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REVIEW ARTICLE

Next-generation disease modeling with direct conversion: a new path to old neurons

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Within just over a decade, human reprogramming-based disease modeling has developed from a rather outlandish idea into an essential part of disease research. While iPSCs are a valuable tool for modeling developmental and monogenetic disorders, their rejuvenated identity poses limitations for modeling age-associated diseases. Direct cell-type conversion of fibroblasts into induced neurons (iNs) circumvents rejuvenation and preserves hallmarks of cellular aging. iNs are thus advantageous for modeling diseases that possess strong age-related and epigenetic contributions and can complement iPSC-based strategies for disease modeling. In this review, we provide an overview of the state of the art of direct iN conversion and describe the key epigenetic, transcriptomic, and metabolic changes that occur in converting fibroblasts. Furthermore, we summarize new insights into this fascinating process, particularly focusing on the rapidly changing criteria used to define and characterize *in vitro*-born human neurons. Finally, we discuss the unique features that distinguish iNs from other reprogramming-based neuronal cell models and how iNs are relevant to disease modeling.

Keywords: aging; cellular reprogramming; direct conversion; disease modeling; epigenetics; geriatric diseases; induced neurons; metabolism; neurodegenerative disorders

Why we need human neurons in a dish

The immense processing power of the human brain is enabled by unique features of the brain's hardware: around 86 billion neurons with each one of them possessing thousands of synaptic connections [1]. It is believed that molecular and cell biological insults to neurons lead to hardware issues and ultimately manifest as neurological disorders. Animal studies, both *in vivo* and *in vitro*, have provided eye-opening insights into the inner workings of neurons and the brain. Animal models of brain disorders have however been found to not necessarily reflect complex human conditions and have unfortunately not been very predictive for the evaluation of drug candidates for several diseases. Alzheimer's disease stands out as a prime example to illustrate this puzzle. Animals, typically mice, can be genetically engineered to reflect central pathological hallmarks of the human disease, but all drug candidates that were developed based on successful animal studies have failed in clinical trials [2,3]. Animal models for other neurodegenerative and neuropsychiatric disorders all face their own individual but similar challenges. This dilemma has promoted the view that human neurons possess their unique biology that might be vital for studying

Abbreviations

ALS, amyotrophic lateral sclerosis; HD, Huntington's disease; iNs, induced neurons; iPSCs, induced pluripotent stem cells; TF, transcription factor.

aspects of many diseases, but the inaccessibility of human brain tissue renders it near-impossible to functionally and molecularly study neurons directly in the human brain. Driven by the idea that human neurons *in vitro* can help to capture and better understand disease-related factors that require a human neuronal cell physiology, human genetics, epigenetic signatures, and age, the generation of human neurons as disease models has received broad attention as a potential game-changer.

Notably, the direct conversion from one cell type into another, often also called fate conversion, direct reprogramming, or transdifferentiation, has been first demonstrated already in the 1980s, where it was shown that overexpression of the transcription factor (TF) MyoD can convert fibroblasts into myoblast-like cells [4]. Interestingly, only 30 years later and a few years after the invention of induced pluripotent stem cells (iPSCs) [5], the direct conversion of fibroblasts into induced neurons (iNs) was discovered [6]. With the invention of iNs, direct conversion strategies, also for other cell types, regained broader interest. From this point onward, direct conversion technologies have grown rapidly, and are today mostly regarded as a subdiscipline of the stem cell field, where they are seen as alternative approaches to generate cell types of interest from human patients and donors for disease modeling or regenerative purposes [7,8]. This boom in applications can be mostly attributed to the explosion of new technologies tailored to iPSC-based systems, most of which are also suitable for directly converted cells. These technologies encompass tools and strategies to harness human donor/patient-specific cells for basic human biology research [9–12], disease modeling [13–17], drug development and safety [18–21], or cell replacement strategies [22]. Although on first sight iNs might appear as 'just another way' to generate neurons in the dish, there are important technical and conceptual differences between iPSC-derived neurons and iNs to be noted. While some of these differential properties cause limitations of the iN technology for certain applications, some properties uniquely qualify iNs to address yet unmet needs. Here, we will review conversion strategies for human somatic cells into iNs, describe mechanistic insights and roadblocks to direct conversion, and discuss current standards and new criteria on how to characterize human neurons. We will further pay particular attention to the conceptual differences between iN conversion and other reprogramming methods and will highlight unique properties that set iNs apart for specific basic and translational applications.

Enabling iN conversion

Unlike neural differentiation protocols starting from iPSCs, direct iN conversion does not follow the concerted chronological stages of development, as one cell type is rather directly transformed into another one [23]. Overexpression of transcription factors (TFs) driving iN conversion (hereafter referred to as conversion TFs) overrides the cell type-specific transcriptional profile of the starting population and instantly activates a neuronal transcriptional program (with a few exceptions), permitting cell-type changes in a very short time [24]. The TFs bind to regulatory elements in the starting cells' genome and jump-start neuronal gene expression. In contrast to stem and progenitor cells, fully differentiated somatic cells possess a tightly regulated epigenetic landscape, with regions specific for other cell types inaccessible for most TFs. Conversion TFs that are sufficient for neuron induction stand out by their ability to bind to largely inaccessible 'neuronal regions' of the genome in differentiated non-neuronal cell types. This ability classifies these factors as pioneer TFs (Fig. 1A); the list of known iN pioneer TFs currently includes Ascl1, Ngn2, and NeuroD1 [25–28]. Although every starting cell type has a unique epigenetic landscape, they all have in common that their chromatin surrounding neuronal gene loci is closed, and a general rule is that most iN strategies involve at least one pioneer TF to access these closed regions. Fact-checking supports the validity of this rule as (a) the vast majority of efficient iN protocols involve at least one pioneer factor (Table 1) [29,30], (b) Ngn2 alone can convert up to 90% of human fibroblasts into iNs, and (c) also Ascl1 alone can induce neuron-like cells from fibroblasts [6,26,31]. Pioneer TFs induce the expression of endogenous secondary pro-neuronal TFs or of factors that repress the starting cell type-specific transcriptome, which further contributes to establishing neuronal identity [27]. Chromatin accessibility and transcriptome data have suggested that Zfp238, Sox8, and Dlx3 are among the most important endogenous secondary TF genes downstream of Ascl1 [32]. Some data indicate that Ngn2, when using an appropriate conversion medium, not only binds most of the Ascl1 binding sites in fibroblasts, but also possesses many additional binding sites [30,33]. However, other data suggest that Ascl1 and Ngn2 possess divergent binding patterns that result in distinct chromatin states and different neuronal fates [34]. While further (meta-)analysis will likely shed more light on these different views, it is not surprising that the most efficient and reliable conversion strategies involve the combined expression of

Ascl1 and Ngn2 [9,10,31,35]. Recently, it has been suggested that a huge variety of TF combinations can be applied to generate subtype-specific iNs from fibroblasts (Table 1), and TF screening studies for iN conversion have led to the identification of additional pro-neuronal factors, such as Brn3a/b/c, Brn4s, and Ezh2 [36,37]. Interestingly, differences in TF choice were noted between species, as, for example, NeuroD1 and Ngn2 were used predominantly in human iN protocols and not in rodent protocols, but no mouse-specific or human-specific TF combinations have been established thus far [6,25]. Further, in some iN studies, the age of the human donor has been negatively correlated with the percentage of iNs obtained [38], and fibroblasts from adult human donors are resistant to Ascl1/Brn2-based conversion, while fetal fibroblasts are highly amenable [38]. RE1-silencing complex (REST), a major neuronal gene repressor in non-neuronal cells, and the aging-associated TF FOXO3 play important roles in controlling neuronal gene expression and show differential activity between fetal and adult/old fibroblasts, resulting in decreased conversion efficacy in aged starting cells [38–40]. Again, the combination of the two pioneer factors Ascl1 and Ngn2, for example, fused via a 2A peptide sequence, has yielded iN efficiencies of typically over 50% across large sample sizes and does not appear to be affected by donor age [9,10,35]. In this setting, however, the presence of a cocktail of small-molecular iN boosters might further mask an age-related inhibitory effect [31,41].

While pioneer TFs are sufficient for iN conversion, they are not essential and several laboratories have successfully obtained iNs without exploiting the classical pioneer TFs (Table 1). Those studies have harnessed the fact that neuronal genes are actively repressed in non-neuronal cells and manipulated this mechanism. Activity of the 'anti-neuro' REST complex is strongly supported by PTB proteins, and PTBs are key targets of the pro-neuronal microRNAs miR-9/9*-124. The PTB-REST-miR-9/9*-124 ensemble is so powerful that mere interference with it, through shRNA-mediated knockdown of PTBs or components of the REST complex or through overexpression of miR-9/9*-124, can replace pioneer transcription factors (Fig. 1A) [38,42–45]. Similar to Ascl1 alone, also shRNA-only and miRNA-only iN protocols are quite inefficient and rely on several 'helper' factors (Fig. 1B) [31,46,47]. For example, overexpression of Myt1l alone is insufficient to induce iNs from fibroblasts, but Myt1l remains one of the most valued iN 'helpers' in various protocols.

Genetic iN boosters

Among the growing number of successful iN-conversion protocols, two main strategies have crystalized that facilitate increased conversion efficiencies and more authentic neuronal outcomes: (a) co-overexpression of pioneer TFs with boosting TFs, and (b) combination of pioneer TFs with media containing cocktails of signaling-pathway modulators (Fig. 1C).

The first published direct conversion strategy was based on an overexpression of the three TFs, namely Ascl1, Brn2, and Myt1l (BAM factors), in mouse fibroblasts [6], which was then extended to BAM with NeuroD1 to convert human fibroblasts to iNs with a similar efficacy [25]. Later studies elaborated on the first paper [10], where it was shown that Ascl1 alone is enough to mediate fibroblast-to-neuron conversion, whereas Brn2 and Myt1l boost conversion efficacy and neuron quality, but are unable to induce reprogramming on their own [31,32,48,49]. Myt1l is a master repressor of non-neuronal genes and a prime example for a boosting TF (Fig. 1A,B) [50]. Albeit lacking pioneer activity, co-overexpression of Myt1l with pioneer TFs dramatically increases iN yields and improves functional properties of iNs in direct conversion protocols, especially those that otherwise fail to yield satisfactory neuronal numbers and properties [6,25,46]. Myt1l can be regarded as the nemesis of the REST complex as it broadly represses non-neuronal fates, such as the myogenic fate that has been identified as a common false diversion of converting cells on their way toward iNs [11,48,51]. Unlike Myt1l, the boosting TF Brn2 acts as a classical activating secondary TF that binds to genomic regions that open in response to pioneer TFs [32]. While not sufficient to induce neurons from fibroblasts, Brn2 alone can convert astrocytes into neuronal cells, indicating some pioneering properties (Fig. 1A) [52]. In sharp contrast to pioneer and secondary TFs, mutations in Myt1l and Brn2 are associated with subtler neurological phenotypes linked to intellectual disability and neuropsychiatric conditions in humans [53–56], whereas the pioneers Ascl1 and Ngn2 are well-established master regulators of nervous system development, the mutation of which causes embryonic lethality.

