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 hearts^{2),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-

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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

Table 1. Examples of the direct reprogramming with defined transcription factors

Source cells	Target cells	Transgenes and/or Treatment	Species	Cell transplantation	Published year	PMID
Neuronal (stem) cells						
Astrocytes	Glutamatergic neurons	Pax6, Mash1, or Ngn2	Mouse		2007	17687043 ²⁵⁾
Embryonic fibroblasts and Hepatocytes	Neuronal cells	Brn2, Ascl1, and Myt1l	Mouse		2010, 2011	20107439 ²⁶⁾ ; 21962918 ²¹²⁾
Astrocytes	GABAergic neurons	Dlx2; Dlx2 and Ascl1	Mouse		2010	20502524 ²¹³⁾
Embryonic fibroblasts and Adult skin fibroblasts	Dopaminergic neurons	Ascl1, Lmx1a, and Nurr1	Mouse & Human	YES	2011	21725324 ²⁷⁾
Fetal fibroblasts and Post-natal 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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Embryonic fibroblasts and Fetal foreskin fibroblasts	Neural stem cells	Sox2	Mouse & Human	YES	2012	22683203 ³¹⁾
Sertoli cells	Neural stem cells	Pax6, Ngn2, Hes1, Id1, Ascl1, Brn2, c-Myc, and Klf4	Mouse	YES	2012	22064700 ³²⁾
Fibroblasts (IMR90 cells)	Dopaminergic neurons	MASH1, NGN2, SOX2, NURR1, and PITX3 + A dominant-negative P53	Human	YES	2014	25129808 ²¹⁴⁾
Non-sensory cochlear epithelial cells	Neuronal cells	Ascl1; Ascl1 and Neurod	Mouse		2014	24928351 ³⁹⁾
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 ⁴⁶⁾
Fibroblasts (3T6 cells)	Neuronal cells	Ascl1, Brn4, and Tcf3	Mouse		2017	29137640 ²¹⁶⁾
Umbilical cord blood cells	Neural stem cells	SOX2 and HMGA2	Human		2017	28844127 ³³⁾
Fibroblasts (3T6 cells)	Neuronal cells	Ascl1, Brn2, and Foxa1	Mouse		2017	28855426 ¹¹⁹⁾
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⁾
Pluripotent stem cell-derived cardiomyocytes	Neuronal cells	Brn2, 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	Neurocytes	SOX2, GATA3, and NEUROD1	Human		2017	28587331 ⁶²⁾
Dermal fibroblasts	Neural precursor cells	SOX2 and PAX6	Human		2018	30450440 ³⁴⁾
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 and LMX1A; SOX2, LMX1A, and FOXA2	Human		2018	30018471 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 ¹⁸¹⁾
Cord blood CD133(+) cells	Neuronal cells	FOXM1, SOX2, MYC, SALL4, and STAT6	Human		2018	29653196 ³⁸⁾

