Somatic Lineage Reprogramming

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Embryonic development and cell specification have been viewed as an epigenetically rigid process. Through accumulation of irreversible epigenetic marks, the differentiation process has been considered unidirectional, and once completed cell specification would be permanent and stable. However, somatic cell nuclear transfer that involved the implantation of a somatic nucleus into a previously enucleated oocyte accomplished in amphibians in the 1950s and in mammals in the late 1990s—resulting in the birth of "Dolly the sheep"—clearly showed that "terminal" differentiation is reversible. In parallel, work on lineage-determining factors like MyoD revealed surprising potential to modulate lineage identity in somatic cells. This work culminated in the discovery that a set of four defined factors can reprogram fibroblasts into induced pluripotent stem (iPS) cells, which were shown to be molecularly and functionally equivalent to blastocyst-derived embryonic stem (ES) cells, thus essentially showing that defined factors can induce authentic reprogramming without the need of oocytes. This concept was further extended when it was shown that fibroblasts can be directly converted into neurons, showing induced lineage conversion is possible even between cells representing two different germ layers. These findings suggest that "everything is possible" (i.e., once key lineage reprogramming factors are identified, cells should be able to convert into any desired lineage).

The initial generation of embryonic stem (ES) cells in the early 1980s was a long sought-after accomplishment (Evans and Kaufman 1981; Martin 1981). These cells were successfully derived from the inner-cell mass cells of the early mouse blastocyst, which are the cells that are giving rise to the germ line and all somatic lineages of the body as well as the nonmaternal extraembryonic tissues. Remarkably, cultured ES cells maintained this unique pluripotent property as they are able to generate germ and all somatic lineages on injection into the early blastocyst, forming a truly chimeric animal. Second and importantly, ES cells can also be propagated for many cell divisions without losing their pluripotency bestowing their stem cell property. With the breakthrough of somatic cell nuclear transfer in mammalian adult cells (Wilmut et al. 1997), it was shown reprogramming through nuclear transfer is not limited to amphibians (Gurdon 1962).

The ability to generate ES cells would now allow the derivation of individualized ES cells. Such cells, derived from a patient's own cell source, could be further differentiated into a clinically relevant cell type for therapeutic transplantation purposes, coined "therapeutic cloning." These concepts moved closer to reality

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when human ES cells were successfully derived from normal blastocysts (Thomson et al. 1998) and when somatic cell nuclear transfer was accomplished with human cells and human oocytes (Tachibana et al. 2013).

However, when translated to human cells, the derivation of nuclear transfer ES cells does require the use of dozens or more human oocytes and involves the destruction of a cloned human embryo. Thus, the procedure faced substantial logistical problems if it were to be used in a clinical setting. Moreover, the cloned embryo is arguably generated artificially but regardless represents an early human embryo with the potential to develop into a human being. Therefore, its needed destruction also raised ethical concerns.

Given the successful nuclear transfer experiment and trusting that the enucleated oocyte does not represent a miracle, but in the end is composed of defined chemical matter, scientists reasoned it ought to be possible that reprogramming somatic cells to pluripotent cells using defined factors. Following a systematic screen of candidate pluripotency factors, it was Takahashi and Yamanaka that succeeded to convert mouse fibroblasts into cells with many pluripotent features, termed induced pluripotent stem (iPS) cells (Takahashi and Yamanaka 2006a).

These initially described cells did not reach full pluripotent potential as they were not able to generate newborn chimeric animals when injected into blastocysts. Not long after the initial discovery, three independent groups including Yamanaka's group showed the formation of iPS cells with full pluripotent potential (Maherali et al. 2007; Okita et al. 2007; Wernig et al. 2007), and not long after that the first human iPS cells were generated (Takahashi et al. 2007; Yu et al. 2007). In light of these breakthrough discoveries, the question arose whether a similar concept of direct lineage conversion could also be extended to other, nonpluripotent lineages, such as conversion between distantly related somatic cell types. Of note, transcription factors had been identified that were able to induce a specific lineage identity in closely related cells such as B-lymphocytes to macrophages, fibroblasts to myocytes, exocrine to endocrine pancreatic cells, and astrocytes to neurons, but di-

rect conversions between distantly related cell types had not been attempted or were not successful yet (Davis et al. 1987; Xie et al. 2004; Berninger et al. 2007; Zhou et al. 2008).

It was our group that then was able to identify three defined transcription factors that were sufficient to convert mouse fibroblasts to functional neurons by way of true transdifferentiation (Vierbuchen et al. 2010), a finding replicated and extended to human fibroblasts the following year by numerous groups including ours, inspiring additional cell-type conversion attempts (Vierbuchen et al. 2010; Yin et al. 2010; Ambasudhan et al. 2011; Caiazzo et al. 2011; Huang et al. 2011; Pang et al. 2011; Pfisterer et al. 2011; Yoo et al. 2011; Margariti et al. 2012; Qian et al. 2012; Agley et al. 2013; Kogiso et al. 2013; Okita et al. 2013; Chakraborty et al. 2014; Victor et al. 2014; Colasante et al. 2015; Jayawardena et al. 2015; Li et al. 2015; Morita et al. 2015; Sayed et al. 2015; Boularaoui et al. 2018).

THE DERIVATION OF INDUCED PLURIPOTENT STEM CELLS

Yamanaka and others screened 24 candidate pluripotency factors for their ability to induce pluripotency in mouse fibroblasts (Takahashi and Yamanaka 2006a). Among those, no single factor was able to induce pluripotency. However, the pooled infection of all 24 factors did. Attempting to identify the critical factors, the group did something very clever. They omitted each factor and tested all 23-factor pools. These experiments showed that four factors, Oct4, Sox2, Klf4, and c-Myc were critical for the reprogramming process. Combining these four factors were then sufficient to induce iPS cells. To this day, these factors remain the basis for inducing pluripotency from somatic cells. Various variations and additional facilitating factors have been identified over the years. In the following we will go through these classes of reprogramming factors in more detail (Hong et al. 2009; Li et al. 2009a).

Oct-3/4

Oct-3/4 (also known as Pou5f1) is a member of the family octamer (oct) transcription factors and is essential to induce and maintain pluripo-

tency and is essential for early embryonic development (Schöler et al. 1991; Nichols et al. 1998). Its absence leads to spontaneous trophoblast differentiation and its presence is what makes ES cells pluripotent with ability to differentiate. Oct4 is considered the most critical reprogramming factor and has been the only factor known to date to induce pluripotency as single factor (Kim et al. 2009).

Sox Family

Several Sox family of transcription factors, in particular Sox2, are associated with maintaining pluripotency similar to Oct-3/4, although it is more broadly expressed such as in neural stem and progenitor cells. Oct4 has a much more restricted expression pattern and quite tightly associated with pluripotent and germ cells. Remarkably, several Sox family members are able to replace Sox2. For example, Sox1, which is not expressed in pluripotent stem cells, yields iPS cells with a similar efficiency as Sox2, and genes Sox3, Sox15, and Sox18 also generate iPS cells, although with decreased efficiency (Takahashi and Yamanaka 2006b).

Klf Family

Klf4 of the zinc finger domain containing Klf family of transcription factors was one of the original four factors found by Yamanaka to induce iPS cells. Unlike Oct4, and to some degree Sox2, it is not specifically expressed in pluripotent stem cells but to very high levels. It is also prominent for its rapid down-regulation during differentiation of ES cells. Unlike Sox2, Klf4 seems to be rather unique among its family members as its related genes Klf1 and Klf5 were able to replace Klf4 in reprogramming, but with reduced efficiency (Jeon et al. 2016).

Myc Family

The Myc family of transcription factors are among the most important oncogenes implicated in the formation of nearly all human cancers (Stefan and Bister 2017). c-Myc was one of the original four iPS cell reprogramming factors.

However, it was quickly realized that c-Myc is not required to induce pluripotency (Nakagawa et al. 2008; Wernig et al. 2008b). With just the three factors Oct4, Sox2, and Klf4, the reprogramming efficiency was reduced and in particular the reprogramming process was slowed down. Thus it was concluded that c-Myc's main role is to drive continued cell proliferation. Both N-myc and L-myc have been also used instead of c-myc with similar efficiency (Riddell et al. 2014).

Nanog

Nanog was identified as a core transcriptional regulator of pluripotency in ES cells (Chambers et al. 2003; Takahashi and Yamanaka 2006b; Silva et al. 2009; Takashima et al. 2014). It was therefore surprising that Nanog was not among the first set of reprogramming factors. Given its strong pro-pluripotent properties, Nanog was continued to be evaluated and was later shown to support human cell reprogramming in an alternative combination of reprogramming factors (Yu et al. 2007).

LIN28

LIN28 stands out among the iPS cell reprogramming factors in that it is not a transcription factor but rather an mRNA-binding protein. It is highly expressed in ES cells and generally associated with undifferentiated cells (Oka et al. 2010; Heffernan et al. 2011). The original reprogramming factor combination did not contain Lin28, but it was found to reprogram human cells in combination with NANOG, POU5F1, and SOX2 (Yu et al. 2007). In the following section we will discuss the various methods that have been used to reprogram cells.

REPROGRAMMING BY EXOGENOUS TRANSGENE OVEREXPRESSION

The most commonly used method to induce lineage reprogramming is the use of viral vectors to introduce exogenous transgenes into cells. This method was the first successfully used in reprogramming and remains one of the most

reliable and most efficient to this day (Takahashi et al. 2007).

Both lentiviruses and conventional retroviruses are the most used in reprogramming and transdifferentiation studies owing to their ability to effectively integrate DNA directly into the genome of the host cell, which allows for stable gene expression (Merrell and Stanger 2016). On reverse transcription of their RNA genome, their proviral DNA is integrated randomly into the host cell genome. As a result of this stable genomic integration, proviral genomes are passed to daughter cells during cell division. One unique feature of lentiviruses is their ability to infect both nondividing and dividing cell types, whereas infection with conventional onco-retroviruses requires active cell division (Dufait et al. 2012). Although conventional retroviruses and lentivirus can carry and express long segments of DNA, the longer the cDNA the lower the efficiency to generate infectious viral particles. Nevertheless, a multicistronic lentiviral vector that contains all four Yamanaka factors turns out to create a very reliable reprogramming tool (Sommer et al. 2009).