Besides TFs, miRNAs have also been harnessed as iN boosters, and REST inhibition or overexpression of REST-associated miRNAs could highly enhance conversion of adult and diseased fibroblasts in pioneer TF-based settings [38,57]. Furthermore, in keeping with an active cell cycle being a major roadblock to iN-conversion initiation, G1 arrest, for instance, achieved through high-density contact inhibition, is

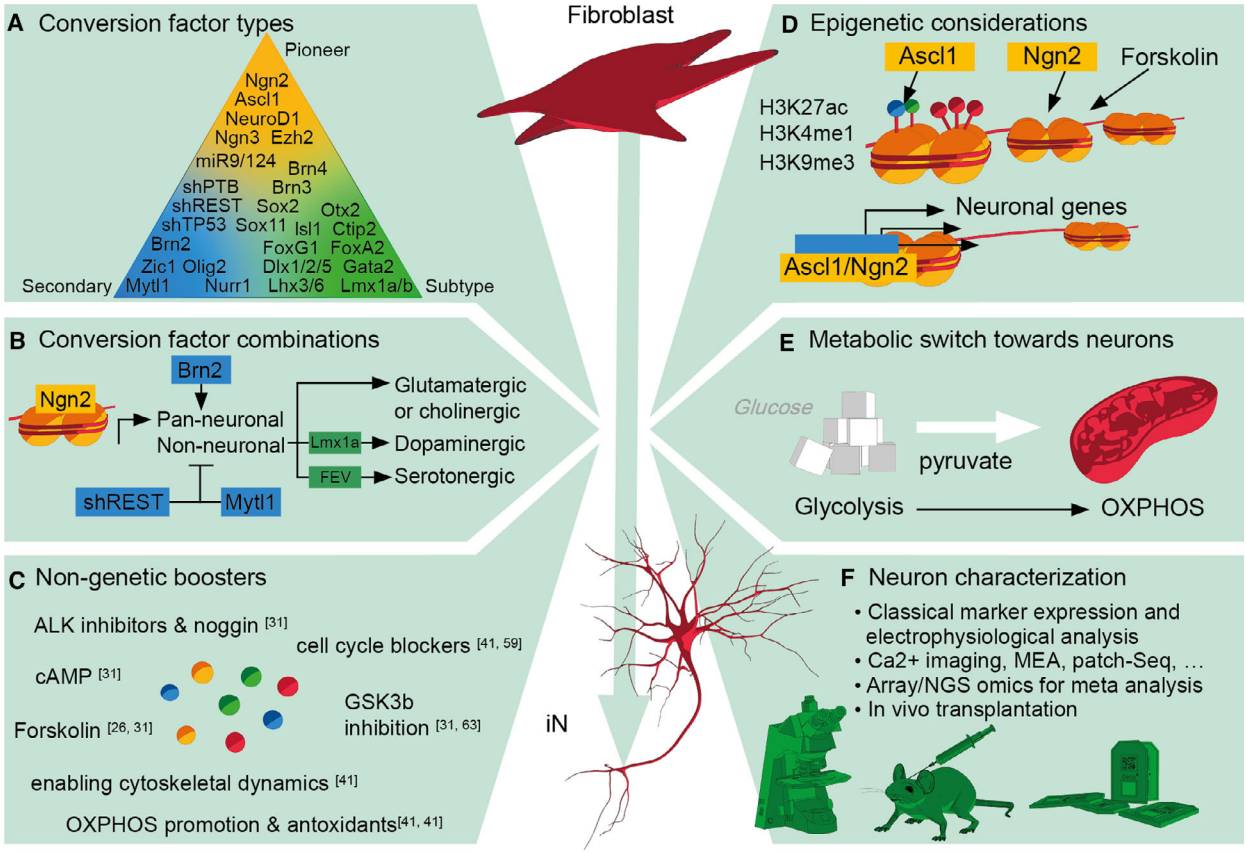

Fig. 1. (A) Factors for direct iN conversion, including transcription factors (TFs), can be classified into pioneer and secondary factors. The TFs Ascl1 and Ngn2 are the two most widely used pioneer factors that can facilitate iN conversion on their own. Secondary factors do not induce conversion on their own and are instead used to achieve increased efficiencies and neuronal qualities. Myt1l is a prime example for a secondary TF, while miR-9/124 and shRNAs against REST or PTB have neuron-inducing capabilities and can be regarded as 'in-between' pioneer and secondary factors. As pioneer factors typically do not (strongly) dictate subtype identity, subtype-specific secondary factors can be added to induce a desired neuronal subtype. Some factors primarily regarded as subtype-specifiers, such as Nurr1, Sox11, and Brn3/4, also display considerable iN boosting efficiencies. (B) First, a pioneer factor induces a broad neuronal transcriptional program, a process that benefits from secondary factors that can help induce a neuronal program either by transactivation activity (e.g., Brn2) or by repressing the non-neuronal program (e.g., Myt1l and REST inhibition). Once a broad epigenetic neuronal context is established, subtype-specific secondary factors (e.g., Lmx1a and FEV) can direct iN toward specific epigenetically stable subtype identities. (C) Nongenetic boosters of iN conversion are used to increase efficiencies and to obtain iNs with better neuronal qualities faster. Typically, chemical boosters are small molecules that block or activate signaling pathways involved in direct conversion or that are known to benefit neuronal differentiation, maturation, or survival. (D) Pioneer TFs can bind and open up closed chromatin regions that are essential to initiate and jump-start iN conversion. However, even pioneer TFs require specific epigenetic marks in order to bind closed chromatin (e.g., trivalent state for Ascl1), and iN boosters (e.g., forskolin) have been found to be directly involved in chromatin remodeling to permit more efficient iN conversion. (E) A radical metabolic switch from glycolysis (the primary source of energy for stem cells and fibroblasts) toward mitochondria-based oxidative phosphorylation (OXPHOS) is a major obstacle for neuronal conversion and iN survival. Enzymatic activity of LDHA (pyruvate to lactate) and reactive oxygen species (a side product of OXPHOS) prohibits iN conversion, whereas promotion of OXPHOS and antioxidant activity enhances iN conversion. (F) Neuronal identity can be assessed using neuronal marker expression or electrophysiological properties, but can be further characterized more profoundly with next-generation transcriptomic, epigenetic, or metabolic analyses.

beneficial for conversion initiation. Consistently, knocking down TP53 could enhance conversion efficacy by decreasing the number of fibroblasts in S phase at the start of neuronal induction [58]. In general, identification and mechanistic characterization of boosting transgenes may not only yield important insights into the process of direct conversion, but also might help to engineer tailored conversion TFs and vectors in the near future [41,59]. However, co-overexpression of multiple TFs is a technical challenge and always harbors the risk to induce bias. For example, TF activity might override important disease-related

Table 1. Direct neuronal conversion strategies.

Cell source	Species	Conversion strategy	Specification	Citation
Fibroblasts	Mouse	**Ascl1**, Brn2, Myt1l (BAM)	First direct conversion from fibroblasts	[6]
		Ascl1 +/− Brn2, Myt1l		[24,32,38,48,49,51]
		B**A**M	Mesoporous silica nanoparticles, dopaminergic	[174]
		B**A**M	CRISPR-based	[59]
		B**A**M	Electroporation, 3D	[175]
		Small molecules		[15,176]
		Ngn2		[33]
		Ascl1, Foxg1, Sox2, Dlx5, Lhx6	GABAergic	[83]
Fibroblasts	Human	B**A**M + **NEUROD1**	First direct conversion from human fibroblasts	[25]
		B**A**M	First direct conversion from adult human fibroblasts	[177]
		ASCL1, NGN2		[9,10,31,34,41]
		ASCL1, NGN2, NKX2.2, FEV, GATA2, LMX1B	Serotonergic	[35]
		ASCL1, FOXA2, LMX1B, FEV	Serotonergic	[75]
		ASCL1, NURR1, LMX1A	Dopaminergic	[178,179]
		ASCL1, PHOX2B, PHOX2A, AP-2A, GATA3, HAND2, NURR1	Noradrenergic	[180]
		ASCL1, KD of P53, HEY2, and PRPX2		[181]
		NGN2/1, BRN3	Sensory neurons	[76]
		Small molecules		[15,182]
		BRN2, MYT1L, FEZF2	Cortical neurons	[81]
		miR-9/9*-124, BCL11B/CTIP2, DLX1, DLX2, MYT1L	Striatal neurons	[7,57]
		miR-9/9*-124 +/− B**A**M		[44,46,47,183]
		miR-9/9*-124, ISL1, LHX3	Motor neurons	[79]
		PTB-KD		[42]
Glia	Mouse	**NeuroD1**		[27]
		NeuroD2	*In vivo*	[184]
		Dlx2	GABAergic neurons	[185]
		Ngn2	*In vivo*	[185]
		Ascl1, Lmx1a, miR-218	*In vivo*, parvalbumin interneurons	[82]
Astrocytes	Mouse	**Ascl1**	*In vitro*/*in vivo*	[64,186]
		Ascl1, Lmx1b, Nurr1	Dopaminergic	[179]
		Ascl1, Phox2a, Phox2b, AP-2a, Gata3, Hand2, Nurr1	Noradrenergic	[181]
		Ascl1, Sox2, Neurod1		[187]
		Ngn2	*In vivo*	[113,185]
		Ascl1, Ngn2	Varying iN subtypes	[73]
		NeuroD1		[80,184]
		Small molecules		[188]
	Human	**NEUROD1, ASCL1,** LMX1A, miR-128	Dopaminergic neurons	[80]
		ASCL1	PTB-mediated	[189]
		Small molecules	Fetal astrocytes	[118,190]
		Small molecules	Adult astrocytes	[191]
Retina	Human	**NEUROD1,** PAX6, BRN2, MYT1L		[192]
Pericytes	Human	**ASCL1**, SOX2		[69]
		ASCL1, MYT1L, BRN2, TLX3, miR-124	Cholinergic	[193]
T cells	Human	B**A**M + **NGN2**		[91]
Cord blood cells	Human	FOXM1, SOX2, MYC, SALL4, STAT6		[194]
Hepatocytes	Mouse	B**A**M		[195]
		Suz12, Ezh2, Meis1, Sry, Smarca4, Esr1, Pparg, Stat3		[194]

Bold font indicates pioneer transcription factors (also see Fig. 1A).