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Hepatocytes	Neuronal cells	Suz12, Ezh2, Meis1, Sry, Smarca4, Esr1, Pparg, and Stat3	Mouse		2018	29653196 ³⁸⁾
Peripheral CD34(+) cells	Neural stem 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 Cebpd	Mouse		2018	29653196 ³⁸⁾
Astrocytes and Foreskin fibroblasts	Noradrenergic neurons	Ascl1, Phox2b, Ap-2a, Gata3, Hand2, Nurr1, and Phox2a	Mouse & Human	YES	2019	31315047 ²²²⁾
Bone marrow-derived cells, Fibroblasts, and Keratinocytes	Neural precursor cells	MSI1, NGN2, and MBD2	Human	YES	2019	31196173 ³⁷⁾
Microglial cells	Neuronal cells	Neurod1	Mouse		2019	30638745 ⁴⁴⁾
Cardiomyocytes						
Cardiac fibroblasts	Cardiomyocytes	Gata4, Mef2c, and Tbx5	Mouse	YES	2010	20691899 ⁴⁹⁾
Cardiac fibroblasts	Cardiomyocytes	Gata4, Mef2c, Tbx5, and Hand2	Mouse		2012	22660318 ⁵⁰⁾
Cardiac fibroblasts and Embryonic fibroblasts	Cardiomyocytes	Mef2c and Tbx5 + Myocd or Gata4	Mouse		2012	22575762 ⁵¹⁾
Cardiac fibroblasts	Cardiomyocytes	GATA4, MEF2C, TBX5, MESP1, and MYOCD	Human		2013	23861494 ⁵²⁾
Embryonic stem cells-derived fibroblasts	Cardiomyocytes	GATA4, MEF2C, TBX5, ESRRG, MESP1, ZFPM2, and MYOCD	Human	YES	2013	24319660 ⁵³⁾
Adult tail-tip fibroblasts and Cardiac fibroblasts	Cardiomyocytes	Mef2c and Tbx5 + Bmi1 knockdown	Mouse		2016	26942853 ¹⁷⁵⁾
Adult fibroblasts	Cardiomyocytes	Gata4, Hand2, Mef2c, Tbx5, and Znf281	Mouse		2017	28982760 ¹⁶⁷⁾
Hepatocytes						
Embryonic fibroblasts and Adult dermal fibroblasts	Hepatocytes	Hnf4a and Foxa1, Foxa2, or Foxa3	Mouse	YES	2011	21716291 ⁵⁵⁾
Caudal fibroblasts	Hepatocytes	Gata4, Hnf1a, and Foxa3 + p19 ^{Arf} knockdown	Mouse	YES	2011	21562492 ⁵⁶⁾
Embryonic fibroblasts	Hepatocytes	HNF1A, HNF4A, HNF6, ATF5, PROX1, CEBPA, and MYC + TP53 knockdown	Human	YES	2014	24582926 ⁵⁷⁾
Fetal and adult fibroblasts and Adipose tissue-derived mesenchymal stem cells	Hepatocytes	FOXA3, HNF1A, and HNF4A + SV40 large T antigen	Human	YES	2014	24582927 ⁵⁸⁾

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Fibroblasts (BJ and MRC-5 cells)	Hepatocytes	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	Foxa3, Gata4, Hnf1a, and Hnf4a	Mouse		2016	26923201 ⁶⁰⁾
Fetal lung fibroblasts	Hepatocytes	ATF5, PROX1, FOXA2, FOXA3, and HNF4A	Human		2017	29192290 ⁶¹⁾
Fibroblasts	Hepatocytes	OCT4, FOXA2, HNF1A, and GATA3	Human		2017	28587331 ⁶²⁾
Embryonic fibroblasts	Hepatocytes	Foxa3, Hnf1a, and Gata4	Mouse	YES	2018	29959867 ⁶³⁾
Embryonic fibroblasts	Hepatocytes	Hnf4a, Foxa3, Klf4, and c-Myc	Mouse	YES	2019	30635054 ⁶⁴⁾
Islet-related cells						
Liver cells <i>in vivo</i>	β cells	Pdx1	Mouse		2000, 2003	10802714 ²⁰⁾ , 12775714 ²¹⁾
Pancreatic exocrine cells <i>in vivo</i>	β cells	Ngn3, Pdx1, and Mafa	Mouse		2008	18754011 ⁶⁵⁾
Hepatocytes	Islet cells	Ngn3	Mouse		2009	19793886 ²⁴⁾
Liver cells	β cells	PDX1, PAX4, and MAFA	Human		2014	24504462 ⁶⁶⁾
Cultured adult pancreatic duct cells	β cells	Pdx1, Ngn3, and Mafa	Mouse		2015	25836667 ⁶⁷⁾
Gallbladder cells	β cells	Pdx1, Ngn, Mafa, and Pax6	Mouse	YES	2016	27833043 ⁶⁸⁾
Hematopoietic system-related cells						
T precursor cells	Macrophages	Cebpa or Cebpb	Mouse		2006	17088084 ²²⁾
T precursor cells	Dendritic cells	Pu.1	Mouse		2006	17088084 ²²⁾
B cells	T cells	Pax5 knockout	Mouse	YES	2007	17851532 ⁷³⁾
Fibroblasts (3T3 cells), Embryonic fibroblasts, and Adult skin fibroblasts	Macrophage-like cells	Pu.1 and Cebpa or Cebpb	Mouse		2008	18424555 ⁷⁴⁾
B cells	Erythroid cells	Gata1, Scl, and Cebpa	Mouse		2012	22968040 ²³⁾
Fibroblasts (3T3 cells) and Adult dermal fibroblasts	Megakaryocyte	Nfe2, Mafg, and Mafk	Mouse & Human	YES	2012	22855609 ⁷⁵⁾
Skin fibroblasts	Monocytes	Spl1, Cebpa, Mnda, and Irf8	Mouse		2012	22428058 ⁷⁶⁾
Embryonic fibroblasts and Adult ear skin fibroblasts	Hematopoietic progenitor cells	Erg, Gata2, Lmo2, Runx1c, and Scl	Mouse	YES	2014	25466247 ⁷⁷⁾
Fibroblasts	Antigen-presenting dendritic cells	Pu.1, Irf8, and Batf3	Mouse & Human		2018	30530727 ⁷⁸⁾
Bone-related cells						
Neonatal foreskin fibroblasts	Chondrogenic cells	c-MYC, KLF4, and SOX9	Human	YES	2013	24146984 ²²⁴⁾