One lentiviral approach taken was the combination with inducible promoters such as the doxycycline-inducible system, which allowed the generation of inducible, "secondary" iPS cells that contain the optimal integration of reprogramming lentiviral transgenes (Wernig et al. 2008c). Integration of viral DNA has the advantage of stable and robust transgene expression, but the disadvantage of altering the host genome by random viral integrations, potentially causing disruptive mutations or activation of proto-oncogenes. Therefore, the search began to explore exogenous transgene expression systems without the need for genomic integration. Lentiviruses can be manipulated to become nonintegrating, but such approaches proved inefficient because of the absence of maintained transgene expression. Similarly, various transfection approaches were successful but overall inefficient and inconsistent (Okita et al. 2008; Yu et al. 2009).

These results were not surprising, as early work had already shown that sustained transgene expression is required for many days and transfection approaches produce only transient expression (Wernig et al. 2007). Consequently, the field then explored other nonintegrating viral delivery methods. Both adenoviruses and Sendai viruses are nonintegrating and Sendai viruses have the ability to produce a more sustained expression by self-replication. Both have been used in reprogramming studies (Ferrari et al. 2008; Ban et al. 2011).

The main advantage of adenoviruses is their large cargo capacity but more importantly, the sustained expression by Sendai viruses through their cytosolic replication proved highly efficient, and for a many years Sendai virus–based reprogramming has been the method of choice for reprogramming, spurring bioscience reagent companies to develop commercial reprogramming kits based on Sendai viruses.

Early on, mRNA transfection was shown to allow iPS cell reprogramming from fibroblasts (Angel and Yanik 2010; Kobayashi et al. 2021). Because the reprogramming factors had to be active for a long period of time, the method required repeated transfections, which was too toxic for cells. An important insight that leads to the ability to successfully reprogram fibroblasts to iPS cells was that blockade of innate immune responses substantially increased cell survival. In combination with optimized media, mRNA transfection has now become a reliable and efficient method to induce reprogramming (Mandal and Rossi 2013).

FACILITATION OF DIRECT CELL REPROGRAMMING BY ENDOGENOUS GENE REGULATION

Silencing Endogenous Genes with CRISPR/Cas9

CRISPR/Cas9 technology has provided a large variety of novel experimental approaches to modulate endogenous gene expression. In particular, CRISPR/Cas9 creates a sequence-specific double-strand break guided to specific genomic loci using the associated guideRNA (gRNA) in the ribonucleic acid–protein complex. The double-strand break triggers DNA repair mechanisms, one of which constitutes the homologous recombination of donor DNA molecules with homologous sequence arms allowing the inser-

tion of any desired DNA molecule at any desired destination in the human genome (Warren et al. 2010; Cong et al. 2013; Qi et al. 2013; Doudna and Charpentier 2014; Doudna 2015; Wen et al. 2018).

The gRNAs are strands of about 20 nucleotides that allow the CRISPR complex to specifically bind to DNA that matches the complementary sequence of the gRNA. Its ability to recognize and bind to specific sequences of DNA with limited off-target effects makes it also a method for the induction or reprogramming and transdifferentiation (Chen et al. 2017).

By its very nature, CRISPR/Cas9 can genetically destroy and thus permanently silence specific genes. As discussed above, most reprogramming requires the overexpression of genes but in some cases elimination of a critical lineage determination factor supports or even induces transdifferentiation such as elimination of the muscle factor MyoD or the B lymphocyte factor Pax5 (Mikkola et al. 2002; Chen et al. 2017).

Up-Regulating Endogenous Genes with dCas9

One important application of Cas9 technology is a variation of the Cas9 protein to induce or repress endogenous gene expression without altering the genetic sequence. dCas9 is a mutant form of Cas9, that has no nuclease activity but retains DNA-binding activity. It can thus be used to enhance or suppress the expression of endogenous genes by way of fusing dCas9 to transcriptional repressing or activating domains.

As mentioned above, gene activation is more relevant for cell reprogramming. Common partner fusion proteins for dCas9 include VP64, VP64-p65-Rta (VPR), histone acetyltransferases (HATs), synergistic activation mediators (SAMs) (Roth et al. 2001; Zhang et al. 2015). VP64 is a transactivator domain that recruits activating transcription factors to up-regulate the gene of interest. VPR domains contain VP64 but also include two additional activating transcription factors p65 and reverse tetracycline transactivator (rTA). All three of these transcription factors are targeted to the same gene, vastly improving its up-regulation in comparison to VP64

alone (Cieślar-Pobuda et al. 2016). dCas9-VPR has been successfully used to induce neuronal reprogramming (Chavez et al. 2015).

HATs, such as p300 and CREB-binding proteins, are enzymes capable of acetylating lysine residues found on histones. Once they become acetylated, the DNA wrapped around the histones is loosened, allowing dCas9 to better access the DNA. dCas9 uses the HAT domain to expose the DNA, then binds to the promoter region of the gene of interest and recruits transcription factors to up-regulate transcription. The use of SAMs is much more direct; instead of altering histone acetylation, SAMs simply contain three components (MS2, p65, and HSF1) that help recruit a wide array of transcription factors. SAMs have the ability to up-regulate genes greatly, as the recruited transcription factors work synergistically to activate the gene of interest.

Cas9-mediated activation (CRISPRa) also offers the possibility of unbiased screens to optimize reprogramming because short gRNA sequences are easier to combine in large numbers than entire genes (Liu et al. 2018).

DIRECT CELL REPROGRAMMING BY PHARMACOLOGICAL AGENTS

Cells have been treated with pharmacological agents that are capable of modifying the genetic and epigenetic environment to promote transdifferentiation. One of the first reprogramming events were in fact induced using such a pharmacological agent. Davis, Weintraub, and Lassar's breakthrough discovery of MyoD in 1987 was based on the previous observation that the DNA methyltransferase inhibitor 5-azacytidine (5-Aza) is able to induce muscle cells from fibroblasts (Jones et al. 1983).

5-Azacytidine is a chemical analog of cytidine. Cells metabolize azacytidine in a cascade of reactions, ultimately incorporating it into DNA. Because of differences in molecular structure, 5-azacytidine is unable to be methylated, thus inhibiting DNA methylation at CpG sequences (Roth et al. 2001). The inhibition of DNA methylation leads to a change in the epigenetic environment, resulting in a change in gene expression. Another DNA methylation inhibitor,

zebularine, supported the transdifferentiation of bone marrow fibroblasts into cardiomyocyte-like cells (Naeem et al. 2013). Although DNA methylation inhibitors have served to gain important insights into epigenetic regulation of reprogramming processes, they also have cytotoxic effects, requiring a narrow dosing and making it difficult to optimize reprogramming while maintaining sufficient cell viability. Another type of pharmacological agent used in support of transdifferentiation is dexamethasone, a glucocorticoid that is capable of activating certain transcription factors to promote the transdifferentiation of several cell types (Naeem et al. 2013; Zhang et al. 2015; Cieślar-Pobuda et al. 2016). Dexamethasone binds to glucocorticoid receptors, which then directly act on chromatin to modulate gene expression (Lu et al. 2006).

To accomplish faithful reprogramming with just small molecules has long been seen as the ultimate and ideal method to convert cells. Accordingly, large compound screens have been performed to explore whether small molecules can replace transcription factors. Indeed, these efforts have led to several small molecules that can boost reprogramming efficiency with smaller groups of transcription factors (Ichida et al. 2009; Li et al. 2009b; Maherali and Hochedlinger 2009). These efforts culminated in the accomplishment of reprogramming fibroblasts to iPS cells exclusively with small molecules (Hou et al. 2013). The efficiency of this method was exceedingly low, however, and appeared not practical for wider applications.

Inspired by these successes, scientists have begun to explore small molecule-based reprogramming of other lineages. Indeed, a direct conversion of fibroblasts into neuronal cells was recently accomplished (Li et al. 2015).

REPROGRAMMING TOWARD NEURONAL FATES

Neuronal cells are one of the most popular targets for transdifferentiation, owing to their limited supply and limited regeneration potential in vivo and important relevance for obtaining access to human cell disease modeling and potentially cell regenerative approaches. Following the discovery of iPS cells and lineage conversion between closely related cells, the demonstration that mouse fibroblasts can be directly converted to functional neurons by Vierbuchen and colleagues was the first example that somatic cell types can be reprogrammed even into distantly related lineages, such as a lineage representing a completely different germ layer. Following a systematic screen of 19 different transcription factors expressed in the neuronal lineage, induced neuronal (iN) cells could be derived from fibroblasts using various transcription factor combinations, most efficiently with the three factors Ascl1, Brn2, and Myt1l (Vierbuchen et al. 2010).

iN cells were shown to express all tested pan-neuronal markers, showing a typical neuronal morphology and the two principal functional properties of neurons, namely, the ability to generate action potentials and the formation functional synapses with other neurons and among each other. Only a year later, this finding was extended to human cells by various other groups (Ambasudhan et al. 2011; Caiazzo et al. 2011; Pang et al. 2011; Pfisterer et al. 2011; Yoo et al. 2011).

In addition to the transcription factors originally described, it was found that microRNAs and regulation of microRNA pathways can greatly facilitate conversion to iN cells (Ambasudhan et al. 2011; Yoo et al. 2011; Xue et al. 2013).

Ensuing work has explored different donor cell types. Surprisingly, some cell types appeared resistant and others permissive to reprogramming, which correlated with a unique chromatin configuration at the Ascl1 target genes (Marro et al. 2011; Wapinski et al. 2013).

More recently, it was shown that even human adult peripheral blood mononuclear cells as well as T lymphocytes can be also used to generate induced neuronal cells, showing that terminally differentiated, mature human cells can be transdifferentiated into a distant lineage efficiently (Tanabe et al. 2018).