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signatures in iN models for neurological disorders [60]. Conceivably, it is a general desire in the field to reduce the number of conversion TFs toward a minimal combination with the maximal effect. Driven by this idea, the alternative use of pathway modulators that repress the identity of the starting population and/or promote neuronal identity represents another strategy to boost iN efficiency and authenticity.

Nongenetic iN boosters

Both purified and recombinant proteins and chemical compounds have been identified to enhance iN generation and have further been helpful to better understand the mechanisms of direct iN conversion (Fig. 1C). Soluble factors are often described to mechanistically 'hit the same spot' as conversion TFs, and thus can replace certain activities of the transgenes. First, and largely adopted from neural differentiation protocols, inhibition of TGF/ALK/SMAD signaling through the application of recombinant Noggin and small-molecule ALK inhibitors, GSK3β inhibition, and cAMP/forskolin have been quickly identified to greatly enhance conversion yields and aid to obtain more authentic neurons (Fig. 1C) [26,31,61,62]. Interestingly, while TGF/ALK/SMAD and GSK3β inhibition is thought to destabilize non-neuronal identities and promote neuronal fate-stabilizing signaling [61], forskolin was shown to directly prime chromatin accessibility for more efficient Ngn2-only conversion, a pioneer-enhancing activity previously ascribed exclusively to TFs (Fig. 1D) [33]. Subsequently, it has been shown that additional pathway modulations, including SIRT1 activation and HDAC inhibition, can be applied to increase the efficacy and maturity of the neuronal population obtained [62]. Next, the cell cycle arrest-promoting activity of Ascl1 and Ngn2 can be enhanced by knocking down *TP53*, serum withdrawal, CDK2 and mTOR inhibition, and chemical JAK/STAT inhibition, which further helps iN conversion by inhibiting both epithelial-to-mesenchymal transition and apoptosis [41,58,63]. Enabling cytoskeletal dynamics through inhibition of integrin signaling facilitates the cell-type switch [41]. Inhibition of HIF-1α improves iN conversion as it promotes the metabolic switch from glycolysis to mitochondria-based oxidative phosphorylation, which is essential for neuronal identity [41,64,65]. While nongenetic boosters are expected to be less invasive than transgenes, they often also possess very powerful neuroprotective functions and thus might, similar to TFs, mask important disease- and epigenetic age-related signatures in iN-based disease models [41].

Subtype-specific iN conversion

In the developing brain, as well as in iPSC differentiation, differentiating neural cells pass through a series of well-defined developmental neural precursor stages, some of which are amenable to regionalizing patterning signals to specify specific neuronal subtypes [66]. In direct iN conversion, these specialized precursor stages are instead skipped [48,67]. Thus, neuronal subtype specification cannot be easily achieved through the addition of patterning factors such as Wnt or Shh in the media, because the responsive cell type is not present at any time point during conversion. The most prominent conversion strategies using BAM, Ascl1/Ngn2, and miR-9/9*-124 typically give rise to a major population of excitatory glutamatergic neurons, and the Ngn2-only protocols lead to excitatory cholinergic neurons (Fig. 1B) [25,26,31]. While these observations somewhat imply a glutamatergic-by-default mechanism, or alternatively have led to the assumption that Ascl1 and Ngn2 are pro-glutamatergic and pro-cholinergic, respectively, reality appears less straightforward. For example, iNs generated from iPSCs through Ngn2-only protocols are predominantly glutamatergic [16,68]. Ascl1/Sox2-based iNs generated from pericytes *in vivo* resemble mixed GABAergic/glutamatergic cultures [69], and Ascl1 has been shown to induce oligodendroglial cells from adult neural stem cells in the dentate gyrus of mice [70]. Also, both TFs are involved in midbrain and hindbrain neuronal differentiation and have varying capacities depending on the regional context [71,72]. Consistently, Ascl1/Ngn2-based conversion of human fibroblasts into iNs leads to a major fraction of glutamatergic iNs, a smaller fraction of GABAergic iNs, and rare dopaminergic and serotonergic cells [10,31,35]. Based on these observations, one might describe Ngn2 and Ascl1 as generally 'pan-neuronal', with little subtype-specifying activity (Fig. 1A). The epigenetic identity of the starting cell population and remnant signaling cues present during the fibroblast-to-iN transition state finally determine subtype identity [23,34]. In highly heterogeneous cell populations, such as astrocytes in the rodent brain or primary human fibroblasts, the subtype of the starting cell type was shown to determine subtype outcomes of the iNs [73]. Both Ngn2 and Ascl1 seem to leave significant 'wiggle room' for subtype specification, which suggests addition of subtype-specifying TFs to the mix for direct iN conversion into the neuronal cell type of interest (Fig. 1A,B). Depending on the combination of the pan-neuronal pioneer TFs with subtype-specific TFs, specific neuronal subtypes with distinct neurotransmitter and channel properties arise,

providing a unique platform for studying specific cells [7,36,74]. This concept has attracted attention beyond the crowd of reprogramming enthusiasts, as it allows generating specific neuronal subtypes that are specifically vulnerable, or resilient, to certain diseases. With the recent advances in subtype-specific direct conversion, various disease-specific neuronal subtypes have become available, such as medium spiny neurons for modeling Huntington's disease (HD) [7], serotonergic neurons for modeling depressive and anxiety disorders [35,75], sensory neurons for studying pain-related diseases [76], motor neurons resembling an amyotrophic lateral sclerosis (ALS)-related phenotype [77–79], dopaminergic neurons to model Parkinson's disease [58,62,80], or cortical [81] and various types of interneurons that appear attractive to model, for example, Tourette's syndrome or paroxysmal dystonia [82,83]. While these advances clearly offer novel tools to better understand neurological diseases, there is very strong evidence that classical neuronal diseases do not only affect neurons, but involve many cell types represented in the human brain [84]. To meet this need to model the human neuron–glia cross talk *in vitro* with patient-specific cells, astrocytes and oligodendrocytes are currently generated via direct conversion from human iPSCs, but direct conversion protocols from fibroblasts into induced astrocytes and oligodendrocytes have yet only been established for rodent cells [85–89]. As an alternative route, direct conversion into induced neural stem cells, which then are amenable to directed 'development-mimicking' differentiation, represents another way to generate glial cell types from human fibroblasts [reviewed in Erharter *et al.*]. Further, peripheral Schwann cells are of great interest for the study and treatment of spinal cord injuries and have been generated from adult human fibroblasts, representing exciting new possibilities for peripheral nerve regeneration [90]. In conclusion, the number of protocols for neuronal subtype-specific direct conversion literally exploded in the last couple of years, and we now have the means to generate a vast variety of neuronal subtypes *in vitro*. The next important steps in the near future would be the development of protocols for the generation of induced glial cell types also from human somatic cells and extension of existing protocols to other, more accessible starting populations, such as human peripheral blood cells [91].

The road toward neurons: a steep and sloppy path?

The famous epigenetic landscape model established by Waddington is an outstanding model to describe cell fate switches during normal development and iPSC differentiation [30,92,93]. Using this model, direct conversion has been depicted as a direct path from one valley to the other, straight over the highest mountains [93]. However, given the apparent easiness of direct conversion and the fact that transition states appear unidirectional and with little developmental potency, a 'tunnel' metaphor might appear more useful. Also, the inherent emphasis on hierarchy in the Waddington model appears less suitable to describe direct conversion and further seems to fall short of describing the roles of stable (quiescent) intermediate and progenitor cell stages during development and plasticity of cell fate in response to external stimuli. As a result, adjusted metaphors such as a highly dynamic epigenetic landscape [94], the Cook island model [95], or the epigenetic disk model [96] have been introduced, all of which attempt to weaken the one-way hierarchal character of the Waddington landscape. All these models have in common that cellular states correspond to valleys or holes, indicating that the epigenome favors certain stable states that correspond to cell types that exist *in vivo* at any given time, but rejects cellular chimeras, which would be cells that transiently share neuronal and starting cell properties, and which are rarely described [48,67].

To explore the differences between the transcriptome path and outcome of stem cell differentiation and direct iN conversion, an elegant study employed single-cell transcriptome analysis of neurons generated through neural stem cell differentiation and direct conversion of neural stem cells into iNs [67]. Interestingly, iNs diverge from the differentiation path early on and do not follow the precise intermediate states of development, but generate a unique intermediate state that is unrelated to the donor and the target cells. However, after this 'shortcut', iNs appear to arrive at the same state as neurons obtained by differentiation, but without losing epigenetic information about age and disease.

Developmental and differentiation protocols are expected to roughly follow similar epigenetic sequences. Starting from somewhere between the zygote and blastocyst stages in development or from embryonic stem cells or iPSCs in differentiation protocols, modifications to the epigenetic landscape follow a hierarchical and highly orchestrated sequence of events that ultimately lead to a differentiated cell [97,98]. To initiate iN conversion however, the already defined and comparably inaccessible chromatin of the starting cell has to be targeted by an overexpressed pioneer TF capable of binding and activating closed chromatin regions [24,28]. Once chromatin remodeling is induced

and the neuronal transcriptional program is started, non-neuronal programs have to be suppressed. In contrast to the very time-consuming and precisely timed process of differentiation, direct conversion induces a neuronal program around three to five days after transgene expression, which can lead to functional synapses already at around three weeks postinduction [6,48]. Converting cells from one germ layer to another, for instance, from mesoderm (fibroblasts) to ectoderm (neurons), requires substantial chromatin remodeling to make target-cell-type-specific genes accessible to TFs. Such changes of cellular identity and the absence of a defined progenitor cell-type stage indicate that iNs do not follow the typical developmental path from stem cell to postmitotic neuron, but rather represent a jump start of a then self-propelled neuronal program [33,41,48,91,99]. In recent years, several studies performing transcriptomic and epigenomic analysis on converting neurons gave rise to new insights and concepts that characterize fibroblast-to-neuron conversion. It has been found that pioneer TFs can be classified as either off-target or on-target TFs. Off-target TFs, such as the Yamanaka factors during iPSC reprogramming, initially bind to target sites in the starting cell type, but, during the process of reprogramming, change their binding sites and end up binding different sites in the reprogrammed cell [100,101]. By contrast, iN-conversion TFs are typically on-target TFs, like Ascl1, Ngn2, and NeuroD1, as they bind specific chromatin regions to induce transdifferentiation and stay at those sites also in the converted iNs, unless turned off in doxycycline-inducible systems [24,27,33,35]. As iN conversion is more efficient than iPSC reprogramming by orders of magnitudes, the difference between off-target iPSC reprogramming and on-target iN conversion has been suggested to underlie the huge difference in reprogramming efficiency. Still, due to epigenetic differences, not every on-target pioneer TF is equally potent in every starting cell type, as, for example, Ascl1 is able to induce conversion of fibroblasts, but not keratinocytes, into iNs [24,30]. This has been somewhat surprising, since the overall less efficient iPSC reprogramming TFs are known to equally reprogram a broad variety of somatic cells into pluripotency [102]. This is explained by the fact that on-target pioneer factors require a more specific epigenetic signature in order to bind to closed chromatin and preexisting histone modifications like a trivalent chromatin state of H3K4me1/H3K27ac/H3K9me3 in fibroblasts, which are one reason why Ascl1 can induce direct iN conversion only in fibroblasts (Fig. 1D) [24].