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Dermal fibroblasts	Osteoblasts	OCT3/4 and OCT6 or OCT9 + L-MYC, c-MYC, or N-MYC	Human		2015	26499074 ⁷⁹⁾
Fibroblasts	Osteoblasts	RUNX2, OCT4, OSTERIX, and L-MYC	Human	YES	2015	25918395 ⁸⁰⁾
Gingival fibroblasts and Adult dermal fibroblasts	Osteoblasts	OCT4, OSTERIX, and L-MYC	Human	YES	2016	26990860 ⁸¹⁾
Embryonic fibroblasts	Osteoblasts	c-Myc, Oct4, and hLMP3	Mouse	YES	2019	30453092 ⁸²⁾
Skeletal muscle-related cells						
Fibroblasts (C3H10T1/2 cells)	Myoblasts	Myod	Mouse		1987	3690668 ¹⁹⁾
Dermal fibroblasts	Myoblasts	MYOD1 and MYCL	Human	YES	2017	28501623 ⁸³⁾
Embryonic fibroblasts	Skeletal muscle progenitor cells	Mef2b and Pitx1 + Pax3 or Pax7	Mouse	YES	2018	28808339 ⁸⁴⁾
Adult fibroblasts	Skeletal muscle progenitor cells	Pax7, Mef2b, and Myod	Mouse	YES	2018	28808339 ⁸⁴⁾
Other cells						
Embryonic fibroblasts and Newborn foreskin fibroblasts	Brown fat cells	Prdm16 and Cebpb	Mouse & Human	YES	2009	19641492 ⁹⁵⁾
Embryonic fibroblasts	Sertoli cells	Nr5a1, Wt1, Dmrt1, Gata4, and Sox9	Mouse	YES	2012	22958931 ⁹⁶⁾
Iris-derived cells	Photoreceptor cells	CRX, RAX, and NEUROD	Human		2012	22558175 ⁸⁵⁾
Embryonic fibroblasts and Adult tail-tip dermal fibroblasts	Melanocytes	Mitf, Sox10, and Pax3	Mouse & Human	YES	2014	25510211 ⁹⁷⁾
Adipose tissue-derived stromal cells	Endothelial cells	SOX18	Human		2014	25290189 ⁹³⁾
Dermal fibroblasts	Photoreceptor cells	CRX, RAX, OTX2, and NEUROD	Human		2014	24456169 ⁸⁶⁾
Embryonic fibroblasts	Thymic epithelial cells	Foxn1	Mouse	YES	2014	25150981 ⁹⁸⁾
Fibroblasts	Sweat gland cells	NF- κ B and LEF-1	Human	YES	2015	26566868 ⁹¹⁾
Amniotic fluid stem cells	Pluripotent stem cells	OCT4	Human		2016	27019637 ²²⁵⁾
Cardiac mesenchymal progenitors	Adipocytes	Klf4 and c-Myc	Mouse		2016	27077806 ⁸⁷⁾
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 ⁹⁹⁾
Endothelial progenitor cells	Smooth muscle cells	MYOCD	Human		2016	26874281 ⁸⁹⁾

