** | **β cells** | PDX1 + EGF and nicotinamide treatment | Human | YES | 2005 | 15899968<sup>134</sup> |
| Adult dermal fibroblasts and Neonatal foreskin fibroblasts | Multilineage blood progenitor cells | OCT4 + hematopoietic cytokines treatment | Human | YES | 2010 | 21057492<sup>135</sup> |
| Embryonic fibroblasts | Cardiomyocytes | OSKM + cytokines (including Bmp4) treatment | Mouse | 2011 | 21278734<sup>136</sup> |
| Adult skin fibroblasts | Neural stem cells | OSKM + neurosphere culture | Mouse | YES | 2012 | 22467474<sup>137</sup> |
| Amniocytes | Vascular endothelial cells | ET2V, FLI1, and ERG1 + TGF β inhibitor treatment | Human | YES | 2012 | 23084400<sup>139</sup> |
| Embryonic stem cells-derived fibroblasts | Neural crest cells | SOX10 + Environmental cues including WNT activation | Human | YES | 2014 | 25158936<sup>140</sup> |
| Newborn fibroblasts | Hepatocytes | OSK + Soluble factors | Human | YES | 2014 | 24572354<sup>126</sup> |
| Dermal fibroblasts | Oligodendrocyte progenitor cells | Oct4 + OPC induction medium | Mouse | YES | 2015 | 26497893<sup>141</sup> |
| Embryonic fibroblasts | Keratinocytes | OSK + Bmp4 and RA treatment | Mouse | YES | 2016 | 27473056<sup>142</sup> |
| Skin fibroblasts | Neural stem cells | OCT3/4, SOX2, KLF4, L-MYC, and LIN28 + P53 knockdown + Neurotrophins treatment | Human | 2016 | 27822179<sup>138</sup> |

**miRNAs (with or without transcription factors)**

| Cardiac fibroblasts | Cardiomyocytes | miR-1, miR-133, miR-208, and miR-499 | Mouse | 2012 | 22539765<sup>147</sup> |
| Embryonic fibroblasts | Neuronal cells | Ptb knockdown | Mouse | 2013 | 23313552<sup>143</sup> |
| Cardiac fibroblasts and Embryonic fibroblasts | Cardiomyocytes | Gata4, Mef2c, and Tbx5 + miR-133; Gata4, Mef2c, Tbx5, Mesp1, and Myocd + miR-133 | Mouse & Human | 2014 | 24920580<sup>149</sup> |
| Adult dermal fibroblasts, Umbilical cord blood cells, and Senescent somatic cells | Neural stem cells | SOX2 + HMGA2 expression or LET-7B inhibition | Human | YES | 2015 | 25600877<sup>144</sup> |
| Fibroblasts | Cardiomyocytes | GATA4, MEF2C, TBX5, ESRRG, MESP1, MYOCARDIN, ZFPM2, and HAND2 + miR-1 | Human | 2017 | 28796841<sup>149</sup> |
| Adult fibroblasts | Neuronal cells | miR-9/9* and miR-124 | Human | 2017 | 28886366<sup>145</sup> |
| Adult fibroblasts | Spinal cord motor neurons | ISL1 and LHX3 + miR-9/9* and miR-124 | Human | 2017 | 28886366<sup>145</sup> |
| Brain vascular pericytes | Cholinergic neuronal cells | ASCL1, MYT1L, BRN2, and TLX3 + miR-124 | Human | YES | 2018 | 29453933<sup>146</sup> |

**Physical stimulation (with or without transcription factors)**

| Embryonic fibroblasts | Neuronal cells | Ascl1, Brn2, and Myt1l + Substrate topography | Mouse | 2014 | 24709523<sup>153</sup> |
| Fibroblasts | Cardiomyocytes | Myocardin, Tbx5, and Mef2c + Microgroove | Mouse | 2015 | 26302234<sup>154</sup> |