One exciting application of neuronal lineage reprogramming is in the in vivo conversion of nonneuronal brain cells into neurons. Several efforts have shown promising results and provided convincing evidence that indeed new neurons can be formed in the adult brain by tran-

INDUCING HEPATOCYTES

Generation of hepatocytes has long been of scientific interest because of the large clinical need for hepatocyte transplantation. Not surprisingly, hepatocytes were thus another cell type rapidly attempting to generate by transdifferentiation. Soon after the description of iN cells in 2011, two groups independently identified transcription factor combinations to convert mouse fibroblasts to induced hepatocytes (iHeps) (Huang et al. 2011; Sekiya and Suzuki 2011). Importantly, similar factors allowed to produce functional iHeps from human fibroblasts (Huang et al. 2014). These human iHeps are already in clinical evaluation for ex vivo application (Lim et al. 2016).

With regard to mechanism, the general consensus is that the overexpression of Hnf4α and Hnf1α in conjunction with Foxa1, 2, or 3 is sufficient to drive the transdifferentiation of fibroblasts into hepatocyte-like cells (Huang et al. 2014). This process was further developed and refined by also targeting the transcription factor Kdm2b, which promoted greater conversion efficiencies as well as more prominent hepatocyte features (Huang et al. 2011). Shen et al. (2003) successfully transdifferentiated murine pancreatic cells into hepatocyte-like cells using dexamethasone and oncostatin M, which both play a role in activating C/EBPs (Shen et al. 2003; Huang et al. 2011; Sekiya and Suzuki 2011; Huang et al. 2014; Masserdotti et al. 2015; Lim et al. 2016). These transdifferentiated cells performed key hepatocyte functions, such as storing glycogen and secreting albumin (Shen et al. 2003; Huang et al. 2011; Sekiya and Suzuki 2011; Huang et al. 2014; Masserdotti et al. 2015; Lim et al. 2016; Pan et al. 2016).

INDUCTION OF SKELETAL AND HEART MYOCYTES

As alluded to earlier, the conversion of fibroblasts to skeletal muscle cells by MyoD and other myo-genic bHLH factors was one of the first examples of lineage reprogramming induced by forced expression of exogenous factors (Davis et al. 1987). Since then, both the mechanism as well as alternative reprogramming methods have been developed (Berkes et al. 2004; Conerly et al. 2016). For instance, Chakraborty et al. (2014) used dCas9 to create functional skeletal myocytes by up-regulating endogenous MyoD in fibroblasts. The induction of MyoD was correlated with the up-regulation of other skeletal myocyte protein markers, and reprogrammed myocytes showed functional properties. Unexpectedly, the CRISPR activation approach yielded a higher percentage of Myod1^{+} and Myog^{+} cells, implying that the dCas9 system resulted in a greater conversion efficiency (Boularaoui et al. 2018). Boularaoui et al. (2018) investigated subjecting the fibroblasts to TGF-β inhibition, WNT-signaling activation, EGF, and IGF1, all of which promoted a significant increase in transdifferentiation efficiency and yield. Tissue culture plastic coated with type I collagen, laminin, or fibronectin also resulted in an increase in transdifferentiation efficiency by promoting cell proliferation, migration, and lineage specification (Ginsberg et al. 2015; Qazi et al. 2015).

Perhaps of higher clinical relevance than skeletal muscle cells are cardiomyocytes, given that apart from infectious diseases, cardiovascular diseases are still one of the most frequent causes of death. In 2012, two groups independently reported the in vivo conversion of cardiac fibroblasts into functional muscle cells (Inagawa et al. 2012; Qian et al. 2012; Song et al. 2012). Importantly, the treatments resulted in significant clinical improvements following cardiac ischemia showing improvement of muscle strength on hypoxic tissue damage.

DIRECT REPROGRAMMING INTO PANCREATIC CELLS

Exocrine pancreatic cells represent an attractive source for potential transdifferentiation into insulin-producing cells. Indeed, Zhou et al. (2008) generated β-like cells in situ from nonendocrine pancreatic cells by expressing three key transcription factors encoded as adenoviruses in mice. The reprogrammed cells resemble the

shape, size, and ultrastructure of β cells. PCR analysis revealed that they also express several genes that are essential for β-cell functions, and —most importantly—secreted insulin in a glucose concentration-dependent manner into the bloodstream (Zhou et al. 2008). In the following work, Lemper et al. (2015) generated β-like cells by transducing human adult exocrine cells with lentiviral vectors coding for MAPK and STAT3. MAPK and STAT3 overexpression caused a large up-regulation in neurogenin 3, a transcription factor that drives undifferentiated pancreatic cells toward the β-cell lineage and up-regulates many other endocrine markers (Zhou et al. 2008). Furthermore, culturing the cells in a 3D matrix of Matrigel increased the efficiency of the transdifferentiation process, likely by increasing cell–cell contact. When these cells were engrafted in immunocompromised mice, they successfully produced insulin and acquired select functions of β cells, marked by the increased expression of proteins vital to the regulation of blood glucose levels (Zhou et al. 2008).

DIRECT REPROGRAMMING TOWARD OTHER LINEAGES

Many more cell lineages have been targeted to be generated by direct lineage reprogramming. These include endothelial-like cells (ETVECs), which were successfully generated from human adult fibroblasts (HAFs) by lentiviral expression of ETV2, a transcription factor responsible for the early development of endothelial cells (Ginsberg et al. 2015; Morita et al. 2015; Qazi et al. 2015; Lee et al. 2017). Cartilage injuries are common in athletes, difficult to treat, and therefore another goal to generate by direct reprogramming. Outani et al. (2013) generated induced chondrogenic (iChon) cells from human dermal fibroblasts (HDFs). Cell reprogramming using transcription factors has also been successful in generating hematopoietic stem progenitor-like cells (HSPCs), which has a great potential to generate different bone marrow cell lineage. Direct conversion efforts have further successfully generated multipotent neural progenitor cells, oligodendrocyte progenitor cells, brown fat cells, and even keratinocytes and many other cell types

(Kajimura et al. 2009; Lujan et al. 2012; Thier et al. 2012; Najm et al. 2013; Pereira et al. 2013; Yang et al. 2013; Riddell et al. 2014; Daniel et al. 2019; Zhao et al. 2020).

APPLICATIONS OF REPROGRAMMING

There are two main applications of reprogramming: the cells' use for regenerative medicine purposes such as cell-based therapies and disease modeling. Both individual cells or engineered three-dimensional tissues could be used for these applications.

Regenerative Medicine

Transdifferentiation, that is, the conversion of one somatic cell type into another somatic cell type and reprogramming involving the induction of iPS cells usually followed by differentiation, allows the generation of cells that are either not available for transplantation or the alternative sources are logistically, ethically, or technically inferior to cells obtained by reprogramming. For instance, as discussed above, nuclear transfer has provided a first strategy to potentially develop patient-derived pluripotent stem cells. However, dozens if not hundreds of human oocytes would be needed to generate nuclear transfer ES cells for each individual, which represents a challenging logistic problem and, in addition, raises ethical concerns, because the only current methodology to obtain human oocytes is from an otherwise healthy female donor in reproductive age by an invasive procedure. Therefore, soon after their discovery, iPS cells were explored for a variety of clinical diseases. The advantages are manifold: iPS cells can be generated from many different cell types including readily accessible cells such as skin, urinary epithelial cells, hair follicle cells, and blood cells. Moreover, they can be generated from individuals of any age. Because of their stem cell properties, iPS cells have a virtually unlimited proliferation potential without losing their relevant pluripotent features. Thus, in principle, any desired number of cells can be generated in culture and the only limitation is the availability of resources. For many clinical applications, the abil-

ity to scale donor cell grafts is critical for a meaningful clinical impact. Challenges associated with the iPS cell reprogramming route are inherently linked to the nature of iPS cells. Their pluripotency represents an intriguing opportunity but also a challenge, because in most cases, one will want to implant a defined, differentiated cell, not a mix of different cell types. Therefore, it will be of utmost importance to devise strategies that allow the differentiation of iPS cells into defined somatic lineages in high purity. Similarly, their strong cell proliferation capacity is an advantage for scaling, but potentially dangerous after transplantation as these cells when contaminating the graft in too high concentrations can cause tumor formation, again speaking for the necessity to use efficient differentiation that is devoid of contaminating undifferentiated cells. Finally, their expandability enables high yields but the cell expansion involves DNA replication and no biological process is completely error-free. Therefore, assessing genomic stability during iPS cell expansion and the overall cell manufacturing process will be a critical safety parameter. Decisions will have to be made based on the kind of mutations found and whether they pose a safety risk following transplantation. Certainly, one of the main categories of mutations that will have to be closely monitored and presumably excluded from any clinical cell graft are known cancer-driving mutations.

One prominent example of a disease with a long history of cell transplantation therapies is Parkinson's disease, the second most common neurodegenerative disease. It has been one of the main foci of regenerative medicine efforts because of the largely well-characterized degeneration of a specific dopaminergic neuron subpopulation in the ventral midbrain. Thus, the replacement of dopamine neurons is a very attractive idea. Since the first proof-of-concept study that iPS cell–derived dopamine neurons can rescue motor behavior in preclinical models, a large number of groups have devoted efforts into the clinical application (Dell'Anno et al. 2014; Barker et al. 2017; Barker and TRANSEURO Consortium 2019; Doi et al. 2020; Schweitzer et al. 2020; Parmar et al. 2021; Piao et al. 2021).

In addition to neurons, cell types targeted for cell transplantation include oligodendrocytes, as-trocytes, insulin-producing cells, hematopoietic cells, hepatocytes, skeletal muscle cells, cardiac muscle cells, skin cells, chondrocytes, and many others (Barker et al. 2017; Barker and TRANSEURO Consortium 2019; Doi et al. 2020; Schweitzer et al. 2020; Parmar et al. 2021; Piao et al. 2021).