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Embryonic stem cells	Trophoblast stem cells	Cdx2, Arid3a, and Gata3	Mouse		2017	28973471 ¹⁰²⁾
Embryonic fibroblasts and Adult tail-tip dermal fibroblasts	Leydig cells	Dmrt1, Gata4, and Nr5a1	Mouse	YES	2017	28017657 ¹⁰¹⁾
Postnatal dermal fibroblasts	Endothelial cells	ER71/ETV2 (ETS variant 2)	Human	YES	2017	28003219 ⁹⁴⁾
Dermal fibroblasts	Adipocytes	PPARG2	Human		2017	28982679 ⁸⁸⁾
Embryonic fibroblasts and Umbilical vein endothelial cells	Intestine progenitor cells	Hnf4a, Foxa3, Gata6, and Cdx2	Mouse & Human	YES	2017	28943029 ¹⁰⁰⁾
Embryonic fibroblasts and Adult dermal fibroblasts	Smooth muscle cells	Myocd, Gata6, and Mef2c	Mouse & Human		2018	30026272 ⁹⁰⁾
Epidermal cells	Sweat gland cells	Foxc1	Mouse	YES	2019	30894517 ⁹²⁾
Renal proximal tubular epithelial (HK2) cells	Nephron progenitor cells	SNAI2, EYA1, and SIX1	Human		2019	30827514 ¹⁰³⁾

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].

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 *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)–37)} 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,⁶⁰⁾ 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.^{20),21),24),65)–71)} 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.^{72),73)} Therefore, these studies were reported earlier than 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.^{74)–78)} 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 1987,¹⁹⁾ a vast number of different direct

reprogramming techniques have been reported; for example, osteoblasts,^{79)–82)} myoblasts,^{19),83)} skeletal muscle progenitor cells,⁸⁴⁾ photoreceptor cells,^{85),86)} adipocytes,^{87),88)} smooth muscle cells,^{89),90)} sweat gland cells,^{91),92)} and endothelial cells^{93),94)} have been induced with several combinations of transcription factors. In addition, the direct conversion of various types of cells, such as brown fat cells,⁹⁵⁾ Sertoli cells,⁹⁶⁾ melanocytes,⁹⁷⁾ thymic epithelial cells,⁹⁸⁾ renal tubular epithelial cells,⁹⁹⁾ intestine progenitor cells,¹⁰⁰⁾ Leydig cells,¹⁰¹⁾ trophoblast stem cells,¹⁰²⁾ and nephron progenitor cells,¹⁰³⁾ 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.^{88),104),105)}

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.^{106)–110)} 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

Table 2. Examples of the direct reprogramming with various methods

Source cells	Target cells	Transgenes and/or Treatment	Species	Cell transplantation	Published year	PMID
Integration-free transduction						
Fibroblasts (BJ and MRC-5 cells)	Hepatocytes	HNF1A and Any two of the three factors: FOXA1, FOXA3, and HNF4A (mRNA transfection)	Human		2014	24963715 ⁵⁹⁾
Non-sensory cochlear epithelial cells	Neuronal cells	Ascl1; Ascl1 and Neurod (plasmid electroporation)	Mouse		2014	24928351 ³⁹⁾
Cardiac fibroblasts	Cardiomyocytes	Gata4, Mef2c, and Tbx5 (peptide-enhanced mRNA transfection)	Mouse		2015	25834424 ¹¹⁶⁾
Astrocytes	Neural stem cells	Sox2 (Leu3p- α IPM system)	Mouse		2016	27148066 ¹²¹⁾
Fetal and embryonic fibroblasts and Brain cells <i>in vivo</i>	Neuronal cells	Ascl1, Brn2, and Myt1l (mRNA transfection with a GO-PEI-based reagent <i>in vitro</i> and <i>in vivo</i>)	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	26990860 ⁸¹⁾
Fibroblasts	Hepatocytes	OCT4, FOXA2, HNF1A, and GATA3 (protein transduction with HVJ-E)	Human		2017	28587331 ⁶²⁾
Fibroblasts	Neurocytes	SOX2, GATA3, and NEUROD1 (protein transduction with HVJ-E)	Human		2017	28587331 ⁶²⁾
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 ³⁴⁾
Embryonic fibroblasts	Hepatocytes	Hnf4a and Foxa3 (modified mRNA transfection)	Mouse	YES	2018	30327781 ¹¹⁵⁾