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| Cell Type                          | Gene Targets/Methods                                                                 | Organism | Year  | GenBank ID |
|-----------------------------------|-------------------------------------------------------------------------------------|-----------|-------|------------|
| Embryonic fibroblasts             | Ascl1, Pitx3, Nurr1, and Lmx1a + Nanogroove                                       | Mouse     | 2015  | 25662493   |
| Dermal fibroblasts                | ETV2 + Hypoxia                                                                     | Human     | 2016  | 27488544   |
| Neonatal tail-tip fibroblasts     | Gata4, Mef2c, and Tbx5 + Microgroove                                               | Mouse     | 2016  | 27376554   |
| Fibroblasts                       | Gata4, Mef2c, and Tbx5 + Hypoxia                                                   | Mouse     | 2016  | 26757100   |
| Dermal fibroblasts                | Nanogroove                                                                          | Mouse     | 2019  | 31005261   |
| CRISPR/Cas9-based methods         | Neuronal cells CRISPR/Cas9-based transcriptional activators + gRNAs for Brm2, Ascl1, and Myt1 | Mouse     | 2016  | 27524438   |
| Liver cells in vivo β cells       | CRISPR/Cas9-based transcriptional activators + gRNA for Pdx1 (in vivo infection)  | Mouse     | YES   | 29224785   |
| Protein engineering (with transcription factors) | Gata4, Tbx5, and Hand2 + Mef2c fused with M3 domain of Myod | Mouse     | 2013  | 23794713   |
| Embryonic fibroblasts             | Cardiomyocytes Gata4, Mef2c, and Tbx5 + TGFβ inhibitors or ROCK inhibitors          | Mouse     | 2015  | 26354680   |
| Pancreatic exocrine cells β cells | Pdx1, Ngn3, and Mafa (synthetic mRNA transfection) + 5-Aza treatment                | Mouse     | 2016  | 27187823   |
| Neonatal cardiac and tail-tip fibroblasts | miR-1, miR-133, miR-208, and miR-499 + three-dimensional culture                  | Mouse     | 2016  | 27941890   |
| Neonatal cardiac and tail-tip fibroblasts | miR-1, miR-133, miR-208, and miR-499 + Reprogramming medium                      | Mouse     | 2016  | 26975336   |
| Adipose-derived stem cells and Neonatal foreskin fibroblasts | iPSC induction followed by TRA-1-60-/SSEA4–selection | Human     | YES   | 27569063   |
| Fibroblasts                       | OCT4 and C/EBPB (protein transduction with HVJ-E) + Adipogenic differentiation medium | Human     | 2017  | 28587331   |
section, a wide variety of technologies used in direct reprogramming studies are introduced.

3.1. Genome integration-free gene transduction. Even now, transcription factors are one of the most powerful and useful tools for direct reprogramming. Therefore, several genome integration-free transduction methods have been applied for direct reprogramming instead of conventional viral vectors, such as retrovirus and lentivirus vectors. Sendai virus (SeV; hemagglutinating virus of Japan, HVJ) is an RNA virus that does not enter the nucleus and replicates in the cytoplasm. Therefore, vector systems using this virus are not at risk of genome integration of the transgenes. In addition to the induction of iPSCs, SeV vectors have been used for direct induction of photoreceptor cells from peripheral blood cells. Additionally, a method for in vivo cardiac reprogramming using SeV vectors achieved high reprogramming efficiency. These results suggested that the SeV vector system can be an alternative tool to induce cellular reprogramming based on the gene transduction method. Moreover, the envelope particle of the virus (HVJ-E) is a useful carrier of genes, siRNAs, proteins, and drugs. Human hepatocytes and neurocytes were induced from fibroblasts through the protein transduction technique with the HVJ-E carrier. The PiggyBac system is a transposon-based gene transduction technique. Although transgenes are integrated into the host genome, these sequences can be removed without any footprints with transposase. Some studies have employed this system and induced mouse hepatocytes and human nephron progenitor cells from somatic cells. mRNA transfection is an alternative gene transduction technique that is safer than previous methods. Since higher animals, including humans, do not have reverse transcriptase, there are no risks of integration of the transgenes mRNAs into their genome. Many studies using mRNA transfection have been reported, e.g., hepatocytes, cardiomyocytes, neuronal cells, endothelial progenitor cells, and neural stem/precursor cells. Nevertheless, several difficulties of this method, such as stability and transfection efficiency, remain. Solutions to these difficulties, such as the chemical modification of the mRNA and specific transfection reagents, have already been adopted in mRNA transfection studies. In addition to these methods, plasmid transfection has also been widely tested. Although there is a risk of genome integration and a problem of low transduction efficiency, novel tools, such as a polysaccharide-based gene co-delivery system, a Leu3p-αIPM system, or episomal vectors, might improve the plasmid-based transduction technologies.