Transdifferentiation typically results in rapid intersomatic cell conversion with less cell proliferation compared with iPS cell reprogramming. Genomic stability may therefore be less of a concern. However, on the flip side, potential donor tissue may be harder to generate in large enough quantities. One attractive application of transdifferentiation is the conversion of cells in situ, which may not require excessive cell expansion and such "in vivo transdifferentiation" may have large clinical effect sizes by converting large numbers of resident cells. A prominent example of this approach has been shown in the heart. Cardiac fibroblasts, which are numerous in the normal and diseased heart, have been successfully transdifferentiated into induced cardiomyocytes capable of improving cardiac function after a myocardial infarction in murine models (Qian et al. 2012). In other examples, there may not even be a need for large numbers of therapeutic cells. In another study, adenoviruses were used to transdifferentiate Sox9^{+} cells, commonly found in the small bile ducts around the liver, into insulin-producing cells that were able to correct features of diabetes in mice models (Banga et al. 2012).

Disease Modeling

Reprogramming and the generation of human iPS cells has a potential to generate human cells for disease modeling. Studying cell biology in human cell models provides the advantage of working with cells that possess the human genetic background. Moreover, cells can be derived from a variety of people including patients, allowing the study of disease processes in the correct genetic background. Many diseases have been modeled using a variety of different cell types (Zeltner and Studer 2015; Wu et al. 2016; Li and Izpisua Belmonte 2019).

Disease modeling applications can be accomplished following various principal approaches:

Table 1. Comparison of various reprogramming methods

Virus	Method	Advantages	Disadvantages
Yes	Conventional Retrovirus	High efficiency	Risk of insertional mutagenesis transgene reactivation and residual expression Risk of presence of proviral genome Can only be used in divided cells
Yes	Lentivirus	High efficiency Can be used in both dividing and nondividing cells	Risk of insertional mutagenesis transgene reactivation and residual expression Risk of presence of proviral genome
Yes	Adenovirus	No/small risk of integration	Moderate efficiency Need for repeated transductions Risk of presence of viral particles
Yes	Sendai virus	High efficiency No viral integration	Risk of presence of viral particles
No	Conventional and episomal plasmids	No integration No viral particles	Very low efficiency Need for repeated transfections
No	Minicircle	Efficiency higher than conventional plasmids No integration No viral particles No bacterial backbone	Low efficiency (lower than viral methods) Need for repeated transfections
No	piggyBac transposon	No viral particles Possibility to excise transgenes after reprogramming	Low efficiency Extra excision step Not imprint-free Risk of insertional mutagenesis transgene reactivation and residual expression Possible interactions between piggyBac system and endogenous transposon systems
No	mRNA	Very high efficiency Nontransgene, no integration No viral particles	Need for repeated transfections Requires specified reprogramming media
No	Protein	Nontransgene, no integration, no exogenous nucleic acids No viral particles	Very low efficiency
No	Small molecule compounds	Nontransgene, no integration, no exogenous nucleic acids No viral particles Moderate efficiency	Extremely low efficiency
No	Direct transfection of mature miRNA	Nontransgene, no integration, No viral particles	Low and inconsistent efficiency

(1) cell types can be derived directly through transdifferentiation; (2) patient and control subject donor cells can be first reprogrammed to iPS cells, which in turn can be differentiated into the cells of interest; (3) pluripotent stem cells can be genetically engineered to contain a disease-associated mutation and then compared with non-engineered lines; and, finally, (4) a conditional mutation can be engineered into one pluripotent stem cell line, which allows for the generation of mutant and control cells from one and the same cell line (Saha and Jaenisch 2009; Dolmetsch and Geschwind 2011; Passier et al. 2016; Hong et al. 2017; Engle et al. 2018; Sharma et al. 2020).

Table 2. A small selection of reprogramming approaches to yield various cell types in vitro

Cell source	Transdifferentiation method	Target cell type	Reprogramming factors	References
Human adult dermal fibroblast	Viral vectors	Glutamatergic neurons	Brn2, Mty1 l, miRNA-124	Ambasudhan et al. 2011
Human adult peripheral blood mononuclear cells	Electroporation		Ascl1, Brn2, Myt1 l, Ngn2	Tanabe et al. 2018
Human striatum astrocytes	Viral vectors		Ascl1, Brn2, Myt1l	Torper et al. 2013
Murine embryonic and postnatal fibroblasts	Viral vectors		Ascl1, Brn2, Myt1l	Vierbuchen et al. 2010
Human embryonic and postnatal fibroblasts	Viral vectors		Ascl1, Brn2, Myt1 l, Neurod1	Pang et al. 2011; Yoo et al. 2011
		Dopamine neurons		Caiazzo et al. 2011; Pfisterer et al. 2011
		GABAergic neurons		Victor et al. 2014; Colasante et al. 2015
		Motor neurons		Son et al. 2011
Mouse fibroblasts	Small molecules			Li et al. 2015
Aged human fibroblasts	Viruses	Glutamatergic neurons	Ascl1, Ngn2	Mertens et al. 2015
Human pericytes				Karow et al. 2018
Murine bone marrow stromal cells	Pharmacological agents	Hepatocytes	Dimethylsulphoxide, butylated hydroxy-anisole, KCl, valproic acid, forskolin, hydrocortisone, insulin	Zurita et al. 2008
Human neonatal fibroblasts	Viral vectors		Foxa2, Hnf4α, C/EBPβ, c-Myc	Kogiso et al. 2013
Human embryonic fibroblasts	Viral vectors		Hnf1α, Hnf4α, Foxa3	Huang et al. 2014
Murine pancreatic cells	Pharmacological agents		Dexamethasone, oncostatin M	Shen et al. 2003
Human adult fibroblasts	Viral vectors	Endothelial cells	ETV2	Morita et al. 2015
Murine amniotic cells	Viral vectors		Sox17	Schachterle et al. 2017
Human newborn dermal and lung fibroblasts	Viral vectors		Oct4, Sox2, KLF4, c-Myc bFGF, βME	Margariti et al. 2012
Human newborn foreskin fibroblasts	Pharmacological agents		Polyinosinic:polycytidylic acid	Sayed et al. 2015

Continued

Table 2. *Continued*

Cell source	Transdifferentiation method	Target cell type	Reprogramming factors	References
Murine embryonic fibroblasts	Pharmacological agents	Skeletal myocytes	5-azacytidine, Myod1	Davis et al. 1987
Murine embryonic fibroblasts	CRISPR/dCas9		Myod1	Chakraborty et al. 2014
Human dermal fibroblasts	Viral vectors		Myod1	Boularaoui et al. 2018
	Pharmacological agents		SB431542, Chir99021, EGF, IGF1	
Human dermal fibroblasts	Pharmacological agents	Chondrocytes	Cartilage-derived morphogenetic protein 1	Yin et al. 2010
Mouse dermal fibroblast	Viral vectors		c-Myc, KLF4, Sox9	Outani et al. 2013
Murine adult pancreatic exocrine cells	Viral vectors (in situ)	Pancreatic β-cells	Pdx1, Ngn3, Mafa	Zhou et al. 2008
Human pancreatic exocrine cells	Viral vectors		MAPK, STAT3	Lemper et al. 2015
Murine cardiac fibroblasts	Viral vectors (in situ)	Cardiomyocytes	Gata4, Mef2c, Tbx5	Qian et al. 2012
Murine bone marrow mesenchymal stem cells	Pharmacological agents		5-azacytidine, zebularine	Naeem et al. 2013
Murine cardiac fibroblasts	Pharmacological agents		miRNA-1, miRNA-133, miRNA-208, miRNA-499	Jayawardena et al. 2015
Murine myoblasts	CRISPR/Cas9	Adipocytes	Myod1	Wang et al. 2019
Human skeletal muscle fibroblasts	Pharmacological agents		Calcitriol, dexamethasone, ascorbic acid, and β-glycerophosphate	Agley et al. 2013
Human subcutaneous adipocytes	Pharmacological agents	Osteoblasts	Calcitriol, dexamethasone, ascorbic acid, and β-glycerophosphate	Justesen et al. 2004
Murine adipose tissue-derived stem cells	Viral vectors		Runx2	Zhang et al. 2006
Murine preadipocytes	Viral vectors		Runx2, MKP-1	Takahashi 2011

Tissue and Organ Engineering

The combination of stem cell differentiation, reprogramming, and tissue engineering provides a powerful approach to generate human functional tissues and organs. Vessel formation is one area of intensive investigation. Hong et al. (2017) generated functional endothelial cells from smooth muscle cells and seeded a decellularized vascular graft with the original smooth muscle cells on the exterior and the reprogrammed endothelial cells on the interior. When cultured in a bioreactor, the reprogrammed endothelial cells formed a complete monolayer and the surrounding layers of smooth muscle cells maintained blood pressure and vessel homeostasis, showing the graft's ability to emulate physiological vasculature (Metz et al. 2012).

Another example is research focusing on liver tissue engineering and efforts to improve functionality of hepatocytes to make them a more viable option for use in humans. Liver tissue engineering has been instrumental in improving disease modeling such as to study viral infections (Bhatia et al. 2014).

A related approach that relies on the remarkable properties of early embryonic-like cell types to self-pattern is the use of organoid cultures. Working with pluripotent stem cells, Yoshiki Sasai had shown that entire optical eye cap structures can spontaneously form by self-organization without the need to specifically construct the tissue by engineering (Sasai et al. 2008, 2012). This concept has been embraced by the scientific community and organoid cultures have now not only applied to neural tissue but extended to many other tissues (Sasai et al. 2008, 2012; Eiraku et al. 2011; Chiaradia and Lancaster 2020; Iwasawa and Takebe 2021; Marsee et al. 2021).