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Foreskin fibroblasts	Neuronal cells	ASCL1 + miR-124 + P53 knock-down (episomal plasmid transfection)	Human		2019	30539819 ¹²²⁾
Bone marrow-derived cells, Fibroblasts, and Keratinocytes	Neural precursor cells	MSI1, NGN2, and MBD2 (plasmid transfection)	Human	YES	2019	31196173 ³⁷⁾
Renal proximal tubular epithelial (HK2) cells	Nephron progenitor cells	SNAI2, EYA1, and SIX1 (PiggyBac transposon)	Human		2019	30827514 ¹⁰³⁾
Chemical compounds (with or without transcription factors)						
Fibroblasts (IMR90 or MRC5 cells)	Cholinergic neurons	NGN2 + Forskolin and dorso-morphin treatment	Human		2013	23873306 ¹³⁰⁾
Embryonic fibroblasts and Neonatal tail-tip fibroblasts	Cardiomyocytes	CHIR99021, RepSox, Forskolin, Valproic acid, Parnate, and TTNPB treatment	Mouse		2015	26292833 ¹²³⁾
Embryonic fibroblasts and Adult tail-tip fibroblasts	Neural stem cells	Valproic acid, Bix01294, RG108, PD0325901, CHIR99021, Vitamin C, and A83-01 treatment	Mouse	YES	2015	26788068 ¹²⁴⁾
Fibroblasts	Neuronal cells	Valproic acid, CHIR99021, RepSox, Forskolin, SP600125, GO6983, and Y-27632 treatment	Human		2015	26253202 ¹²⁵⁾
Fibroblasts	Neuronal cells	ISX9, SB431542, Forskolin, CHIR99021, and I-BET151 treatment	Mouse		2015	26253201 ¹²⁶⁾
Embryonic fibroblasts	Neural stem cells	A83-01, Purmorphamine, Valproic acid, and Thiazovivin treatment	Mouse		2017	27207831 ¹³¹⁾
Urine-derived cells	Hepatocytes	FOXA3 and HNF1A or HNF4A + CHIR99021, RepSox, Valproic acid, Parnate, TTNPB, and Dznep treatment	Human		2018	30315254 ¹²⁷⁾
Embryonic and adult fibroblasts	Skeletal muscle progenitor cells	Myod + CHIR99021, RepSox, and Forskolin treatment	Mouse	YES	2018	29742392 ¹²⁸⁾
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, Tranylcypromine treatment	Human	YES	2018	30581079 ¹³²⁾
Striatal neurons	Dopaminergic neurons	Sox2, Nurr1, Lmx1a, and Foxa2 + Valproic acid treatment (<i>in vivo</i> infection)	Mouse		2018	30318292 ⁴²⁾
Glioblastoma cells (U87MG cells)	Neuronal cells	Forskolin, ISX9, CHIR99021, I-BET151, and DAPT treatment	Human		2018	30091580 ¹²⁹⁾
Dermal fibroblasts	Neuronal cells	NGN2 and ASCL1 + Pyrintegrin, ZM336372, AZ960, and KC7F2 treatment	Human		2019	31099332 ¹³³⁾