In fibroblast-to-iN conversion, Ascl1 exerts its function already a few hours after induction, acting as a transcriptional activator inducing neuronal- and muscle-related gene expression [32]. Until day 5, Ascl1 alone is responsible for 80% of chromatin changes occurring during the whole reprogramming process, leading to an upregulation of genes involved in neuronal processes, neuronal network formation, and early genes of neuronal maturation [32]. Downstream of Ascl1, TFs like Zfp238 additionally influence the expression of genes involved in chromatin remodeling, including methylases [24,103]. Some iN protocols that are not quite efficient can result in cells that lack epigenetic marks of mature neurons [48,51]. Modifying the epigenetic landscape, for instance, with the help of small molecules like forskolin, or overexpressing additional factors like Brn2 and Myt1l, aids to efficiently generate mature neurons [33,48,51]. The importance of methylation remodeling in neuronal maturation is further supported by the inability to generate mature neurons upon knockout of genes involved in histone methylation, which control accessibility to genes involved in synapse formation and neuronal function [103]. Another milestone achievement in epigenetics has been mouse microglia-to-iN conversion [27]. Here, the pioneer TF NeuroD1 binds unmethylated CpG-rich regions and rearranges the bivalent H3K4me3/H3K27me3 state toward a monovalent H3K4me3 state during conversion. Interestingly, the secondary TFs Prdm8, Bhlhe22, and Brn2, which are direct target genes of NeuroD1 in microglia, can also facilitate microglia-to-iN conversion by either inducing neuronal gene expression (Brn2) or repressing microglial genes (Bhlhe22, Prdm8) [27]. Further, cell type-specific epigenetic characteristics that pose a hurdle for direct conversion from keratinocytes also hinder conversion of human cardiomyocytes, which have been shown to only marginally convert into neurons with BAM/NeuroD1 [104]. Similarly, the development of robust protocols for iN generation from stored human blood samples has been as much anticipated as it has been challenging. In a proof of principle, adult human peripheral blood mononuclear cells have been successfully converted into functional iNs using electroporation of BAM/NeuroD1 [91], which is a less efficient, but also a less immunogenic TF delivery method than viruses. Further, albeit the use of well-selected small-molecular boosters and coculture with primary mouse glial cells, blood-to-iN conversion in comparison still faces low efficiencies [91].

You are what you eat: Metabolic hallmarks of iN conversion

Originally regarded more as a mere characteristic of neurons, a growing body of evidence now suggests a central role for metabolic regulation in differentiation and direct iN conversion [95,105]. Following the initiation of neuronal transcriptional programs, converting cells are forced to adapt neuronal metabolism (Fig. 1E) [9,106,107]. Compared to fibroblasts or astrocytes, neurons rely heavily on oxidative phosphorylation to meet their high demand of energy [108–110]. The metabolic switch from aerobic glycolysis to neuron-specific oxidative phosphorylation is crucial for the generation of neurons. Inhibiting oxidative phosphorylation or overexpressing glycolysis genes such LDHA or HK2 results in diminished neuronal differentiation [65]. While during neural stem cell differentiation cells can slowly adapt to the metabolic switch, direct conversion forces fibroblasts to rapidly switch to oxidative phosphorylation, resulting in increased oxidative stress [64]. This observation has led to the view that the metabolic switch represents a major roadblock in direct conversion as it triggers cell death in more than 80% of transgene-induced cells [64]. This vast amount of stress-induced cell death during conversion could be prevented by either overexpressing anti-apoptotic protein Bcl-2 or adding molecules implicated in the anti-oxidative stress response, which further resulted in a more effective and faster conversion from astrocytes [64]. In consistence with the view that the metabolic shift from fibroblasts to neurons represents a major roadblock for successful conversion, chemical derepression of oxidative phosphorylation using the Hif-1α translation inhibitor KC7F2 during conversion leads to increased mitochondrial membrane potentials and more robust and efficient iN conversion from adult and old human donor fibroblasts [41].

Updating our criteria to define neurons

'Well well well, so you want to be a *real* neuron…'. Since the first neurons were generated from human embryonic stem cells, an evergreen question has been if obtained cells should be called neurons, or if they should rather be described as neuronal-like cells. While some argue that human stem cell-derived neurons should be called a neuron once they meet a set of electrophysiological characteristics, others suggest to rather call them neuronal-like cells to avoid confusion with primary neurons [111]. While the question of naming seems to be merely of interest to nomenclature aficionados, it is out of the question that it is vital to possess a wide range of relevant criteria, as well as a well-stocked toolbox to measure a cell's neuronal identity. While reprogrammed neurons might always be distinguishable from *in vivo*-born cells, recent work has demonstrated that the environment of cells appears to be more important for cellular identity than the origin of cells, indicating that cell identity is plastic and highly environment-dependent [112,113]. Further, unlike for most other human cell types, primary human neuronal cultures of defined brain regions are not available for direct comparison, which further complicates the definition of gold standard criteria. By any means, the value of cell models should be measured by how well they perform their respective tasks, and these tasks might range from teaching us more about a complex disease, or how well they integrate into a neuronal circuit following transplantation [6,33–38,114,115].

With the establishment of (epi)genomic sequencing and epigenetic array technologies, single-cell technologies, and new insights into the complexity of neurological diseases, the historic neuron-electrophysiology-centric view on neuronal identity, function, and disease is fading [35,51,69,116]. Thus, also a broadened set of criteria on how to characterize a neuron using such new omics technologies in addition to the classical cell biological and electrophysiological measures is uprising (Fig. 1F). Still, the most common way to assess neuronal cultures is to perform immunocytochemical analysis with markers like β-tubulin, MAP2, DCX, synapsin I, or tau and to assess neuronal morphology according to soma size and number and length of dendrites, as it is standard practice in the stem cell field [15,41,81,97,117]. Additionally, electrophysiological analysis has proven valuable to define functional neurons, as neurons have very specific electrophysiological features like hyperpolarized membrane potential and spontaneous and triggered action potentials [32,46,49]. For many studies that are not neuronal function-centered, Ca^{2+} imaging and multielectrode arrays can be used as surrogates for electrophysiology and can further deliver insights into neuronal network activity *in vitro* [20,21,119,120]. In this regard, iNs have been shown to express neuronal markers, show mature neuronal morphology, and possess both spontaneous and induced postsynaptic currents [15,57,81,118,119]. Differentiating neurons in the developing brain and in iPSC differentiation pass through stages that specifically allow them to participate in functional circuits [121], while iNs skip these stages. This raises the question as to whether directly converted neurons have the neuron-specific ability to create and integrate into a

neuronal network. It has been shown that iNs, despite following a different developmental path than neurons in the brain, can survive and differentiate after transplantation into the mouse brain or cultivation on organotypic brain slices [57,81]. Outstandingly, iNs are capable of long-distance axonal outgrowth, following axonal growth cues specific for the neuronal subtype and connecting with target brain regions, providing evidence of a stable conversion of cells into functional, fully differentiated neurons [57].

While classical cell biological and electrophysiological criteria are a gold standard for characterizing such cells in culture, and transplantation experiments have a strong relevance and broad implications, they do not tell us much about more general measures that define cell identity. Neurons do not only have unique electrical properties, but also have unique epigenetic, proteomic, metabolic, and other characteristics that might be equally important for the roles they play in the brain. While marker expression and electrophysiological characteristics often correlate with cell identity, they are considered to be downstream of epigenetic and proteomic changes and likely follow earlier pathogenic events in neurological diseases. Immunocytochemical data are further quite variable between experiments, antibody batches, and laboratories, thus not well-quantifiable, low-dimensional, and thus less ideal to be directly compared by statistically powerful (meta-)analytical means. Conversely, gene expression changes related to synapse formation, voltage-gated potassium channels, or mitochondrial oxidative phosphorylation critically define mature neuronal identity [122], and transcriptomes of single human iPSC-derived neurons can clearly predict neuronal functionality [123]. As epigenomic, transcriptomic, proteomic, and metabolic profiles of human brain samples are emerging, we can now use these data and map iN and iPSC-derived data to them. A prime source for these data is transcriptome databases of the adult and developing human brain [124–126], which have been exploited by stem cell scientists to learn more about the underlying identity of cultured neurons. For example, Stein *et al.* [127] developed a machine learning approach that can help to identify the developmental maturity and regional identity of *in vitro* models, which has detected marked differences between laboratories and differentiation protocols. Nayler et al. [128] have compared the transcriptomes of iPSC-derived cerebellar neurons to the Allen Brain Atlas to demonstrate that their cells are transcriptionally similar to discrete regions of the human cerebellum at the second trimester of development. Camp et al. [129] compared single-cell transcriptome data derived from iPSC cerebral organoids to *in vivo* data and found that the cells recapitulate gene expression trajectories that correspond specifically to human fetal neocortical development. This concept was later extended toward new insights into cellular diversity, once also *in vivo* single-cell data of the developing human brain became available [130–133]. In the iN field, Vadodaria *et al.* [35] used a transcriptome comparison to confirm serotonergic iN identity, as they found a high similarity of serotonergic iNs to the transcriptomes of the human raphe nucleus. While these types of analysis should not be considered to fully replace marker expression and functional analyses, they are highly complementary and clearly add additional value.