3.2. Chemical compounds. Small chemical compounds that work as agonists or antagonists for various cellular reactions, such as signal transduction, the cell cycle, post-translational modification, apoptosis, epigenetics, and metabolism, have many advantages compared with gene transduction methods: 1) They are relatively more stable than biomolecules in vitro and in vivo. If they are not stable, chemical modifications can improve their stability. 2) Most can penetrate cells or organelles, unlike biopolymers. 3) There is no risk of genome integration of foreign sequences. 4) Suitable conditions for the chemicals are flexible and controllable.

Most chemicals used in the direct reprogramming studies have positive or negative effects for various signal transduction pathways, e.g., Wnt (CHIR99021), cAMP (Forskolin), TGFβ (RepSox), SB431542, Ca2+ (ISX9), Shh (Purmorphamine), Retinoic acid (TTNPB), JAK/STAT (AZ960, SP600125), Notch (DAPT), AMPK (dorsomorphin), PCK (GO6983), HIF (KC7F2), MAPK (PD0325901, ZM336372), Notch (DAPT), AMPK (dorsomorphin), PCK (GO6983), HIF (KC7F2), MAPK (PD0325901, ZM336372), β1-integrin (Pyrintegrin), and Rho/ROCK (Thiazovivin, Y-27632) signaling pathways, and epigenetic modifications, e.g., histone lysine deacetylation (Valproic acid), histone lysine acetylation recognition (I-BET151), histone lysine methylation (Bix01294, Dznep), DNA methylation (RG108), and DNA demethylation (Vitamin C). Although several types of cells, such as neuronal cells, hepatocytes, cardiomyocytes, and skeletal muscle progenitor cells, have been induced with chemicals, half of the reported studies used gene transduction in conjunction with the chemical treatments. Precise analysis of the functions of each transcription factor is required to compensate their functions with chemical compounds.

3.3. Partial reprogramming with specific culture conditions. Some studies have utilized gene transduction combinations and external signals from culture media. In particular, initializing factors of iPSCs have frequently been used in combination with external signals. A wide variety of cells, e.g., β cells, blood progenitor cells, cardiomyo-
cytes,\textsuperscript{136} neural stem cells,\textsuperscript{137,138} vascular endothelial cells,\textsuperscript{139} neural crest cells,\textsuperscript{140} hepatocytes,\textsuperscript{140} oligodendrocyte progenitor cells,\textsuperscript{141} and keratinocytes,\textsuperscript{142} were previously generated and transplanted into model animals. Although these protocols are time- and cost-effective compared with iPSC-employing methods, the risk of tumorigenesis caused by cell initialization remains to be elucidated.