Finally, reprogramming and stem cell biology can be used to generate entire organs from patient-derived cells. Owing to their pluripotency, iPS cells can be injected into blastocysts and contribute to organ and tissue development. To translate to human organs, iPS cells will have to be injected into blastocysts from other species. The Nakauchi group provided the first proof-of-concept result and reported that it is possible to generate an entire rat pancreas in mice (Kobayashi et al. 2010). Following this work, several other organs or tissues have been successfully generated in this manner (Kobayashi et al. 2010; Usui et al. 2012; Rashid et al. 2014; Yamaguchi et al. 2017; Hamanaka et al. 2018; Mori et al. 2019; Kobayashi et al. 2021; Nishimura et al. 2021).

CONCLUDING REMARKS AND OUTLOOK

The field of reprogramming and pluripotent stem cells has provided a completely new area of investigation both for mechanistic insights into lineage specification and important applications for modeling disease and potentially treating disease. Reprogramming technologies have added a new and complementary approach to existing animal models and provides a rich source for new scientific endeavors. As the control over cell-type specification and maturation improves and tissue engineering is more integrated into reprogrammed human cell models, more complex scientific questions will become addressable. The potential of human reprogramming for cell-based therapies has just begun to be realized and there is no question that this area will witness a large growth in various applications (see Tables 1 and 2; Grath and Dai 2019).

REFERENCES

Agley CC, Rowlerson AM, Velloso CP, Lazarus NR, Harridge SDR. 2013. Human skeletal muscle fibroblasts, but not myogenic cells, readily undergo adipogenic differentiation. *J Cell Sci* **126:** 5610–5625.

Ambasudhan R, Talantova M, Coleman R, Yuan X, Zhu S, Lipton SA, Ding S. 2011. Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. *Cell Stem Cell* **9:** 113–118. doi:10.1016/j.stem.2011.07.002

Angel M, Yanik MF. 2010. Innate immune suppression enables frequent transfection with RNA encoding reprogramming proteins. *PLoS ONE* **5:** e11756. doi:10.1371/journal.pone.0011756

Arlotta P, Magavi SS, Macklis JD. 2003. Induction of adult neurogenesis: molecular manipulation of neural precursors in situ. *Ann NY Acad Sci* **991:** 229–236. doi:10.1111/j.1749-6632.2003.tb07479.x

Ban H, Nishishita N, Fusaki N, Tabata T, Saeki K, Shikamura M, Takada N, Inoue M, Hasegawa M, Kawamata S, et al. 2011. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. *Proc Natl Acad Sci* **108:** 14234–14239. doi:10.1073/pnas.1103509108

Banga A, Akinci E, Greder LV, Dutton JR, Slack JMW. 2012. In vivo reprogramming of Sox9$^+$ cells in the liver to insulin-secreting ducts. *Proc Natl Acad Sci* **109:** 15336–15341. doi:10.1073/pnas.1201701109

Barker RA; TRANSEURO Consortium. 2019. Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease. *Nat Med* **25:** 1045–1053. doi:10.1038/s41591-019-0507-2

Barker RA, Parmar M, Studer L, Takahashi J. 2017. Human trials of stem cell-derived dopamine neurons for Parkinson's disease: dawn of a new era. *Cell Stem Cell* **21:** 569–573. doi:10.1016/j.stem.2017.09.014

Berkes CA, Bergstrom DA, Penn BH, Seaver KJ, Knoepfler PS, Tapscott SJ. 2004. Pbx marks genes for activation by MyoD indicating a role for a homeodomain protein in establishing myogenic potential. *Mol Cell* **14:** 465–477. doi:10.1016/S1097-2765(04)00260-6

Berninger B, Costa MR, Koch U, Schroeder T, Sutor B, Grothe B, Gotz M. 2007. Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. *J Neurosci* **27:** 8654–8664. doi:10.1523/JNEUROSCI.1615-07.2007

Bhatia SN, Underhill GH, Zaret KS, Fox IJ. 2014. Cell and tissue engineering for liver disease. *Sci Transl Med* **6:** 245sr2. doi:10.1126/scitranslmed.3005975

Boularaoui SM, Abdel-Raouf KMA, Alwahab NSA, Kondash ME, Truskey GA, Teo JCM, Christoforou N. 2018. Efficient transdifferentiation of human dermal fibroblasts into skeletal muscle. *J Tissue Eng Regen Med* **12:** 918–936. doi:10.1002/term.2415

Caiazzo M, Dell'Anno MT, Dvoretskova E, Lazarevic D, Taverna S, Leo D, Sotnikova TD, Menegon A, Roncaglia P, Colciago G, et al. 2011. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* **476:** 224–227. doi:10.1038/nature10284

Chakraborty S, Ji H, Kabadi AM, Gersbach CA, Christoforou N, Leong KW. 2014. A CRISPR/Cas9-based system for reprogramming cell lineage specification. *Stem Cell Reports* **3:** 940–947. doi:10.1016/j.stemcr.2014.09.013

Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, Smith A. 2003. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* **113:** 643–655. doi:10.1016/S0092-8674(03)00392-1

Chavez A, Scheiman J, Vora S, Pruitt BW, Tuttle M, Iyer PR, Lin S, Kiani S, Guzman CD, Wiegand DJ, et al. 2015. Highly efficient Cas9-mediated transcriptional programming. *Nat Methods* **12:** 326–328. doi:10.1038/nmeth.3312

Chen Z, Li S, Subramaniam S, Shyy JYJ, Chien S. 2017. Epigenetic regulation: a new frontier for biomedical engineers. *Annu Rev Biomed Eng* **19:** 195–219. doi:10.1146/annurev-bioeng-071516-044720

Chiaradia I, Lancaster MA. 2020. Brain organoids for the study of human neurobiology at the interface of in vitro and in vivo. *Nat Neurosci* **23:** 1496–1508. doi:10.1038/s41593-020-00730-3

Cieślar-Pobuda A, Rafat M, Knoflach V, Skonieczna M, Hudecki A, Małecki A, Urasińska E, Ghavami S, Łos MJ. 2016. Human induced pluripotent stem cell differentiation and direct transdifferentiation into corneal epithelial-like cells. *Oncotarget* **7:** 42314–42329. doi:10.18632/oncotarget.9791

Colasante G, Lignani G, Rubio A, Medrihan L, Yekhlef L, Sessa A, Massimino L, Giannelli SG, Sacchetti S, Caiazzo M, et al. 2015. Rapid conversion of fibroblasts into functional forebrain GABAergic interneurons by direct genetic reprogramming. *Cell Stem Cell* **17:** 719–734. doi:10.1016/j.stem.2015.09.002

Conerly ML, Yao Z, Zhong JW, Groudine M, Tapscott SJ. 2016. Distinct activities of Myf5 and MyoD indicate separate roles in skeletal muscle lineage specification and differentiation. *Dev Cell* **36:** 375–385. doi:10.1016/j.devcel.2016.01.021

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Xu X, Jiang W, Marraffini LA, et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science* **339:** 819–823. doi:10.1126/science.1231143

Daniel MG, Sachs D, Bernitz JM, Fstkchyan Y, Rapp K, Satija N, Law K, Patel F, Gomes AM, Kim HS, et al. 2019. Induction of human hemogenesis in adult fibroblasts by defined factors and hematopoietic coculture. *FEBS Lett* **593:** 3266–3287. doi:10.1002/1873-3468.13621

Davis RL, Weintraub H, Lassar AB. 1987. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51:** 987–1000. doi:10.1016/0092-8674(87)90585-X

Dell'Anno MT, Caiazzo M, Leo D, Dvoretskova E, Medrihan L, Colasante G, Giannelli S, Theka I, Russo G, Mus L, et al. 2014. Remote control of induced dopaminergic neurons in parkinsonian rats. *J Clin Invest* **124:** 3215–3229. doi:10.1172/JCI74664

Doi D, Magotani H, Kikuchi T, Ikeda M, Hiramatsu S, Yoshida K, Amano N, Nomura M, Umekage M, Morizane A, et al. 2020. Pre-clinical study of induced pluripotent stem cell-derived dopaminergic progenitor cells for Parkinson's disease. *Nat Commun* **11:** 3369. doi:10.1038/s41467-020-17165-w

Dolmetsch R, Geschwind DH. 2011. The human brain in a dish: the promise of iPSC-derived neurons. *Cell* **145:** 831–834. doi:10.1016/j.cell.2011.05.034

Doudna JA. 2015. Genomic engineering and the future of medicine. *JAMA* **313:** 791–792. doi:10.1001/jama.2015.287

Doudna JA, Charpentier E. 2014. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science* **346:** 1258096. doi:10.1126/science.1258096

Dufait I, Liechtenstein T, Lanna A, Bricogne C, Laranga R, Padella A, Breckpot K, Escors D. 2012. Retroviral and lentiviral vectors for the induction of immunological tolerance. *Scientifica (Cairo)* **2012:** 1–14. doi:10.6064/2012/694137

Eiraku M, Takata N, Ishibashi H, Kawada M, Sakakura E, Okuda S, Sekiguchi K, Adachi T, Sasai Y. 2011. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472:** 51–56. doi:10.1038/nature09941

Engle SJ, Blaha L, Kleiman RJ. 2018. Best practices for translational disease modeling using human iPSC-derived neurons. *Neuron* **100:** 783–797. doi:10.1016/j.neuron.2018.10.033

Evans MJ, Kaufman MH. 1981. Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292:** 154–156. doi:10.1038/292154a0

Falkner S, Grade S, Dimou L, Conzelmann KK, Bonhoeffer T, Götz M, Hübener M. 2016. Transplanted embryonic neurons integrate into adult neocortical circuits. *Nature* **539:** 248–253. doi:10.1038/nature20113

Ferrari R, Pellegrini M, Horwitz GA, Xie W, Berk AJ, Kurdistani SK. 2008. Epigenetic reprogramming by adenovirus e1a. *Science* **321:** 1086–1088. doi:10.1126/science.1155546

Ginsberg M, Schachterle W, Shido K, Rafii S. 2015. Direct conversion of human amniotic cells into endothelial cells without transitioning through a pluripotent state. *Nat Protoc* **10:** 1975–1985. doi:10.1038/nprot.2015.126

Grath A, Dai G. 2019. Direct cell reprogramming for tissue engineering and regenerative medicine. *J Biol Eng* **13:** 14. doi:10.1186/s13036-019-0144-9

Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G. 2014. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. *Cell Stem Cell* **14:** 188–202. doi:10.1016/j.stem.2013.12.001

Gurdon JB. 1962. The transplantation of nuclei between two species of *Xenopus*. *Dev Biol* **5:** 68–83. doi:10.1016/0012-1606(62)90004-0

Hamanaka S, Umino A, Sato H, Hayama T, Yanagida A, Mizuno N, Kobayashi T, Kasai M, Suchy FP, Yamazaki S, et al. 2018. Generation of vascular endothelial cells and hematopoietic cells by blastocyst complementation. *Stem Cell Reports* **11:** 988–997. doi:10.1016/j.stemcr.2018.08.015

Heffernan C, Sumer H, Verma PJ. 2011. Generation of clinically relevant "induced pluripotent stem" (iPS) cells. *J Stem Cells* **6:** 109–127.