Further, biological or epigenetic age can be well measured using the DNA methylation profile exploiting a growing number of epigenetic clock algorithms [134–136]. The original epigenetic clock used the methylation values of 353 specific CpG loci to calculate the age of a human sample, and Huh *et al.* have employed this tool to demonstrate for the first time that iNs from adult donors are indeed (epigenetically) adult cells, while iPSCs and their derivatives are typically rejuvenated into prenatal epigenetic ages [134,137,138]. Further along epigenomic criteria, and in contrast to CpG methylations, non-CpG methylation (mCH) marks are a typical characteristic of neurons [139,140]. In mature neurons, mCH marks are accumulated at particularly high levels, which was not observed in any other tissue before [139]. mCH marks play an essential role in cell type-specific fine-tuning of transcription, being responsible for dynamic expression patterns during early differentiation, and later represent stable repressors in mature neurons [51]. These methylation marks are strong indicators of mature neurons and may further contribute to the vast diversity of neuronal subtypes in the human brain [140,141]. Luo *et al.* recently showed that in contrast to iPSC-derived neurons, BAM-based iNs are the first cellular neuronal model system displaying this epigenetic hallmark of mature adult neurons [51,142,143]. Overall, as neurons are generally defined by their marker expression and electrophysiological properties, the ascent of next-generation sequencing methods has allowed to carefully characterize iNs according to their gene expression profile and epigenetic landscape, representing more detailed and reliable validations. We expect such criteria to become more and more popular, extending our knowledge of cell identity, and that they will eventually largely replace classical means of neuronal characterization.

The direct path to the end: Specifics of iN and their consequences for disease modeling

It is primarily due to the inaccessibility of live human brain tissue that most studies on complex age-related neurodegenerative disorders have primarily relied on transgenic animal models that, while yielding important insights, have also revealed limitations regarding transferability to human physiology. *In vitro* generation of patient-specific neurons from iPSCs for modeling diseases of the brain has evolved into an integral part of neuroscience [144–146], and employing human iPSC technology to investigate aspects of age-related neurodegenerative diseases in a patient-specific genetic context at a cellular level has yielded important human neuron-specific insights [147–150]. However, iPSC-based studies could not shed much new light on the causes of sporadic age-related diseases, because iPSC reprogramming is known to reset the epigenetic state of the cell and erases most of the epigenetic memory, including those that stem from potentially important environmental influences (Fig. 2) [10,151–156]. The rejuvenation effect of iPSC reprogramming is a major drawback when attempting to model late-onset diseases [17,157,158], and artificial induction of the factor age by overexpressing progerin [151], shortening of telomeres [159], or exposing cells to age-related stressors [12,160] is an upcoming and widely used strategy to elicit a relevant phenotype in iPSC models for neurodegenerative diseases [12,148,161–163]. Direct iN conversion of human fibroblasts from elderly human patients and healthy donors into iNs circumvents this issue and has attracted broad attention. Old human donor-derived iNs show stark transcriptomic signatures of aging, as well as nuclear pore and transport-associated aging [10,164], metabolic and mitochondrial aging [9,137], epigenetic aging [137], and other aspects of cellular aging (Fig. 2) [12,17,157]. Human iNs thus stand out as a highly attractive patient-specific model system for age-related neurological diseases and thus significantly extend the possibilities offered by iPSCs. For example, while iPSC-derived neurons from genetically defined amyotrophic lateral sclerosis (ALS) or Huntington's disease (HD) patients are a promising model for investigating these devastating diseases [165–167], both HD and ALS show an adult-onset pathology that is also influenced by aging. Because iPSC-derived neurons have a fetal developmental identity and because age-related cellular defects are erased, the study of a disease-related pathology in these cells is challenging and external stressors are typically used to provoke phenotypes [167–169]. iNs, however, bypass these stages of fetal development, preserve disease-relevant defects associated with cellular aging, and recapitulate disease-related phenotypes including morphological, survival, and functional defects (Fig. 2) [77]. The key advantage of such cellular models over postmortem brain tissue is that they permit to experimentally test cause–consequence relationships via functional studies, and to evaluate molecular intervention with potential drug-like molecules.

Importantly, the rejuvenating effect of iPSC reprogramming should mostly be regarded as an additional advantage, because comparing phenotypically young and phenotypically old neurons from the same donor (isogenic rejuvenated control) can help to reduce donor-specific bias and allows assessing the contribution of age to the disease phenotype. Although it is a new concept, harnessing the power of combining iPSC differentiation and direct iN conversion has already been published. Tang *et al.* [157] have generated induced motor neurons, as well as iPSC-derived motor neurons from three young (0–3 years) and three old (53–71 years) healthy donors, as well as from four familial ALS patients carrying SOD1 or FUS mutations. As expected, the rejuvenated iPSC-derived motor neurons did not show age-related differences, while iN-converted, age-equivalent induced motor neurons showed nuclear envelope defects, increased signs of DNA damage/repair (γH2AX), and age-dependent deterioration of the cellular aging markers Lap2α, H3K9me3, and HP1γ, which are likely downstream of a defective nuclear envelope [151]. While this study did not assess any age-related differences between patient- and control-derived induced motor neurons, an impressive study by Victor *et al.* [7] demonstrated the importance of modeling old age as a disease-relevant factor in a model consisting of both iPSC-derived and fibroblast-derived medium spiny iNs. Mutated huntingtin protein spontaneously aggregated in HD iNs, but not in control iNs, HD fibroblasts, or HD iPSC-derived neurons. In consistence with the observation that HD iPSC-derived neurons need external stressors to display this disease phenotype, the authors showed that an age-related collapse in proteostasis triggered huntingtin aggregation in an age- and repeat-length-dependent manner [7]. In the context of a multiple hit theory of age-related diseases, where at least one essential 'hit' is old age and other 'hits' are of either genomic or environmental nature, these data suggest two major contributors for HD, namely age and repeat length, that together determine onset and severity of the pathology. However, in addition to age, it appears likely that also other epigenetic signatures of the donor that relate to environmental signals are also preserved

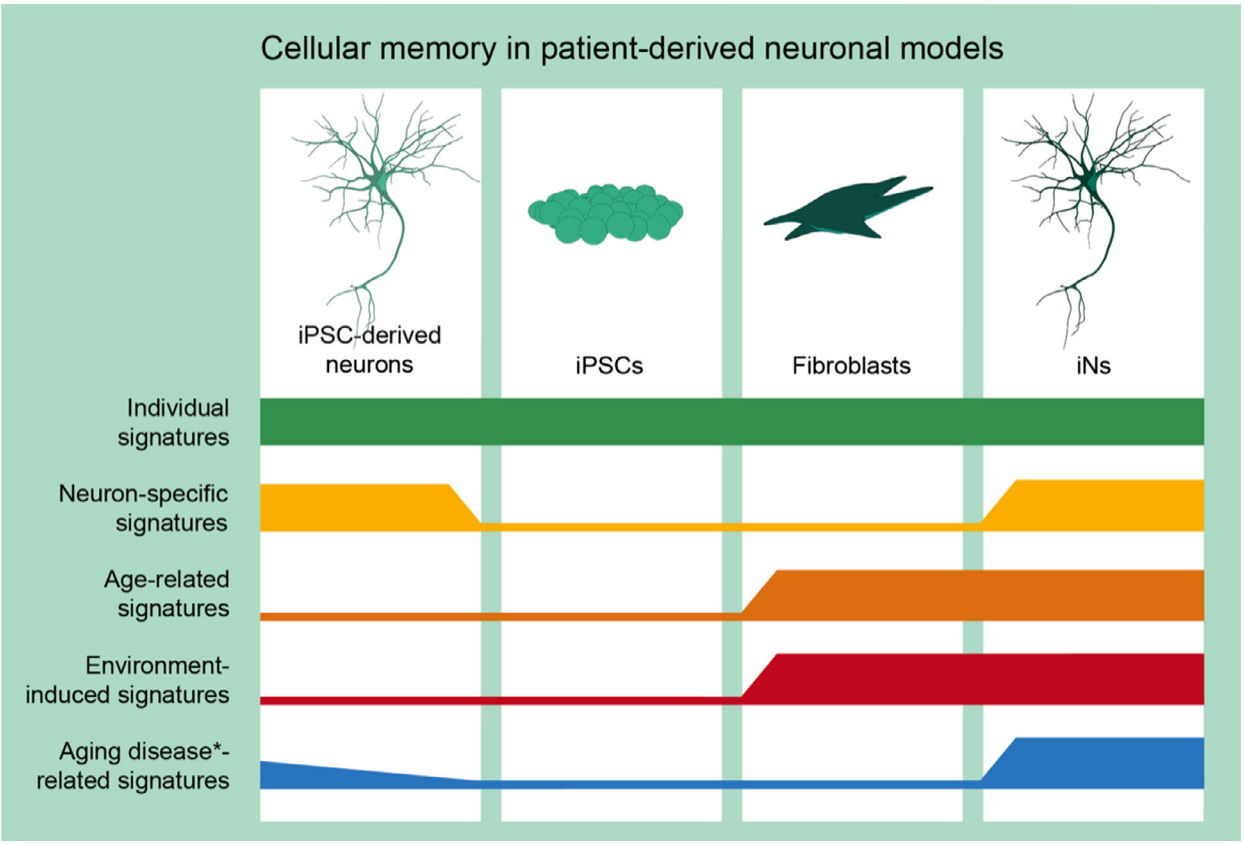

Fig. 2. Human patient-specific models are representative of the individual's genetic and epigenetic signatures. These individual signatures may vary between cell types, but are present in fibroblasts, iPSCs, iPSC-derived neurons, and iNs likewise. Neuron-specific signatures are present only in iPSC-derived neurons and iNs, but not in fibroblasts or iPSCs. Contrary to iPSC reprogramming, direct iN conversion preserves signatures of donor age and likely also captures environment-induced signatures, which might or might not be relevant for the disease model. In the context of a multiple hit theory for age-related diseases, it appears conceivable that features of such diseases might only emerge in iN models and not in iPSC-based models, because they require all the individual signatures, neuron-specific signatures, and age-related signatures to unfold in the cells. Artificial induction of age in iPSC-based models might help to elicit such features also in a rejuvenated context.

in iNs. For the resulting iN model, such environment-induced signatures might be irrelevant or even artifact-causing (e.g., if they relate to sun exposure of the skin), but they probably also involve important disease-related signatures that might be encoded within fibroblasts, but stay without stark transcriptional consequences until they are brought into a neuronal context via iN (Fig. 2).

While most studies imply that iN conversion is particularly useful for modeling age-associated diseases, direct reprogramming approach is not always the method of choice for disease modeling, especially not when it comes to developmental diseases such as autism spectrum disorder. Schafer *et al.* have used iPSC differentiation and iN conversion starting from iPSCs in parallel, and only iPSC differentiation revealed a disease-related phenotype, while iN conversion did not. Specifically, the study explored developmental transcriptomic and epigenomic trajectories in autism spectrum disorder using classical neural differentiation and cerebral organoids. As opposed to the most widely believed assumption that the first disease phenotypes emerge in immature neurons, they found heterochronic trajectories in patient cells that were already epigenetically primed for acceleration at the neural stem cell stage [16]. To pinpoint the origin of this phenotype to neural stem cells, the authors also made use of direct Ngn2-based iN conversion starting from iPSCs to skip (jump over) the neural stem cell stage and consequently found no autism spectrum disorder-related neuronal phenotypes in iNs.