### 3.4. MicroRNAs (miRNAs)

miRNAs regulate the expression of specific mRNAs post-transcriptionally. Therefore, miRNAs can be used for direct reprogramming instead of or in combination with gene transduction. In a previous study, somatic cells were converted into several types of neuronal cells,\textsuperscript{143–146} and cardiomyocytes,\textsuperscript{147–149} miRNA induction avoids the problem of transgene genome integration because the miRNA genes can be induced into cells as RNA. However, most of the reported studies employed gene transduction simultaneously for conversion. Complete miRNA-based reprogramming methods are expected to be developed in future.

### 3.5. Physical stimulations

Several physical stimulations have been used as additional stimulations for direct reprogramming. Hypoxia conditions have been frequently employed in stem cell biology and control stem cell functions through the HIF signaling pathway.\textsuperscript{150} In a study involving direct reprogramming, these conditions were also used to accelerate conversion.\textsuperscript{151,152} On the other hand, micro- or nano-imprinted patterns, \textit{e.g.}, microgrooves, of cultured cell substrates are known as enhancers of reprogramming in biomaterials science. Biochemical and molecular biology studies are required to understand the effect of imprinted patterns for reprogramming.\textsuperscript{153–156} Of note, the electrical stimulation-induced direct reprogramming of fibroblasts into chondrocytes without any soluble factors or transgenes was reported recently.\textsuperscript{157} Elucidating the molecular mechanism of the reprogramming phenomenon may aid in the development of direct reprogramming technology.

### 3.6. Other methods

Several other innovative approaches have been applied in direct reprogramming studies, such as a CRISPR/Cas9-based method. A modified Cas9 protein, which has a domain for transcriptional activation and guides RNAs for the endogenous transcription factor genes, is introduced into source cells to activate endogenous transcription factors.\textsuperscript{151,158} The genome integration-free protocols of the CRISPR/Cas9 system have already been established, thus, this method is also an integration-free method. Studies utilizing CRISPR-based direct reprogramming will increase because this method is straightforward and suitable for screening reprogramming factors.

Rational protein engineering of transcription factors is a unique approach translated from another field, \textit{i.e.}, biomolecular engineering. Functional protein domains, such as VP16 or M3 of Myod, are attached to transcription factors to improve their transcriptional activities. Neuronal cells and cardiomyocytes have been induced successfully by using artificial transcription factors.\textsuperscript{159,160}

Additionally, the number of hybrid methods using the techniques above are increasing.\textsuperscript{161–166} In future, engineering-based approaches optimizing the reprogramming process will be considerably more important compared with previous stages.

### 4. Mechanism analyses and future applications of direct reprogramming

To make direct reprogramming technology more efficient and safer for medical and industrial applications, analyses of the molecular and cellular mechanisms of cell fate conversion, \textit{i.e.}, the transition of the transcriptome, the behavior of transcription factors, epigenetic remodeling, and the heterogeneity of cell populations, are essential (Fig. 2). In addition, applied research on direct reprogramming technology in medicine and industry has increased in recent years toward practical use (Table 3). Therefore, representatives of molecular and cellular mechanism analyses are introduced in this section. In addition, novel applications of direct reprogramming, including non-medical uses, are described.

#### 4.1. Analyses of the molecular and cellular mechanisms of direct reprogramming

Regarding the mechanisms of direct cell fate conversion, the reprogramming processes of cardiomyocytes,\textsuperscript{148,149,165,167–179} neuronal cells,\textsuperscript{39,43,48,143,145,160,180–188} glial cells,\textsuperscript{195} photoreceptor cells,\textsuperscript{196} β cells,\textsuperscript{197} renal tubular epithelial cells,\textsuperscript{198} trophoblast stem cells,\textsuperscript{102} and mesodermal cells,\textsuperscript{199,200} have been reported.