Hong H, Takahashi K, Ichisaka T, Aoi T, Kanagawa O, Nakagawa M, Okita K, Yamanaka S. 2009. Suppression of induced pluripotent stem cell generation by the p53–p21 pathway. *Nature* **460:** 1132–1135. doi:10.1038/nature08235

Hong X, Margariti A, Le Bras A, Jacquet L, Kong W, Hu Y, Xu Q. 2017. Transdifferentiated human vascular smooth muscle cells are a new potential cell source for endothelial regeneration. *Sci Rep* **7:** 5590. doi:10.1038/s41598-017-05665-7

Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, Zhao T, Ye J, Yang W, Liu K, et al. 2013. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science* **341:** 651–654. doi:10.1126/science.1239278

Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L. 2011. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* **475:** 386–389. doi:10.1038/nature10116

Huang P, Zhang L, Gao Y, He Z, Yao D, Wu Z, Cen J, Chen X, Liu C, Hu Y, et al. 2014. Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. *Cell Stem Cell* **14:** 370–384. doi:10.1016/j.stem.2014.01.003

Ichida JK, Blanchard J, Lam K, Son EY, Chung JE, Egli D, Loh KM, Carter AC, Di Giorgio FP, Koszka K, et al. 2009. A small-molecule inhibitor of Tgf-β signaling replaces sox2 in reprogramming by inducing *Nanog*. *Cell Stem Cell* **5:** 491–503. doi:10.1016/j.stem.2009.09.012

Inagawa K, Miyamoto K, Yamakawa H, Muraoka N, Sadahiro T, Umei T, Wada R, Katsumata Y, Kaneda R, Nakade K, et al. 2012. Induction of cardiomyocyte-like cells in infarct hearts by gene transfer of Gata4, Mef2c, and Tbx5. *Circ Res* **111:** 1147–1156. doi:10.1161/CIRCRESAHA.112.271148

Iwasawa K, Takebe T. 2021. Organogenesis in vitro. *Curr Opin Cell Biol* **73:** 84–91. doi:10.1016/j.ceb.2021.06.007

Jayawardena TM, Finch EA, Zhang L, Zhang H, Hodgkinson CP, Pratt RE, Rosenberg PB, Mirotsou M, Dzau VJ. 2015. MicroRNA induced cardiac reprogramming in vivo: evidence for mature cardiac myocytes and improved cardiac function. *Circ Res* **116:** 418–424. doi:10.1161/CIRCRESAHA.116.304510

Jeon H, Waku T, Azami T, Khoa LTP, Yanagisawa J, Takahashi S, Ema M. 2016. Comprehensive identification of Krüppel-like factor family members contributing to the self-renewal of mouse embryonic stem cells and cellular reprogramming. *PLoS ONE* **11:** e0150715. doi:10.1371/journal.pone.0150715

Jones PA, Taylor SM, Wilson V. 1983. DNA modification, differentiation, and transformation. *J Exp Zool* **228:** 287–295. doi:10.1002/jez.1402280212

Justesen J, Pedersen SB, Stenderup K, Moustapha K. 2004. Subcutaneous adipocytes can differentiate into bone-forming cells in vitro and in vivo. *Tissue Eng* **10:** 381–391. doi:10.1089/107632704323061744

Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gygi SP, Spiegelman BM. 2009. Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-β transcriptional complex. *Nature* **460:** 1154–1158. doi:10.1038/nature08262

Karow M, Camp JG, Falk S, Gerber T, Pataskar A, Gac-Santel M, Kegeyama J, Brazovskaja A, Garding A, Fan W, et al. 2018. Direct pericyte-to-neuron reprogramming via unfolding of a neural stem cell-like program. *Nat Neurosci* **21:** 932–940.

Kim JB, Sebastiano V, Wu G, Araúzo-Bravo MJ, Sasse P, Gentile L, Ko K, Ruau D, Ehrich M, van den Boom D, et al. 2009. Oct4-induced pluripotency in adult neural stem cells. *Cell* **136:** 411–419. doi:10.1016/j.cell.2009.01.023

Kobayashi T, Yamaguchi T, Hamanaka S, Kato-Itoh M, Yamazaki Y, Ibata M, Sato H, Lee YS, Usui JI, Kniesley AS, et al. 2010. Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. *Cell* **142:** 787–799. doi:10.1016/j.cell.2010.07.039

Kobayashi T, Goto T, Oikawa M, Sanbo M, Yoshida F, Terada R, Niizeki N, Kajitani N, Kazuki K, Kazuki Y, et al. 2021. Blastocyst complementation using *Prdm14*-deficient rats enables efficient germline transmission and generation of functional mouse spermatids in rats. *Nat Commun* **12:** 1328.154. doi:10.1038/s41467-021-21557-x

Kogiso T, Nagahara H, Otsuka M, Shiratori K, Dowdy SF. 2013. Transdifferentiation of human fibroblasts into hepatocyte-like cells by defined transcriptional factors. *Hepatol Int* **7:** 937–944. doi:10.1007/s12072-013-9432-5

Lee S, Park C, Han JW, Kim JY, Cho K, Kim EJ, Kim S, Lee SJ, Oh SY, Tanaka Y, et al. 2017. Direct reprogramming of human dermal fibroblasts into endothelial cells using ER71/ETV2. *Circ Res* **120**: 848–861. doi:10.1161/CIRCRESAHA.116.309833

Lemper M, Leuckx G, Heremans Y, German MS, Heimberg H, Bouwens L, Baeyens L. 2015. Reprogramming of human pancreatic exocrine cells to β-like cells. *Cell Death Differ* **22**: 1117–1130. doi:10.1038/cdd.2014.193

Li M, Izpisua Belmonte JC. 2019. Organoids—preclinical models of human disease. *N Engl J Med* **380**: 569–579. doi:10.1056/NEJMra1806175

Li H, Collado M, Villasante A, Strati K, Ortega S, Cañamero M, Blasco MA, Serrano M. 2009a. The *Ink4/Arf* locus is a barrier for iPS cell reprogramming. *Nature* **460**: 1136–1139. doi:10.1038/nature08290

Li W, Wei W, Zhu S, Zhu J, Shi Y, Lin T, Hao E, Hayek A, Deng H, Ding S. 2009b. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. *Cell Stem Cell* **4**: 16–19. doi:10.1016/j.stem.2008.11.014

Li X, Zuo X, Jing J, Ma Y, Wang J, Liu D, Zhu J, Du X, Xiong L, Du Y, et al. 2015. Small-molecule-driven direct reprogramming of mouse fibroblasts into functional neurons. *Cell Stem Cell* **17**: 195–203. doi:10.1016/j.stem.2015.06.003

Lim KT, Lee SC, Gao Y, Kim KP, Song G, An SY, Adachi K, Jang YU, Kim J, Oh KJ, et al. 2016. Small molecules facilitate single factor-mediated hepatic reprogramming. *Cell Rep* **15**: 814–829. doi:10.1016/j.celrep.2016.03.071

Liu Y, Yu C, Daley TP, Wang F, Cao WS, Bhate S, Lin X, Still C, Liu H, Zhao D, et al. 2018. CRISPR activation screens systematically identify factors that drive neuronal fate and reprogramming. *Cell Stem Cell* **23**: 758–771.e8. doi:10.1016/j.stem.2018.09.003

Lu NZ, Wardell SE, Burnstein KL, Defranco D, Fuller PJ, Giguere V, Hochberg RB, McKay L, Renoir JM, Weigel NL, et al. 2006. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. *Pharmacol Rev* **58**: 782–797. doi:10.1124/pr.58.4.9

Lujan E, Chanda S, Ahlenius H, Sudhof TC, Wernig M. 2012. Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. *Proc Natl Acad Sci* **109**: 2527–2532. doi:10.1073/pnas.1121003109

Maherali N, Hochedlinger K. 2009. Tgfβ signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. *Curr Biol* **19**: 1718–1723. doi:10.1016/j.cub.2009.08.025

Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachenchko R, Tchieu J, Jaenisch R, et al. 2007. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* **1**: 55–70. doi:10.1016/j.stem.2007.05.014

Mandal PK, Rossi DJ. 2013. Reprogramming human fibroblasts to pluripotency using modified mRNA. *Nat Protoc* **8**: 568–582. doi:10.1038/nprot.2013.019

Margariti A, Winkler B, Karamariti E, Zampetaki A, Tsai Tn, Baban D, Ragoussis J, Huang Y, Han JDJ, Zeng L, et al. 2012. Direct reprogramming of fibroblasts into endothelial cells capable of angiogenesis and reendothelialization in tissue-engineered vessels. *Proc Natl Acad Sci* **109**: 13793–13798. doi:10.1073/pnas.1205526109

Marro S, Pang ZP, Yang N, Tsai MC, Qu K, Chang HY, Südhof TC, Wernig M. 2011. Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. *Cell Stem Cell* **9**: 374–382. doi:10.1016/j.stem.2011.09.002

Marsee A, Roos FJM, Verstegen MMA; HPB Organoid Consortium; Gehart H, de Konig E, Lemaigre F, Forbes SJ, Peng WC, Huch M, et al. 2021. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. *Cell Stem Cell* **28**: 816–832. doi:10.1016/j.stem.2021.04.005

Martin GR. 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci* **78**: 7634–7638. doi:10.1073/pnas.78.12.7634

Masserdotti G, Gillotin S, Sutor B, Drechsel D, Irmler M, Jørgensen H, Sass S, Theis FJ, Beckers J, Berninger B, et al. 2015. Transcriptional mechanisms of proneural factors and REST in regulating neuronal reprogramming of astrocytes. *Cell Stem Cell* **17**: 74–88. doi:10.1016/j.stem.2015.05.014

Merrell AJ, Stanger BZ. 2016. Adult cell plasticity in vivo: de-differentiation and transdifferentiation are back in style. *Nat Rev Mol Cell Biol* **17**: 413–425. doi:10.1038/nrm.2016.24

Mertens J, Paquola ACM, Ku M, Hatch E, Böhnke L, Ladjevardi S, McGrath S, Campbell B, Lee H, Herdy JR, et al. 2015. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. *Cell Stem Cell* **17**: 705–718.