There are probably more meaningful conceptual differences between iN-based and iPSC-derived models that only wait to be explored and that have the potential to significantly improve *in vitro* disease modeling in the future. For example, iPSC derivatives have been

shown to resemble fetal stages of brain development, and it remains an open question to what extent iNs from fetal, neonatal, and adult donor fibroblasts would resemble neurons of the according developmental stages. These questions must be addressed with state-of-the-art tools for defining and comparing cell identities. A fetal identity of neurons after iPSC differentiation, however, does not implicate a functionally immature cellular phenotype, as both directly converted neurons and iPSC-derived neurons can show mature neuronal markers and features [10,81,157,170].

Additionally, iNs exhibit various technical advantages as well as critical disadvantages compared to iPSC reprogramming, which have been discussed more in detail elsewhere [17]. One of the major limiting factors of direct conversion models is that the starting material is finite, and no expandable stem cell stages are involved in the process. As a result, iN cell numbers are low and the ability to scale up the system (e.g., through immortalization of fibroblasts) harbors risks of introducing artifacts that might invalidate their use as a model for aging. Thus, material-intensive technologies or big screens are challenging with iNs [171]. One practical and obvious advantage of iNs is that the procedure is faster, easier, and cheaper than iPSC reprogramming and differentiation, and many iN papers have stood out by comparatively large numbers of human donors [7,9,10,137]. Biological variability between human samples and cell lines of different genetic backgrounds has been identified as a major challenge in human iPSC-based disease modeling [60]. While more variability between humans than inbred mice had definitely to be expected, variability has apparently caught the disease modeling field by surprise and has caused doubt about the technology. Here, iNs provide the opportunity to advance the reproducibility and relevance of human disease modeling studies, as higher patient and control numbers can be used, thereby making the application of powerful statistical/bioinformatical tools for data analysis useful [172]. iNs further do not only represent interindividual variability, but also the fibroblast culture of each patient exhibits a certain degree of heterogeneity (mosaicism) compared to the clonal identity of iPSCs. This difference might be regarded as a disadvantage, for instance, for the generation of isogenic control lines, or as an advantage when cell mosaicism may play a role, such as in aging or psychiatric conditions [173].

Conclusion

Direct iN conversion offers a valuable addition to iPSCs to study the fundamentals of cell identity, investigate human neuronal function, and model neurological diseases, and as a new strategy for *in vivo* cell replacement therapies. Unlike iPSC-based differentiation to neurons, iNs circumvent the known paths of neurodevelopment, and we have started to better understand the mechanics of this process in the recent years. The unique characteristics of iNs already let them stand out as a valuable tool for many applications. Among the best-known phenomena is the age-preserving characteristic of iNs, which makes them a useful complement to iPSC models of brain aging and age-related diseases. Today is only the dawn of this technology, and the development of more direct conversion protocols and applications can be expected in the near future. Application of iN technology profits from the rapid developments in the iPSC field, and iNs already leave their mark in the current process of redefining how we think about cell fate, neuronal identity, and possibilities to deconstruct and reconstruct the inner workings of the human brain.

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References

1 Azevedo FAC, Carvalho LRB, Grinberg LT, Farfel JM, Ferretti REL, Leite REP, Jacob Filho W, Lent R and Herculano-Houzel S (2009) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol* **513**, 532–541.

2 Coimbra JRM, Marques DFF, Baptista SJ, Pereira CMF, Moreira PI, Dinis TCP, Santos AE and Salvador JAR (2018) Highlights in BACE1 inhibitors for Alzheimer's disease treatment. *Front Chem* **6**, 178.

3 Hu X, Das B, Hou H, He W and Yan R (2018) BACE1 deletion in the adult mouse reverses preformed amyloid deposition and improves cognitive functions. *J Exp Med* **215**, 927–940.

4 Davis RL, Weintraub H and Lassar AB (1987) Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51**, 987–1000.
5 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K and Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872.
6 Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC and Wernig M (2010) Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035–1041.
7 Victor MB, Richner M, Olsen HE, Lee SW, Monteys AM, Ma C, Huh CJ, Zhang B, Davidson BL, Yang XW *et al.* (2018) Striatal neurons directly converted from Huntington's disease patient fibroblasts recapitulate age-associated disease phenotypes. *Nat Neurosci* **21**, 341–352.
8 Heinrich C, Bergami M, Gascón S, Lepier A, Viganò F, Dimou L, Sutor B, Berninger B and Götz M (2014) Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. *Stem Cell Rep* **3**, 1000–1014.
9 Kim Y, Zheng X, Ansari Z, Bunnell MC, Herdy JR, Traxler L, Lee H, Paquola ACM, Blithikioti C, Ku M *et al.* (2018) Mitochondrial aging defects emerge in directly reprogrammed human neurons due to their metabolic profile. *Cell Rep* **23**, 2550–2558.
10 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.
11 Mall M, Kareta MS, Chanda S, Ahlenius H, Perotti N, Zhou B, Grieder SD, Ge X, Drake S, Euong Ang C *et al.* (2017) Myt1l safeguards neuronal identity by actively repressing many non-neuronal fates. *Nature* **544**, 245–249.
12 Böhnke L, Traxler L, Herdy JR and Mertens J (2019) Human neurons to model aging: a dish best served old. *Drug Discov Today Dis Model* **27**, 43–49.
13 Liu C, Oikonomopoulos A, Sayed N and Wu JC (2018) Modeling human diseases with induced pluripotent stem cells: from 2D to 3D and beyond. *Development* **145**, dev156166. https://doi.org/10.1242/dev.156166
14 Koch P, Breuer P, Peitz M, Jungverdorben J, Kesavan J, Poppe D, Doerr J, Ladewig J, Mertens J, Tüting T *et al.* (2011) Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. *Nature* **480**, 543–546.
15 Hu W, Qiu B, Guan W, Wang Q, Wang M, Li W, Gao L, Shen L, Huang Y, Xie G *et al.* (2015) Direct conversion of normal and Alzheimer's disease human fibroblasts into neuronal cells by small molecules. *Cell Stem Cell* **17**, 204–212.
16 Schafer ST, Paquola ACM, Stern S, Gosselin D, Ku M, Pena M, Kuret TJM, Liyanage M, Mansour AA, Jaeger BN *et al.* (2019) Pathological priming causes developmental gene network heterochronicity in autistic subject-derived neurons. *Nat Neurosci* **22**, 243–255.
17 Mertens J, Reid D, Lau S, Kim Y and Gage FH (2018) Aging in a dish: iPSC-derived and directly induced neurons for studying brain aging and age-related neurodegenerative diseases. *Annu Rev Genet* **52**, 271–293.
18 Rajasingh S, Isai DG, Samanta S, Zhou Z, Dawn B, Kinsey WH, Czirok A and Rajasingh J (2018) Manipulation-free cultures of human iPSC-derived cardiomyocytes offer a novel screening method for cardiotoxicity. *Acta Pharmacol Sin* **39**, 1590–1603.
19 Mathur A, Loskill P, Shao K, Huebsch N, Hong S, Marcus SG, Marks N, Mandegar M, Conklin BR, Lee LP *et al.* (2015) Human iPSC-based cardiac microphysiological system for drug screening applications. *Sci Rep* **5**, 8883.
20 Mertens J, Wang Q-W, Kim Y, Yu DX, Pham S, Yang B, Zheng Y, Diffenderfer KE, Zhang J, Soltani S *et al.* (2015) Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature* **527**, 95–99.
21 Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D *et al.* (2011) Modelling schizophrenia using human induced pluripotent stem cells. *Nature* **473**, 221–225.
22 Kikuchi T, Morizane A, Doi D, Magotani H, Onoe H, Hayashi T, Mizuma H, Takara S, Takahashi R, Inoue H *et al.* (2017) Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. *Nature* **548**, 592–596.
23 Chambers SM and Studer L (2011) Cell fate plug and play: direct reprogramming and induced pluripotency. *Cell* **145**, 827–830.
24 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.
25 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.
26 Liu M-L, Zang T, Zou Y, Chang JC, Gibson JR, Huber KM and Zhang C-L (2013) Small molecules enable neurogenin 2 to efficiently convert human fibroblasts into cholinergic neurons. *Nat Commun* **4**, 2183.
27 Matsuda T, Irie T, Katsurabayashi S, Hayashi Y, Nagai T, Hamazaki N, Adefuin AMD, Miura F, Ito