Several methodologies have been employed for the molecular biological analysis of direct reprogramming. Comprehensive transcriptome analyses using RNA-seq have been performed frequently, and differential expression gene analysis between
Table 3. Applications of the directly reprogrammed cells

| Source cells | Target cells | Transgenes and/or Treatment | Species | Published year | PMID |
|--------------|--------------|-----------------------------|---------|----------------|------|
| Liver cells  | β cells      | Pdx1 (an adenoviral vectors) | Mouse   | 2000, 2003     | 10802714, 12775714 |
| Pancreatic exocrine cells | β cells | Ngn3, Pdx1, and Mafa (an adenoviral vector) | Mouse | 2008 | 18754011 |
| Cardiac fibroblasts | Cardiomyocytes | Gata4, Mef2c, Tbx5, and Hand2 (a retrovirus vector) | Mouse | 2012 | 22660318 |
| Cardiac fibroblasts | Cardiomyocytes | miR-1, miR-133, miR-208, and miR-499 (a lentiviral vector) | Mouse | 2012 | 22539765 |
| Non-sensory cochlear epithelial cells | Neuronal cells | Ascl1; Ascl1 and Neurod (in vivo plasmid electroporation) | Mouse | 2014 | 24928351 |
| Liver cells in mouse models of chronic liver disease | Hepatocytes | Foxa3, Gata4, Hnf1α, and Hnf4α (an AAV vector) | Mouse | 2016 | 26923201 |
| Brain cells in vivo | Neuronal cells | Ascl1, Brn2, and Myt11 (mRNA transfection with a GO-PEI-based reagent) | Mouse | 2016 | 28145631 |
| Bile duct cells | β cells | Pdx1, Ngn3, and Mafa (an adenoviral vector) | Mouse | 2017 | 28363269 |
| Hepatocytes | β cells | Pdx1, Ngn3, and Mafa (hydrodynamics tail vein injection) | Mouse | 2017 | 28100951 |
| Resident glial cells | Neuronal cells | Ascl1, Lmx1α, and Nur71 (an AAV vector) | Mouse | 2017 | 28844656 |
| Liver cells in vivo | β cells | CRISPR/Cas9-based transcriptional activators + gRNA for Pdx1 (an AAV vector) | Mouse | 2017 | 29224783 |
| Striatal neurons | Dopaminergic neurons | Sox2, Nur71, Lmx1α, and Foxa2 (a lentiviral vector) + Valproic acid treatment | Mouse | 2018 | 30318292 |
| Resident glial cells | Interneurons | Ascl1, Lmx1α, and Nur71 (an AAV vector) | Mouse | 2019 | 31259901 |

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source and target cells has provided a considerable amount of information regarding essential regulator genes and signaling pathways during conversion. Epigenetic remodeling studies during cell-fate conversions have also been performed in various reprogrammed cells. Histone modification and DNA methylation data clarified the drastic and rapid change in chromatin states in reprogrammed cells. In addition, detailed analysis of transduced and endogenously upregulated transcription factors was a critical task for understanding direct reprogramming. Several methods, such as loss-of-function, ChIP-seq, and mass spectrometry, have been used to analyze the function and behavior of transcription factors. Transcription factor studies indicated that almost all of the reprogramming-related transcription factors played vital roles during the reprogramming process, e.g., by remodeling histone and DNA modifications, initiating the target transcription network, and erasing the transcriptional signature of the source cells. Recently, the activities of specific transcription factors, termed pioneer factors, seem pivotal for remodeling the chromatin states of cell conversion. For example, Ascl1 was identified as a pioneer factor for the induction of iN cells, which