Metz RP, Patterson JL, Wilson E. 2012. Vascular smooth muscle cells: isolation, culture, and characterization. *Methods Mol Biol* **843**: 169–176. doi:10.1007/978-1-61779-523-7_16

Mikkola I, Heavey B, Horcher M, Busslinger M. 2002. Reversion of B cell commitment upon loss of *Pax5* expression. *Science* **297**: 110–113. doi:10.1126/science.1067518

Mori M, Furuhashi K, Danielsson JA, Hirata Y, Kakiuchi M, Lin CS, Ohta M, Riccio P, Takahashi Y, Xu X, et al. 2019. Generation of functional lungs via conditional blastocyst complementation using pluripotent stem cells. *Nat Med* **25**: 1691–1698. doi:10.1038/s41591-019-0635-8

Morita R, Suzuki M, Kasahara H, Shimizu N, Shichita T, Sekiya T, Kimura A, Sasaki Ki, Yasukawa H, Yoshimura A. 2015. ETS transcription factor ETV2 directly converts human fibroblasts into functional endothelial cells. *Proc Natl Acad Sci* **112**: 160–165. doi:10.1073/pnas.1413234112

Naeem N, Haneef K, Kabir N, Iqbal H, Jamall S, Salim A. 2013. DNA methylation inhibitors, 5-azacytidine and zebularine potentiate the transdifferentiation of rat bone marrow mesenchymal stem cells into cardiomyocytes. *Cardiovasc Ther* **31**: 201–209. doi:10.1111/j.1755-5922.2012.00320.x

Najm FJ, Lager AM, Zaremba A, Wyatt K, Caprariello AV, Factor DC, Karl RT, Maeda T, Miller RH, Tesar PJ. 2013. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte pro-

genitor cells. *Nat Biotechnol* **31:** 426–433. doi:10.1038/nbt .2561

Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. 2008. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* **26:** 101–106. doi:10.1038/nbt1374

Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Schöler H, Smith A. 1998. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95:** 379–391. doi:10.1016/S0092-8674(00)81769-9

Nishimura T, Suchy FP, Bhadury J, Igarashi KJ, Charlesworth CT, Nakauchi H. 2021. Generation of functional organs using a cell-competitive niche in intra- and interspecies rodent chimeras. *Cell Stem Cell* **28:** 141–149 e143. doi:10.1016/j.stem.2020.11.019

Oka Y, Nakajima K, Nagao K, Miura K, Ishii N, Kobayashi H. 2010. 293FT cells transduced with four transcription factors (OCT4, SOX2, NANOG, and LIN28) generate aberrant ES-like cells. *J Stem Cells Regen Med* **6:** 149–156. doi:10.46582/jsrm.0603008

Okita K, Ichisaka T, Yamanaka S. 2007. Generation of germline-competent induced pluripotent stem cells. *Nature* **448:** 313–317. doi:10.1038/nature05934

Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. 2008. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* **322:** 949–953. doi:10.1126/science.1164270

Okita K, Yamakawa T, Matsumura Y, Sato Y, Amano N, Watanabe A, Goshima N, Yamanaka S. 2013. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. *Stem Cells* **31:** 458–466. doi:10.1002/stem .1293

Outani H, Okada M, Yamashita A, Nakagawa K, Yoshikawa H, Tsumaki N. 2013. Direct induction of chondrogenic cells from human dermal fibroblast culture by defined factors. *PLoS ONE* **8:** e77365. doi:10.1371/journal.pone .0077365

Pan XP, Wang YN, Yu XP, Zhu CX, Li JZ, Du WB, Zhang YM, Cao HC, Zhang YH, Zhu DH, et al. 2016. Efficient generation of functional hepatocyte-like cells from mouse liver progenitor cells via indirect co-culture with immortalized human hepatic stellate cells. *Hepatobiliary Pancreat Dis Int* **15:** 173–179. doi:10.1016/S1499-3872(16) 60074-7

Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Südhof TC, et al. 2011. Induction of human neuronal cells by defined transcription factors. *Nature* **476:** 220–223. doi:10.1038/nature10202

Parmar M, Björklund A, Björklund T. 2021. In vivo conversion of dopamine neurons in mouse models of Parkinson's disease—a future approach for regenerative therapy? *Curr Opin Genet Dev* **70:** 76–82. doi:10.1016/j.gde .2021.06.002

Passier R, Orlova V, Mummery C. 2016. Complex tissue and disease modeling using hiPSCs. *Cell Stem Cell* **18:** 309–321. doi:10.1016/j.stem.2016.02.011

Pereira CF, Chang B, Qiu J, Niu X, Papatsenko D, Hendry CE, Clark NR, Nomura-Ktabayashi A, Kovacic JC,

Ma'ayan A, et al. 2013. Induction of a hemogenic program in mouse fibroblasts. *Cell Stem Cell* **13:** 205–218. doi:10 .1016/j.stem.2013.05.024

Pfisterer U, Wood J, Nihlberg K, Hallgren O, Bjermer L, Westergren-Thorsson G, Lindvall O, Parmar M. 2011. Efficient induction of functional neurons from adult human fibroblasts. *Cell Cycle* **10:** 3311–3316. doi:10.4161/cc .10.19.17584

Piao J, Zabierowski S, Dubose BN, Hill EJ, Navare M, Claros N, Rosen S, Ramnarine K, Horn C, Fredrickson C, et al. 2021. Preclinical efficacy and safety of a human embryonic stem cell-derived midbrain dopamine progenitor product, MSK-DA01. *Cell Stem Cell* **28:** 217–229.e7. doi:10.1016/j.stem.2021.01.004

Qazi TH, Mooney DJ, Pumberger M, Geißler S, Duda GN. 2015. Biomaterials based strategies for skeletal muscle tissue engineering: existing technologies and future trends. *Biomaterials* **53:** 502–521. doi:10.1016/j.bio materials.2015.02.110

Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* **152:** 1173–1183. doi:10.1016/j.cell .2013.02.022

Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D. 2012. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* **485:** 593–598. doi:10.1038/nature 11044

Qian H, Kang X, Hu J, Zhang D, Liang Z, Meng F, Zhang X, Xue Y, Maimon R, Dowdy SF, et al. 2020. Reversing a model of Parkinson's disease within situ converted nigral neurons. *Nature* **582:** 550–556. doi:10.1038/s41586-020-2388-4

Rashid T, Kobayashi T, Nakauchi H. 2014. Revisiting the flight of Icarus: making human organs from PSCs with large animal chimeras. *Cell Stem Cell* **15:** 406–409. doi:10 .1016/j.stem.2014.09.013

Riddell J, Gazit R, Garrison BS, Guo G, Saadatpour A, Mandal PK, Ebina W, Volchkov P, Yuan GC, Orkin SH, et al. 2014. Reprogramming committed murine blood cells to induced hematopoietic stem cells with defined factors. *Cell* **157:** 549–564. doi:10.1016/j.cell.2014.04.006

Roth SY, Denu JM, Allis CD. 2001. Histone acetyltransferases. *Annu Rev Biochem* **70:** 81–120. doi:10.1146/annurev .biochem.70.1.81

Rouaux C, Bhai S, Arlotta P. 2012. Programming and reprogramming neuronal subtypes in the central nervous system. *Dev Neurobiol* **72:** 1085–1098. doi:10.1002/dneu .22018

Saha K, Jaenisch R. 2009. Technical challenges in using human induced pluripotent stem cells to model disease. *Cell Stem Cell* **5:** 584–595. doi:10.1016/j.stem.2009.11.009

Sasai Y, Ogushi M, Nagase T, Ando S. 2008. Bridging the gap from frog research to human therapy: a tale of neural differentiation in *Xenopus* animal caps and human pluripotent cells. *Dev Growth Differ* **50:** S47–S55. doi:10 .1111/j.1440-169X.2008.01010.x

Sasai Y, Eiraku M, Suga H. 2012. In vitro organogenesis in three dimensions: self-organising stem cells. *Development* **139:** 4111–4121. doi:10.1242/dev.079590

Sayed N, Wong WT, Ospino F, Meng S, Lee J, Jha A, Dexheimer P, Aronow BJ, Cooke JP. 2015. Transdifferentiation of human fibroblasts to endothelial cells role of innate immunity. *Circulation* **131**: 300–309. doi:10.1161/CIRCULATIONAHA.113.007394

Schachterle W, Badwe CR, Palikuqi B, Kunar B, Ginsberg M, Lis R, Yokoyama M, Elemento O, Scandura JM, Rafii S. 2017. Sox17 drives functional engraftment of endothelium converted from non-vascular cells. *Nat Commun* **8**: 13963.