T, Kimura H *et al.* (2019) Pioneer factor NeuroD1 rearranges transcriptional and epigenetic profiles to execute microglia-neuron conversion. *Neuron* **101**, 472–485.e7.
28 Iwafuchi-Doi M and Zaret KS (2014) Pioneer transcription factors in cell reprogramming. *Genes Dev* **28**, 2679–2692.
29 Morris SA (2016) Direct lineage reprogramming via pioneer factors; a detour through developmental gene regulatory networks. *Development* **143**, 2696–2705.
30 Colasante G, Rubio A, Massimino L and Broccoli V (2019) Direct neuronal reprogramming reveals unknown functions for known transcription factors. *Front Neurosci* **13**, 283.
31 Ladewig J, Mertens J, Kesavan J, Doerr J, Poppe D, Glaue F, Herms S, Wernet P, Kögler G, Müller F-J *et al.* (2012) Small molecules enable highly efficient neuronal conversion of human fibroblasts. *Nat Methods* **9**, 575–578.
32 Wapinski OL, Lee QY, Chen AC, Li R, Corces MR, Ang CE, Treutlein B, Xiang C, Baubet V, Suchy FP *et al.* (2017) Rapid chromatin switch in the direct reprogramming of fibroblasts to neurons. *Cell Rep* **20**, 3236–3247.
33 Smith DK, Yang J, Liu M-L and Zhang C-L (2016) Small molecules modulate chromatin accessibility to promote NEUROG2-mediated fibroblast-to-neuron reprogramming. *Stem Cell Rep* **7**, 955–969.
34 Aydin B, Kakumanu A, Rossillo M, Moreno-Estellés M, Garipler G, Ringstad N, Flames N, Mahony S and Mazzoni EO (2019) Proneural factors Ascl1 and Neurog2 contribute to neuronal subtype identities by establishing distinct chromatin landscapes. *Nat Neurosci* **22**, 897–908.
35 Vadodaria KC, Mertens J, Paquola A, Bardy C, Li X, Jappelli R, Fung L, Marchetto MC, Hamm M, Gorris M *et al.* (2016) Generation of functional human serotonergic neurons from fibroblasts. *Mol Psychiatry* **21**, 49–61.
36 Tsunemoto R, Lee S, Szűcs A, Chubukov P, Sokolova I, Blanchard JW, Eade KT, Bruggemann J, Wu C, Torkamani A *et al.* (2018) Diverse reprogramming codes for neuronal identity. *Nature* **557**, 375–380.
37 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.
38 Drouin-Ouellet J, Lau S, Brattås PL, Rylander Ottosson D, Pircs K, Grassi DA, Collins LM, Vuono R, Andersson Sjöland A, Westergren-Thorsson G *et al.* (2017) REST suppression mediates neural conversion of adult human fibroblasts via microRNA-dependent and -independent pathways. *EMBO Mol Med* **9**, 1117–1131.
39 Ahlenius H, Chanda S, Webb AE, Yousif I, Karmazin J, Prusiner SB, Brunet A, Südhof TC and Wernig M (2016) FoxO3 regulates neuronal reprogramming of cells from postnatal and aging mice. *Proc Natl Acad Sci USA* **113**, 8514–8519.
40 Webb AE, Pollina EA, Vierbuchen T, Urbán N, Ucar D, Leeman DS, Martynoga B, Sewak M, Rando TA, Guillemot F *et al.* (2013) FOXO3 shares common targets with ASCL1 genome-wide and inhibits ASCL1-dependent neurogenesis. *Cell Rep* **4**, 477–491.
41 Herdy J, Schafer S, Kim Y, Ansari Z, Zangwill D, Ku M, Paquola A, Lee H, Mertens J and Gage FH (2019) Chemical modulation of transcriptionally enriched signaling pathways to optimize the conversion of fibroblasts into neurons. *eLife* **8**, e41356. https://doi.org/10.7554/eLife.41356
42 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.
43 Xue Y, Qian H, Hu J, Zhou B, Zhou Y, Hu X, Karakhanyan A, Pang Z and Fu X-D (2016) Sequential regulatory loops as key gatekeepers for neuronal reprogramming in human cells. *Nat Neurosci* **19**, 807–815.
44 Lau S, Ottosson DR, Jakobsson J and Parmar Correspondence M (2014) Direct neural conversion from human fibroblasts using self-regulating and nonintegrating viral vectors in brief. *Cell Rep* **9**, 1673–1680.
45 Hu J, Qian H, Xue Y and Fu X-D (2018) PTB/nPTB: master regulators of neuronal fate in mammals. *Biophys Rep* **4**, 204–214.
46 Ambasudhan R, Talantova M, Coleman R, Yuan X, Zhu S, Lipton SA and Ding S (2011) Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. *Cell Stem Cell* **9**, 113–118.
47 Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW and Crabtree GR (2011) MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature* **476**, 228–231.
48 Treutlein B, Lee QY, Camp JG, Mall M, Koh W, Shariati SAM, Sim S, Neff NF, Skotheim JM, Wernig M *et al.* (2016) Dissecting direct reprogramming from fibroblast to neuron using single-cell RNA-seq. *Nature* **534**, 391–395.
49 Chanda S, Ang CE, Davila J, Pak C, Mall M, Lee QY, Ahlenius H, Jung SW, Südhof TC and Wernig M (2014) Generation of induced neuronal cells by the single reprogramming factor ASCL1. *Stem Cell Rep* **3**, 282–296.
50 Mall M and Wernig M (2017) The novel tool of cell reprogramming for applications in molecular medicine. *J Mol Med* **95**, 695–703.

51 Luo C, Lee QY, Wapinski O, Castanon R, Nery JR, Mall M, Kareta MS, Cullen SM, Goodell MA, Chang HY *et al.* (2019) Global DNA methylation remodeling during direct reprogramming of fibroblasts to neurons. *eLife* **8**, e40197. https://doi.org/10.7554/eLife.40197

52 Zhu X, Zhou W, Jin H and Li T (2018) Brn2 alone is sufficient to convert astrocytes into neural progenitors and neurons. *Stem Cells Dev* **27**, 736–744. https://doi.org/10.1089/scd.2017.0250

53 De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Ercument Cicek A, Kou Y, Liu L, Fromer M, Walker S *et al.* (2014) Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature* **515**, 209–215.

54 Wang T, Guo H, Xiong B, Stessman HAF, Wu H, Coe BP, Turner TN, Liu Y, Zhao W, Hoekzema K *et al.* (2016) De novo genic mutations among a Chinese autism spectrum disorder cohort. *Nat Commun* **7**, 13316.

55 Loid P, Mäkitie R, Costantini A, Viljakainen H, Pekkinen M and Mäkitie O (2018) A novel *MYT1L* mutation in a patient with severe early-onset obesity and intellectual disability. *Am J Med Genet Part A* **176**, 1972–1975.

56 Marchetto MC, Belinson H, Tian Y, Freitas BC, Fu C, Vadodaria KC, Beltrao-Braga PC, Trujillo CA, Mendes APD, Padmanabhan K *et al.* (2017) Altered proliferation and networks in neural cells derived from idiopathic autistic individuals. *Mol. Psychiatry* **22**, 820–835.

57 Victor MB, Richner M, Hermanstyne TO, Ransdell JL, Sobieski C, Deng P-Y, Klyachko VA, Nerbonne JM and Yoo AS (2014) Generation of human striatal neurons by microRNA-dependent direct conversion of fibroblasts. *Neuron* **84**, 311–323.

58 Jiang H, Xu Z, Zhong P, Ren Y, Liang G, Schilling HA, Hu Z, Zhang Y, Wang X, Chen S *et al.* (2015) Cell cycle and p53 gate the direct conversion of human fibroblasts to dopaminergic neurons. *Nat Commun* **6**, 10100.

59 Black JB, Adler AF, Wang H-G, D'Ippolito AM, Hutchinson HA, Reddy TE, Pitt GS, Leong KW and Gersbach CA (2016) Targeted epigenetic remodeling of endogenous loci by CRISPR/Cas9-based transcriptional activators directly converts fibroblasts to neuronal cells. *Cell Stem Cell* **19**, 406–414.

60 Mertens J, Marchetto MC, Bardy C and Gage FH (2016) Evaluating cell reprogramming, differentiation and conversion technologies in neuroscience. *Nat Rev Neurosci* **17**, 424–437.

61 Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M and Studer L (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* **27**, 275–280.

62 Pfisterer U, Ek F, Lang S, Soneji S, Olsson R and Parmar M (2016) Small molecules increase direct neural conversion of human fibroblasts. *Sci Rep* **6**, 38290.

63 Park NI, Guilhamon P, Desai K, McAdam RF, Langille E, O'Connor M, Lan X, Whetstone H, Coutinho FJ, Vanner RJ *et al.* (2017) ASCL1 reorganizes chromatin to direct neuronal fate and suppress tumorigenicity of glioblastoma stem cells. *Cell Stem Cell* **21**, 209–224.e7.

64 Gascón S, Murenu E, Masserdotti G, Ortega F, Russo GL, Petrik D, Deshpande A, Heinrich C, Karow M, Robertson SP *et al.* (2016) Identification and successful negotiation of a metabolic checkpoint in direct neuronal reprogramming. *Cell Stem Cell* **18**, 396–409.

65 Zheng X, Boyer L, Jin M, Mertens J, Kim Y, Ma L, Ma L, Hamm M, Gage FH and Hunter T (2016) Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. *eLife* **5**, e13374. https://doi.org/10.7554/eLife.13374

66 Tao Y and Zhang SC (2016) Neural Subtype Specification from Human Pluripotent Stem Cells. *Cell Stem Cell* **19**, 573–586. https://doi.org/10.1016/j.stem.2016.10.015.

67 Briggs JA, Li VC, Lee S, Woolf CJ, Klein A and Kirschner MW (2017) Mouse embryonic stem cells can differentiate via multiple paths to the same state. *eLife* **6**, e26945. https://doi.org/10.7554/eLife.26945

68 Zhang Y, Pak C, Han Y, Ahlenius H, Zhang Z, Chanda S, Marro S, Patzke C, Acuna C, Covy J *et al.* (2013) Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron* **78**, 785–798. https://doi.org/10.1016/j.neuron.2013.05.029

69 Karow M, Camp JG, Falk S, Gerber T, Pataskar A, Gac-Santel M, Kageyama 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.

70 Jessberger S, Toni N, Clemenson GD Jr, Ray J and Gage FH (2008) Directed differentiation of hippocampal stem/progenitor cells in the adult brain. *Nat Neurosci* **11**, 888–893.

71 Kele J, Simplicio N, Ferri ALM, Mira H, Guillemot F, Arenas E and Ang S-L (2006) Neurogenin 2 is required for the development of ventral midbrain dopaminergic neurons. *Development* **133**, 495–505.

72 Parras CM, Schuurmans C, Scardigli R, Kim J, Anderson DJ and Guillemot F (2002) Divergent functions of the proneural genes Mash1 and Ngn2 in the specification of neuronal subtype identity. *Genes Dev* **16**, 324–338.

73 Chouchane M, Melo de Farias AR, Moura DMS, Hilscher MM, Schroeder T, Leão RN and Costa MR (2017) Lineage reprogramming of astroglial cells from different origins into distinct neuronal subtypes. *Stem Cell Rep* **9**, 162–176.