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Culture condition and soluble factors (with transcription factors)						
Liver cells <i>in vivo</i>	β cells	PDX1 + EGF and nicotinamide treatment	Human	YES	2005	15899968 ¹³⁴⁾
Adult dermal fibroblasts and Neonatal foreskin fibroblasts	Multilineage blood progenitor cells	OCT4 + hematopoietic cytokines treatment	Human	YES	2010	21057492 ¹³⁵⁾
Embryonic fibroblasts	Cardiomyocytes	OSKM + cytokines (including Bmp4) treatment	Mouse		2011	21278734 ¹³⁶⁾
Adult skin fibroblasts	Neural stem cells	OSKM + neurosphere culture	Mouse	YES	2012	22467474 ¹³⁷⁾
Amniocytes	Vascular endothelial cells	ETV2, FLI1, and ERG1 + TGF β inhibitor treatment	Human	YES	2012	23084400 ¹³⁹⁾
Embryonic stem cells-derived fibroblasts	Neural crest cells	SOX10 + Environmental cues including WNT activation	Human	YES	2014	25158936 ¹⁴⁰⁾
Newborn fibroblasts	Hepatocytes	OSK + Soluble factors	Human	YES	2014	24572354 ²²⁶⁾
Dermal fibroblasts	Oligodendrocyte progenitor cells	Oct4 + OPC induction medium	Mouse	YES	2015	26497893 ¹⁴¹⁾
Embryonic fibroblasts	Keratinocytes	OSK + Bmp4 and RA treatment	Mouse	YES	2016	27473056 ¹⁴²⁾
Skin fibroblasts	Neural stem cells	OCT3/4, SOX2, KLF4, L-MYC, and LIN28 + P53 knockdown + Neurotrophins treatment	Human		2016	27822179 ¹³⁸⁾
miRNAs (with or without transcription factors)						
Cardiac fibroblasts	Cardiomyocytes	miR-1, miR-133, miR-208, and miR-499	Mouse		2012	22539765 ¹⁴⁷⁾
Embryonic fibroblasts	Neuronal cells	Ptb knockdown	Mouse		2013	23313552 ¹⁴³⁾
Cardiac fibroblasts and Embryonic fibroblasts	Cardiomyocytes	Gata4, Mef2c, and Tbx5 + miR-133; Gata4, Mef2c, Tbx5, Mesp1, and Myocd + miR-133	Mouse & Human		2014	24920580 ¹⁴⁸⁾
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 ¹⁴⁴⁾
Fibroblasts	Cardiomyocytes	GATA4, MEF2C, TBX5, ESRRG, MESP1, MYOCARDIN, ZFPM2, and HAND2 + miR-1	Human		2017	28796841 ¹⁴⁹⁾
Adult fibroblasts	Neuronal cells	miR-9/9* and miR-124	Human		2017	28886366 ¹⁴⁵⁾
Adult fibroblasts	Spinal cord motor neurons	ISL1 and LHX3 + miR-9/9* and miR-124	Human		2017	28886366 ¹⁴⁵⁾
Brain vascular pericytes	Cholinergic neuronal cells	ASCL1, MYT1L, BRN2, and TLX3 + miR-124	Human	YES	2018	29453933 ¹⁴⁶⁾
Physical stimulation (with or without transcription factors)						
Embryonic fibroblasts	Neuronal cells	Ascl1, Brn2, and Myt1l + Substrate topography	Mouse		2014	24709523 ¹⁵³⁾
Fibroblasts	Cardiomyocytes	Myocardin, Tbx5, and Mef2c + Microgroove	Mouse		2015	26302234 ¹⁵⁴⁾

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Embryonic fibroblasts	Dopaminergic neurons	Ascl1, Pitx3, Nurr1, and Lmx1a + Nanogroove	Mouse		2015	25662493 ¹⁵⁵⁾
Dermal fibroblasts	Endothelial progenitor cells	ETV2 + Hypoxia	Human		2016	27488544 ¹⁵¹⁾
Neonatal tail-tip fibroblasts	Cardiomyocytes	Gata4, Mef2c, and Tbx5 + Microgroove	Mouse		2016	27376554 ¹⁵⁶⁾
Fibroblasts	Cardiomyocytes	Gata4, Mef2c, and Tbx5 + Hypoxia	Mouse		2016	26757100 ¹⁵²⁾
Dermal fibroblasts	Chondrocytes	Electrical stimulation	Human		2019	31005261 ¹⁵⁷⁾
CRISPR/Cas9-based methods						
Embryonic fibroblasts	Neuronal cells	CRISPR/Cas9-based transcriptional activators + gRNAs for Brn2, Ascl1, and Myt1l	Mouse		2016	27524438 ¹⁵⁸⁾
Liver cells <i>in vivo</i>	β cells	CRISPR/Cas9-based transcriptional activators + gRNA for Pdx1 (<i>in vivo</i> infection)	Mouse	YES	2017	29224783 ⁷¹⁾
Protein engineering (with transcription factors)						
Embryonic fibroblasts	Cardiomyocytes	Gata4, Tbx5, and Hand2 + Mef2c fused with M3 domain of Myod	Mouse		2013	23794713 ¹⁵⁹⁾
Astrocytes	Neuronal cells	Ascl1, Creb-VP16, and Icer	Mouse		2017	28321434 ¹⁶⁰⁾
Other methods						
Bone marrow stromal cells	Renal proximal tubular epithelial cells	HK2 cell-extracts treatment + streptolysin O	Human	YES	2015	25754206 ¹⁶¹⁾
Embryonic fibroblasts	Cardiomyocytes	Gata4, Hand2, Mef2c, and Tbx5 + miR-1 and miR-133 + TGF β inhibitors or ROCK inhibitors	Mouse		2015	26354680 ¹⁶²⁾
Pancreatic exocrine cells	β cells	Pdx1, Ngn3, and Mafa (synthetic mRNA transfection) + 5-Aza treatment	Mouse		2016	27187823 ¹⁶³⁾
Neonatal cardiac and tail-tip fibroblasts	Cardiomyocytes	miR-1, miR-133, miR-208, and miR-499 + three-dimensional culture	Mouse		2016	27941896 ¹⁶⁴⁾
Neonatal cardiac and tail-tip fibroblasts	Cardiomyocytes	miR-1, miR-133, miR-208, and miR-499 + Reprogramming medium	Mouse		2016	26975336 ¹⁶⁵⁾
Adipose-derived stem cells and Neonatal foreskin fibroblasts	Neural stem cells	iPSC induction followed by TRA-1-60–/SSEA4–selection	Human	YES	2016	27569063 ¹⁶⁶⁾
Fibroblasts	Adipocytes	OCT4 and C/EBPB (protein transduction with HVJ-E) + Adipogenic differentiation medium	Human		2017	28587331 ⁶²⁾