Schöler HR, Ciesiolka T, Gruss P. 1991. A nexus between Oct-4 and E1A: implications for gene regulation in embryonic stem cells. *Cell* **66**: 291–304. doi:10.1016/0092-8674(91)90619-A

Schweitzer JS, Song B, Herrington TM, Park TY, Lee N, Ko S, Jeon J, Cha Y, Kim K, Li Q, et al. 2020. Personalized iPSC-derived dopamine progenitor cells for Parkinson's disease. *N Engl J Med* **382**: 1926–1932. doi:10.1056/NEJMoa1915872

Sekiya S, Suzuki A. 2011. Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. *Nature* **475**: 390–393. doi:10.1038/nature10263

Sharma A, Sances S, Workman MJ, Svendsen CN. 2020. Multi-lineage human iPSC-derived platforms for disease modeling and drug discovery. *Cell Stem Cell* **26**: 309–329. doi:10.1016/j.stem.2020.02.011

Shen CN, Horb ME, Slack JMW, Tosh D. 2003. Transdifferentiation of pancreas to liver. *Mech Dev* **120**: 107–116. doi:10.1016/S0925-4773(02)00337-4

Silva J, Nichols J, Theunissen TW, Guo G, van Oosten AL, Barrandon O, Wray J, Yamanaka S, Chambers I, Smith A. 2009. Nanog is the gateway to the pluripotent ground state. *Cell* **138**: 722–737. doi:10.1016/j.cell.2009.07.039

Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G. 2009. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells* **27**: 543–549. doi:10.1634/stemcells.2008-1075

Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ, Eggan K. 2011. Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* **9**: 205–218.

Song K, Nam YJ, Luo X, Qi X, Tan W, Huang GN, Acharya A, Smith CL, Tallquist MD, Neilson EG, et al. 2012. Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature* **485**: 599–604. doi:10.1038/nature11139

Stefan E, Bister K. 2017. MYC and RAF: key effectors in cellular signaling and major drivers in human cancer. *Curr Top Microbiol Immunol* **407**: 117–151.

Tachibana M, Amato P, Sparman M, Gutierrez NM, Tippner-Hedges R, Ma H, Kang E, Fulati A, Lee HS, Sritanaudomchai H, et al. 2013. Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* **153**: 1228–1238. doi:10.1016/j.cell.2013.05.006

Tai W, Wu W, Wang LL, Ni H, Chen C, Yang J, Zang T, Zou Y, Xu XM, Zhang CL. 2021. In vivo reprogramming of NG2 glia enables adult neurogenesis and functional recovery following spinal cord injury. *Cell Stem Cell* **28**: 923–937.e4. doi:10.1016/j.stem.2021.02.009

Takahashi T. 2011. Overexpression of Runx2 and MKP-1 stimulates transdifferentiation of 3T3-L1 preadipocytes into bone-forming osteoblasts in vitro. *Calcif Tissue Int* **88**: 336–347.

Takahashi K, Yamanaka S. 2006a. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**: 663–676. doi:10.1016/j.cell.2006.07.024

Takahashi K, Yamanaka S. 2006b. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**: 663–676. doi:10.1016/j.cell.2006.07.024

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**: 861–872. doi:10.1016/j.cell.2007.11.019

Takashima Y, Guo G, Loos R, Nichols J, Ficz G, Krueger F, Oxley D, Santos F, Clarke J, Mansfield W, et al. 2014. Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell* **158**: 1254–1269. doi:10.1016/j.cell.2014.08.029

Tanabe K, Ang CE, Chanda S, Olmos VH, Haag D, Levinson DF, Südhof TC, Wernig M. 2018. Transdifferentiation of human adult peripheral blood T cells into neurons. *Proc Natl Acad Sci* **115**: 6470–6475. doi:10.1073/pnas.1720273115

Thier M, Wörsdörfer P, Lakes YB, Gorris R, Herms S, Opitz T, Seiferling D, Quandel T, Hoffmann P, Nöthen MM, et al. 2012. Direct conversion of fibroblasts into stably expandable neural stem cells. *Cell Stem Cell* **10**: 473–479. doi:10.1016/j.stem.2012.03.003

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. 1998. Embryonic stem cell lines derived from human blastocysts. *Science* **282**: 1145–1147. doi:10.1126/science.282.5391.1145

Torper O, Pfisterer U, Wolf DA, Pereira M, Lau S, Jakobsson J, Björklund A, Grealish S, Parmar M. 2013. Generation of induced neurons via direct conversion in vivo. *Proc Natl Acad Sci* **110**: 7038–7043.

Usui J, Kobayashi T, Yamaguchi T, Knisely AS, Nishinakamura R, Nakauchi H. 2012. Generation of kidney from pluripotent stem cells via blastocyst complementation. *Am J Pathol* **180**: 2417–2426. doi:10.1016/j.ajpath.2012.03.007

Victor MB, Richner M, Hermanstyne TO, Ransdell JL, Sobieski C, Deng PY, Klyachko VA, Nerbonne JM, Yoo AS. 2014. Generation of human striatal neurons by microRNA-dependent direct conversion of fibroblasts. *Neuron* **84**: 311–323. doi:10.1016/j.neuron.2014.10.016

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. 2010. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**: 1035–1041. doi:10.1038/nature08797

Wang W, Ishibashi J, Trefely S, Shao M, Cowan AJ, Sakers A, Lim H-W, O'Connor S, Doan MT, Cohen P. 2019. A PRDM16-driven metabolic signal from adipocytes regulates precursor cell fate. *Cell Metab* **30**: 174–189.

Wapinski OL, Vierbuchen T, Qu K, Lee QY, Chanda S, Fuentes DR, Giresi PG, Ng YH, Marro S, Neff NF, et al. 2013. Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. *Cell* **155**: 621–635. doi:10.1016/j.cell.2013.09.028

Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, et al. 2010. Highly

efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* **7:** 618–630. doi:10.1016/j.stem.2010.08.012

Wen W, Cheng X, Fu Y, Meng F, Zhang JP, Zhang L, Li XL, Yang Z, Xu J, Zhang F, et al. 2018. High-level precise Knockin of iPSCs by simultaneous reprogramming and genome editing of human peripheral blood mononuclear cells. *Stem Cell Reports* **10:** 1821–1834. doi:10.1016/j.stemcr.2018.04.013

Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. 2007. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **448:** 318–324. doi:10.1038/nature05944

Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, Broccoli V, Constantine-Paton M, Isacson O, Jaenisch R. 2008a. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci* **105:** 5856–5861. doi:10.1073/pnas.0801677105

Wernig M, Meissner A, Cassady JP, Jaenisch R. 2008b. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* **2:** 10–12. doi:10.1016/j.stem.2007.12.001

Wernig M, Lengner CJ, Hanna J, Lodato MA, Steine E, Foreman R, Staerk J, Markoulaki S, Jaenisch R. 2008c. A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. *Nat Biotechnol* **26:** 916–924. doi:10.1038/nbt1483

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature* **385:** 810–813. doi:10.1038/385810a0

Wu J, Izpisua Belmonte JC. 2016. Stem cells: a renaissance in human biology research. *Cell* **165:** 1572–1585. doi:10.1016/j.cell.2016.05.043

Xie H, Ye M, Feng R, Graf T. 2004. Stepwise reprogramming of B cells into macrophages. *Cell* **117:** 663–676. doi:10.1016/S0092-8674(04)00419-2

Xue Y, Ouyang K, Huang J, Zhou Y, Ouyang H, Li H, Wang G, Wu Q, Wei C, Bi Y, et al. 2013. Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. *Cell* **152:** 82–96. doi:10.1016/j.cell.2012.11.045

Yamaguchi T, Sato H, Kato-Itoh M, Goto T, Hara H, Sando M, Mizuno N, Kobayashi T, Yanagida A, Umino A, et al. 2017. Interspecies organogenesis generates autologous functional islets. *Nature* **542:** 191–196. doi:10.1038/nature21070

Yang N, Zuchero JB, Ahlenius H, Marro S, Ng YH, Vierbuchen T, Hawkins JS, Geissler R, Barres BA, Wernig M. 2013. Generation of oligodendroglial cells by direct lineage conversion. *Nat Biotechnol* **31:** 434–439. doi:10.1038/nbt.2564

Yin S, Cen L, Wang C, Zhao G, Sun J, Liu W, Cao Y, Cui L. 2010. Chondrogenic transdifferentiation of human dermal fibroblasts stimulated with cartilage-derived morphogenetic protein 1. *Tissue Eng Part A* **16:** 1633–1643. doi:10.1089/ten.tea.2009.0570

Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW, Crabtree GR. 2011. MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature* **476:** 228–231. doi:10.1038/nature10323

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, et al. 2007. Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318:** 1917–1920. doi:10.1126/science.1151526

Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA. 2009. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* **324:** 797–801. doi:10.1126/science.1172482

Zeltner N, Studer L. 2015. Pluripotent stem cell-based disease modeling: current hurdles and future promise. *Curr Opin Cell Biol* **37:** 102–110. doi:10.1016/j.ceb.2015.10.008

Zhang X, Yang M, Lin L, Chen P, Ma KT, Zhou CY, Ao YF. 2006. Runx2 overexpression enhances osteoblastic differentiation and mineralization in adipose-derived stem cells in vitro and in vivo. *Calcif Tissue Int* **79:** 169–178.

Zhang Y, Yin C, Zhang T, Li F, Yang W, Kaminski R, Fagan PR, Putatunda R, Young WB, Khalili K, et al. 2015. CRISPR/gRNA-directed synergistic activation mediator (SAM) induces specific, persistent and robust reactivation of the HIV-1 latent reservoirs. *Sci Rep* **5:** 1–14.

Zhao A, Yang Y, Pan X, Cai S. 2020. Generation of keratinocyte stem-like cells from human fibroblasts via a direct reprogramming approach. *Biotechnol Prog* **36:** e2961.

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. 2008. In vivo reprogramming of adult pancreatic exocrine cells to β-cells. *Nature* **455:** 627–632. doi:10.1038/nature07314

Zurita M, Bonilla C, Otero L, Aguayo C, Vaquero J. 2008. Neural transdifferentiation of bone marrow stromal cells obtained by chemical agents is a short-time reversible phenomenon. *Neurosci Res* **60:** 275–280.