| Disease model                                              | Mouse | 2019 | 30638745[43] |
|------------------------------------------------------------|-------|------|--------------|
| Microglial cells Neuronal cells Neurod1 (a lentiviral vector) |       |      |              |
| Fibroblasts from Huntington’s disease patients             |       |      |              |
| Neuronal cells PTB1 knockdown                              | Human | 2014 | 25275533[45] |
| Urine-derived cells from muscle diseases patients          |       |      |              |
| Skeletal muscle cells MYOD                                 | Human | 2016 | 27651888[104] |
| TSC2 gene-inactivated fibroblasts (CRISPR knockout)        |       |      |              |
| Neuronal cells ASCL1, LMX1A, and NURR1                     | Human | 2016 | 27857203[207] |
| Adult skin fibroblasts from ALS patients                   |       |      |              |
| Motor neurons NEUROG2, SOX11, ISL1, and LHX3              | Human | 2016 | 26725112[46] |
| Dermal fibroblasts from metabolic disease patients         |       |      |              |
| Adipocytes PPARG2                                          | Human | 2017 | 28982679[88] |
| Fibroblasts from BMD patients                              |       |      |              |
| Skeletal muscle cells MYOD                                 | Human | 2018 | 30171539[105] |
| Fibroblasts from MERRF patients                            |       |      |              |
| Neuronal cells ASCL1 and BRN2 + REST complex knockdown     | Human | 2019 | 30797798[47] |

| Other animals                                              |       |      |              |
|------------------------------------------------------------|-------|------|--------------|
| Embryonic fibroblasts                                      |       |      |              |
| Neuronal cells ASCL1, BRN2, MYT1L, and NEUROD1             | Marmoset | 2014 | 24694048[211] |
| Skin fibroblasts                                           |       |      |              |
| Cardiomyocytes GATA4, HAND2, TBX5, and MEF2C               | Canine | 2015 | 26681949[209] |
| Cardiac fibroblasts                                        |       |      |              |
| Cardiomyocytes Gata4, Mef2c, and Tbx5 + miR-590            | Rat, Porcine, & Human | 2016 | 27930352[208] |
| Fetal fibroblasts                                          |       |      |              |
| Cardiomyocytes, Neurocytes, Oocytes, and Cumulus granulosa cells | Goat | 2017 | 27629151[210] |

| Drug discovery                                             |       |      |              |
|------------------------------------------------------------|-------|------|--------------|
| Embryonic stem cells                                       |       |      |              |
| Neuronal cells NGN2                                        | Human | 2019 | 31155484[48] |
can preferentially bind to chromatin and allowed the binding of other reprogramming factors used for iN cell induction.\(^{202}\) Additionally, in the case of iHep cell induction, well-known pioneer factors from the Foxa protein family are indispensable.\(^{55,56,58,64,114,115,127,189}\) Therefore, understanding the roles of pioneer factors will improve direct reprogramming technology. Additionally, the function of miRNA in iCMs has been thoroughly analyzed, and, therefore, miRNA-based reprogramming technologies of iCMs have been developed.\(^{148,165,177}\) The analysis of miRNAs in other direct reprogramming methods may result in their improvement. Other molecular and cellular analyses, such as signal transduction,\(^{174,183,197}\) metabolic remodeling,\(^{198}\) time-lapse imaging,\(^{170}\) and cell morphological studies,\(^{181}\) have been performed. Recently, single-cell analyses, such as single-cell RNA sequencing (scRNA-seq), have been performed in many direct reprogramming studies.\(^{149,181,197,199}\) These studies have clarified many features of direct reprogramming processes. For example, a limiting step and an obstruction factor,\(^{187}\) a stem cell-like transition state,\(^{181}\) and multiple trajectories\(^{192}\) of the direct cell fate conversions were discovered using scRNA-seq data. In the near future, other single-cell analysis techniques, such as ATAC-seq,\(^{203}\) ChIL-seq,\(^{204}\) CUT\&RUN,\(^{205}\) and Hi-C\(^{206}\) will reveal the chromatin dynamics and epigenetic remodeling processes in single converting cells during direct reprogramming processes.