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,¹¹¹⁾ SeV vectors have been used for direct induction of photoreceptor cells from peripheral blood cells.¹¹²⁾ Additionally, a method for *in vivo* cardiac reprogramming using SeV vectors achieved high reprogramming efficiency.¹¹³⁾ 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.⁶²⁾ 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 hepatocytes¹¹⁴⁾ and human nephron progenitor cells from somatic cells.¹⁰³⁾ 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 transduced mRNAs into their genome. Many studies using mRNA transduction have been reported, *e.g.*, hepatocytes,^{59),115)} cardiomyocytes,¹¹⁶⁾ neuronal cells,⁴⁰⁾ endothelial progenitor cells,¹¹⁷⁾ and neural stem/precursor cells.^{34),118)} 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^{34),115)} and specific transfection reagents,^{40),116)} have already been adopted in mRNA transfection studies. In addition to these methods, plasmid transfection has also been widely tested.^{27),29),32),78),116),117)} 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,^{119),120)} a Leu3p- α IPM system,¹²¹⁾ or episomal vectors,¹²²⁾ 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),^{123)–129)} cAMP (Forskolin),^{123),125),126),128)–130)} TGF β (RepSox, A83-01, SB431542),^{123)–128),131)} Ca²⁺ (ISX9),^{126),129)} Shh (Purmorphamine),^{131),132)} Retinoic acid (TTNPB),^{123),127)} JAK/STAT (AZ960, SP600125),^{125),133)} Notch (DAPT),¹²⁹⁾ AMPK (dorsomorphin),¹³⁰⁾ PCK (GO6983),¹²⁵⁾ HIF (KC7F2),¹³³⁾ MAPK (PD0325901, ZM336372),^{124),133)} β 1-integrin (Pyrintegrin),¹³³⁾ and Rho/ROCK (Thiazovivin, Y-27632)^{125),131)} signaling pathways, and epigenetic modifications, *e.g.*, histone lysine deacetylation (Valproic acid),^{42),123)–125),127),131)} histone lysine acetylation recognition (I-BET151),^{126),129)} histone lysine methylation (Bix01294, Dznep),^{124),127)} DNA methylation (RG108),¹²⁴⁾ and DNA demethylation (Vitamin C).¹²⁴⁾ 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.^{42),127),128),130),132),133)} 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,¹³⁴⁾ blood progenitor cells,¹³⁵⁾ cardiomyo-

cytes,¹³⁶⁾ neural stem cells,^{137),138)} vascular endothelial cells,¹³⁹⁾ neural crest cells,¹⁴⁰⁾ hepatocytes,¹⁴⁰⁾ oligodendrocyte progenitor cells,¹⁴¹⁾ and keratinocytes,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^{143)–146)} and cardiomyocytes.^{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.¹⁵⁰⁾ In a study involving direct reprogramming, these conditions were also used to accelerate conversion.^{151),152)} On the other hand, micro- or nano-imprinted patterns, *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.^{153)–156)} Of note, the electrical stimulation-induced direct reprogramming of fibroblasts into chondrocytes without any soluble factors or transgenes was reported recently.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.^{71),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, *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.^{159),160)}

Additionally, the number of hybrid methods using the techniques above are increasing.^{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, *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,^{148),149),165),167)–179)} neuronal cells,^{39),45),48),143),145),160),180)–188)} and hepatocytes^{189)–192)} have been well-studied among the various direct reprogramming methods. Additionally, several studies regarding the direct reprogramming of other cell types, *e.g.*, neural stem/progenitor cells,^{193),194)} glial cells,¹⁹⁵⁾ photoreceptor cells,¹⁹⁶⁾ β cells,¹⁹⁷⁾ renal tubular epithelial cells,¹⁹⁸⁾ trophoblast stem cells,¹⁰²⁾ and mesodermal cells,^{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

Fig. 2. (Color online) Different study types for direct reprogramming. Typical study types for direct reprogramming are indicated. Cutting-edge biochemistry and cell biology techniques have been employed for direct reprogramming studies. TF: transcription factor.