4.2. Medical and industrial applications of direct reprogramming. As described above, most applied research for direct reprogramming has focused on clinical medicine, \(i.e.,\) cell transplantation and \textit{in vitro} direct reprogramming therapies. Meanwhile, some non-medical applications have been reported in recent years (Table 3). Direct reprogramming studies of disease model cells, which are induced from patient-derived cells, are necessary for basic studies of different disorders, and these cells can be employed for \textit{in vitro} experiments for drug discovery. Indeed, a human iN cell type, instead of primary neuronal cells, was previously applied to \textit{in vitro} drug treatment experiments.\(^{45}\) Additionally, several directly induced neuronal and skeletal muscle cell types from patients with various diseases have been reported.\(^{45–47,104,105,207}\) Direct reprogramming technology has the potential to change the pharmaceutical industry. In direct reprogramming studies, cells from different species, \(e.g.,\) rats,\(^{208}\) pigs,\(^{208}\) dogs,\(^{209}\) goats,\(^{210}\) and marmosets,\(^{211}\) have been generated. Some of the animals studied were industrial animals, such as livestock or pets, since direct reprogramming technology might contribute to animal industries, such as veterinary medicine.

5. Summary

Direct reprogramming is a promising technology that can convert somatic cells into various terminally differentiated or somatic stem/progenitor cell types for medical and industrial applications. Therefore, the number of studies utilizing this technology has increased rapidly in the last decade. Artificial cell fate conversions can be achieved with targeted expression of defined sets of transcription factors, treatment with chemical compounds as agonists or antagonists for various bioreactions, culturing with soluble factors or specific culture conditions, the induction of miRNA sets, appropriate physical stimulations, among other methods. Various cell types have been induced from somatic cells through direct reprogramming. Among them, neuronal cells, cardiomyocytes, and hepatocytes have been well-studied and their reprogramming mechanisms have been thoroughly analyzed. These technologies are expected to be applied in a variety of industries such as agriculture, biomaterial, healthcare, and medical industries.

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Profile

Kenichi Horisawa was born in Tochigi prefecture, Japan, and graduated from University of Tsukuba in 1997. After 2 years in a scientific publishing company, he entered the Graduate School of Science and Technology, Keio University, and received his Ph.D. degree under the supervision of Prof. Hiroshi Yanagawa in 2005. He worked as a postdoctoral fellow in Prof. Hiroshi Yanagawa’s laboratory from 2005 to 2010, and as an Assistant Professor in Prof. Nobuhide Doi’s laboratory from 2010 to 2013 in the Faculty of Science and Technology, Keio University. In 2013, he joined Prof. Atsushi Suzuki’s laboratory at the Medical Institute of Bioregulation, Kyushu University, as an Assistant Professor. His research interests are molecular mechanisms for the regeneration and direct reprogramming of hepatic cells, primarily on the molecular function and behavior of the master transcription factors for hepatic development.

Profile

Atsushi Suzuki was born in Gunma prefecture, Japan, in 1974 and graduated from Tohoku University in 1998. Then, he moved to University of Tsukuba with an aim to contribute to the development of innovative medicine using the knowledge and experience obtained from the study of basic biology. Subsequently, he succeeded in the prospective isolation of hepatic stem cells from the developing mouse liver and unraveled the mechanisms controlling the properties of hepatic stem cells during liver development. He made his Ph.D. thesis defense in the summer of 2002 and then spent time at the Salk Institute for Biological Studies, San Diego, U.S.A., to learn novel experimental techniques. After being awarded his Ph.D. from University of Tsukuba in March 2003, he continued his work at the Salk Institute until returning to RIKEN/CDB in Japan in 2005. Two and half years later, he moved to Kyushu University to join the tenure track program of “Kyushu University Research Superstar Program (SSP)” and became a principal investigator in his own laboratory. In that time, he decided to start new research projects, and finally succeeded in the induction of direct conversion of skin-derived fibroblasts to hepatocytes using defined transcription factors. He subsequently became an Associate Professor in 2011 and then Professor at Kyushu University in 2013. His ongoing studies are focused on not only liver development, regeneration, and diseases but also the determination and conversion of the fate of cells in various digestive organs, with the hope that these will provide new insights into therapies for diseases in organs of the digestive system.