Table 3. Applications of the directly reprogrammed cells

Source cells	Target cells	Transgenes and/or Treatment	Species	Published year	PMID
<i>In vivo</i> reprogramming (regenerative medicine)					
Liver cells	β cells	Pdx1 (an adenoviral vectors)	Mouse	2000, 2003	10802714 ²⁰⁾ ; 12775714 ²¹⁾
Pancreatic exocrine cells	β cells	Ngn3, Pdx1, and Mafa (an adenoviral vector)	Mouse	2008	18754011 ⁶⁵⁾
Cardiac fibroblasts	Cardiomyocytes	Gata4, Mef2c, Tbx5, and Hand2 (a retrovirus vector)	Mouse	2012	22660318 ⁵⁰⁾
Cardiac fibroblasts	Cardiomyocytes	miR-1, miR-133, miR-208, and miR-499 (a lentiviral vector)	Mouse	2012	22539765 ⁵⁴⁾
Non-sensory cochlear epithelial cells	Neuronal cells	Ascl1; Ascl1 and Neurod (<i>in vivo</i> plasmid electroporation)	Mouse	2014	24928351 ³⁹⁾
Liver cells in mouse models of chronic liver disease	Hepatocytes	Foxa3, Gata4, Hnf1a, and Hnf4a (an AAV vector)	Mouse	2016	26923201 ⁶⁰⁾
Brain cells <i>in vivo</i>	Neuronal cells	Ascl1, Brn2, and Myt1l (mRNA transfection with a GO-PEI-based reagent)	Mouse	2016	28145631 ⁴⁰⁾
Bile duct cells	β cells	Pdx1, Ngn3, and Mafa (an adenoviral vector)	Mouse	2017	28363269 ⁶⁹⁾
Hepatocytes	β cells	Pdx1, Ngn3, and Mafa (hydrodynamics tail vein injection)	Mouse	2017	28100871 ⁷⁰⁾
Resident glial cells	Neuronal cells	Ascl1, Lmx1a, and Nurr1 (an AAV vector)	Mouse	2017	28844658 ⁴¹⁾
Liver cells <i>in vivo</i>	β cells	CRISPR/Cas9-based transcriptional activators + gRNA for Pdx1 (an AAV vector)	Mouse	2017	29224783 ⁷¹⁾
Striatal neurons	Dopaminergic neurons	Sox2, Nurr1, Lmx1a, and Foxa2 (a lentiviral vector) + Valproic acid treatment	Mouse	2018	30318292 ⁴²⁾
Resident glial cells	Interneurons	Ascl1, Lmx1a, and Nurr1 (an AAV vector)	Mouse	2019	31259901 ⁴³⁾

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Microglial cells	Neuronal cells	Neurod1 (a lentiviral vector)	Mouse	2019	30638745 ⁴⁴⁾
Disease model					
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	OSKM + Lineage-specific medium	Goat	2017	27629151 ²¹⁰⁾
Drug discovery					
Embryonic stem cells	Neuronal cells	NGN2	Human	2019	31155484 ⁴⁸⁾

source and target cells has provided a considerable amount of information regarding essential regulator genes and signaling pathways during conversion.^{143),148),176),179),180),200)} Epigenetic remodeling studies during cell-fate conversions have also been performed in various reprogrammed cells.^{44),48),171),175),177),182),188),191),194),195)} 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 spectro-

metry, have been used to analyze the function and behavior of transcription factors.^{102),145),160),167),169),172),173),179),184)–186),190),195),199)} 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.²⁰¹⁾ For example, *Ascl1* was identified as a pioneer factor for the induction of iN cells, which

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,¹⁶⁸⁾ 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),187),192),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 trajectories¹⁹²⁾ 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 *in vivo* 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 *in vitro* experiments for drug discovery. Indeed, a human iN cell type, instead of primary neuronal cells, was previously applied to *in vitro* drug treatment experiments.⁴⁸⁾ 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.*, rat,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 the forced 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.