74 Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ and Eggan K (2011) Cell stem cell article conversion of mouse and human fibroblasts into functional spinal motor neurons. *Stem Cell* **9**, 205–218.
75 Xu Z, Jiang H, Zhong P, Yan Z, Chen S and Feng J (2016) Direct conversion of human fibroblasts to induced serotonergic neurons. *Mol Psychiatry* **21**, 62–70.
76 Blanchard JW, Eade KT, Szűcs A, Lo Sardo V, Tsunemoto RK, Williams D, Sanna PP and Baldwin KK (2015) Selective conversion of fibroblasts into peripheral sensory neurons. *Nat Neurosci* **18**, 25–35.
77 Liu M-L, Zang T and Zhang C-L (2016) Direct lineage reprogramming reveals disease-specific phenotypes of motor neurons from human ALS patients. *Cell Rep* **14**, 115–128.
78 Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ and Eggan K (2011) Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* **9**, 205–218.
79 Abernathy DG, Kim WK, McCoy MJ, Lake AM, Ouwenga R, Lee SW, Xing X, Li D, Lee HJ, Heuckeroth RO *et al.* (2017) MicroRNAs induce a permissive chromatin environment that enables neuronal subtype-specific reprogramming of adult human fibroblasts. *Cell Stem Cell* **21**, 332–348.e9.
80 Rivetti di Val Cervo P, Romanov RA, Spigolon G, Masini D, Martín-Montañez E, Toledo EM, La Manno G, Feyder M, Pifl C, Ng Y-H *et al.* (2017) Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model. *Nature Biotechnol* **35**, 444–452.
81 Miskinyte G, Devaraju K, Grønning Hansen M, Monni E, Tornero D, Woods NB, Bengzon J, Ahlenius H, Lindvall O and Kokaia Z (2017) Direct conversion of human fibroblasts to functional excitatory cortical neurons integrating into human neural networks. *Stem Cell Res Ther* **8**, 207.
82 Pereira M, Birtele M, Shrigley S, Benitez JA, Hedlund E, Parmar M and Ottosson DR (2017) Direct reprogramming of resident NG2 glia into neurons with properties of fast-spiking parvalbumin-containing interneurons. *Stem Cell Rep* **9**, 742–751.
83 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.
84 Oksanen M, Petersen AJ, Naumenko N, Puttonen K, Lehtonen Š, Gubert Olivé M, Shakirzyanova A, Leskelä S, Sarajärvi T, Viitanen M *et al.* (2017) PSEN1 mutant iPSC-derived model reveals severe astrocyte pathology in Alzheimer's disease. *Stem Cell Rep* **9**, 1885–1897.
85 Caiazzo M, Giannelli S, Valente P, Lignani G, Carissimo A, Sessa A, Colasante G, Bartolomeo R, Massimino L, Ferroni S *et al.* (2015) Direct conversion of fibroblasts into functional astrocytes by defined transcription factors. *Stem Cell Rep* **4**, 25–36.
86 Lee E-H and Park C-H (2017) Comparison of reprogramming methods for generation of induced-oligodendrocyte precursor cells. *Biomol Ther* **25**, 362–366.
87 Ehrlich M, Mozafari S, Glatza M, Starost L, Velychko S, Hallmann A-L, Cui Q-L, Schambach A, Kim K-P, Bachelin C *et al.* (2017) Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. *Proc Natl Acad Sci* **114**, E2243–E2252.
88 Canals I, Ginisty A, Quist E, Timmerman R, Fritze J, Miskinyte G, Monni E, Hansen MG, Hidalgo I, Bryder D *et al.* (2018) Rapid and efficient induction of functional astrocytes from human pluripotent stem cells. *Nat Methods* **15**, 693–696.
89 Tchieu J, Calder EL, Guttikonda SR, Gutzwiller EM, Aromolaran KA, Steinbeck JA, Goldstein PA and Studer L (2019) NFIA is a gliogenic switch enabling rapid derivation of functional human astrocytes from pluripotent stem cells. *Nat Biotechnol* **37**, 267–275.
90 Kitada M, Murakami T, Wakao S, Li G and Dezawa M (2019) Direct conversion of adult human skin fibroblasts into functional Schwann cells that achieve robust recovery of the severed peripheral nerve in rats. *Glia* **67**, 950–966.
91 Tanabe K, Ang CE, Chanda S, Olmos VH, Haag D, Levinson DF, Südhof TC and Wernig M (2018) Transdifferentiation of human adult peripheral blood T cells into neurons. *Proc Natl Acad Sci USA* **115**, 6470–6475.
92 Waddington CH (2014) The Strategy of the Genes. Routledge, London, UK, 274pp.
93 Takahashi K (2012) Cellular reprogramming – lowering gravity on Waddington's epigenetic landscape. *J Cell Sci* **125**, 2553–2560. https://doi.org/10.1242/jcs.084822
94 Rajagopal J and Stanger BZ (2016) Plasticity in the adult: how should the waddington diagram be applied to regenerating tissues? *Dev Cell* **36**, 133–137.
95 Masserdotti G, Gascón S and Götz M (2016) Direct neuronal reprogramming: learning from and for development. *Development* **143**, 2494–2510.
96 Ladewig J, Koch P and Brüstle O (2013) Leveling Waddington: the emergence of direct programming and the loss of cell fate hierarchies. *Nat Rev Mol Cell Biol* **14**, 225–236.
97 Shi Y, Kirwan P and Livesey FJ (2012) Directed differentiation of human pluripotent stem cells to

cerebral cortex neurons and neural networks. *Nat Protoc* **7**, 1836–1846.
98 Atlasi Y and Stunnenberg HG (2017) The interplay of epigenetic marks during stem cell differentiation and development. *Nat Rev Genet* **18**, 643–658.
99 Luginbühl J, Kouno T, Nakano R, Chater TE, Sivaraman DM, Kishima M, Roudnicky F, Carninci P, Plessy C and Shin JW (2019) Decoding neuronal diversity by single-cell Convert-seq. *bioRxiv*, 600239.
100 Chronis C, Fiziev P, Papp B, Butz S, Bonora G, Sabri S, Ernst J and Plath K (2017) Cooperative binding of transcription factors orchestrates reprogramming. *Cell* **168**, 442–459.
101 Fu K, Chronis C, Soufi A, Bonora G, Edwards M, Smale ST, Zaret KS, Plath K and Pellegrini M (2018) Comparison of reprogramming factor targets reveals both species-specific and conserved mechanisms in early iPSC reprogramming. *BMC Genom* **19**, 956.
102 Kuno A, Nishimura K and Takahashi S (2018) Time-course transcriptome analysis of human cellular reprogramming from multiple cell types reveals the drastic change occurs between the mid phase and the late phase. *BMC Genom* **19**, 9.
103 Barbagiovanni G, Germain P-L, Zech M, Stewart AF, Winkelmann J and Correspondence GT (2018) KMT2B is selectively required for neuronal transdifferentiation, and its loss exposes dystonia candidate genes. *Cell Rep* **25**, 988–1001.
104 Chuang W, Sharma A, Shukla P, Li G, Mall M, Rajarajan K, Abilez OJ, Hamaguchi R, Wu JC, Wernig M *et al.* (2017) Partial reprogramming of pluripotent stem cell-derived cardiomyocytes into neurons. *Sci Rep* **7**, 44840.
105 Vierbuchen T and Wernig M (2012) Molecular roadblocks for cellular reprogramming. *Mol Cell* **47**, 827–838.
106 Lorenz C and Prigione A (2017) Mitochondrial metabolism in early neural fate and its relevance for neuronal disease modeling. *Curr Opin Cell Biol* **49**, 71–76.
107 Zink A, Priller J and Prigione A (2018) Pluripotent stem cells for uncovering the role of mitochondria in human brain function and dysfunction. *J Mol Biol* **430**, 891–903.
108 Yellen G (2018) Fueling thought: management of glycolysis and oxidative phosphorylation in neuronal metabolism. *J Cell Biol* **217**, 2235–2246.
109 Bélanger M, Allaman I and Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab* **14**, 724–738.
110 Correction: Zhang *et al.*, An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex (2015) *J Neurosci* **35**, 864–866.
111 Yang N, Ng YH, Pang ZP, Südhof TC and Wernig M (2011) Induced neuronal cells: how to make and define a neuron. *Cell Stem Cell* **9**, 517–25.
112 Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, Jaeger BN, O'Connor C, Fitzpatrick C, Pasillas MP *et al.* (2017) An environment-dependent transcriptional network specifies human microglia identity. *Science* **356**, eaal3222.
113 Grande A, Sumiyoshi K, López-Juárez A, Howard J, Sakthivel B, Aronow B, Campbell K and Nakafuku M (2013) Environmental impact on direct neuronal reprogramming in vivo in the adult brain. *Nat Commun* **4**, 2373. https://doi.org/10.1038/ncomms3373
114 Koch P, Kokaia Z, Lindvall O and Brüstle O (2009) Emerging concepts in neural stem cell research: autologous repair and cell-based disease modelling. *Lancet Neurol* **8**, 819–829. https://doi.org/10.1016/S1474-4422(09)70202-9
115 Pereira M, Birtele M and Rylander Ottosson D (2019) In Vivo Direct Reprogramming of Resident Glial Cells into Interneurons by Intracerebral Injection of Viral Vectors. J. Vis, Exp.
116 Luo C, Keown CL, Kurihara L, Zhou J, He Y, Li J, Castanon R, Lucero J, Nery JR, Sandoval JP *et al.* (2017) Single-cell methylomes identify neuronal subtypes and regulatory elements in mammalian cortex. *Science* **357**, 600–604.
117 Kang S, Chen X, Gong S, Yu P, Yau S, Su Z, Zhou L, Yu J, Pan G and Shi L (2017) Characteristic analyses of a neural differentiation model from iPSC-derived neuron according to morphology, physiology, and global gene expression pattern. *Sci Rep* **7**, 12233.
118 Yin J-C, Zhang L, Ma N-X, Wang Y, Lee G, Hou X-Y, Lei Z-F, Zhang F-Y, Dong F-P, Wu G-Y *et al.* (2019) Chemical conversion of human fetal astrocytes into neurons through modulation of multiple signaling pathways. *Stem Cell Rep* **12**, 488–501.
119 McKinney CE (2017) Using induced pluripotent stem cells derived neurons to model brain diseases. *Neural Regen Res* **12**, 1062–1067.
120 Bardy C, van den Hurk M, Eames T, Marchand C, Hernandez RV, Kellogg M, Gorris M, Galet B, Palomares V, Brown J *et al.* (2015) Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. *Proc Natl Acad Sci USA* **112**, E2725–E2734.
121 Agostini M, Romeo F, Inoue S, Niklison-Chirou MV, Elia AJ, Dinsdale D, Morone N, Knight RA, Mak TW and Melino G (2016) Metabolic reprogramming during neuronal differentiation. *Cell Death Differ* **23**, 1502–1514.
122 He Z and Yu Q (2018) Identification and characterization of functional modules reflecting transcriptome transition during human neuron maturation. *BMC Genom* **19**, 262. https://doi.org/10.1186/s12864-018-4649-2

123 Bardy C, van den Hurk M, Kakaradov B, Erwin JA, Jaeger BN, Hernandez RV, Eames T, Paucar AA, Gorris M, Marchand C *et al.* (2016) Predicting the functional states of human iPSC-derived neurons with single-cell RNA-seq and electrophysiology. *Mol Psychiatry* **21**, 1573–1588.
124 Kang HJ, Kawasawa YI, Cheng F, Zhu Y, Xu X, Li M, Sousa AMM, Pletikos M, Meyer KA, Sedmak G *et al.* (2011) Spatio-temporal transcriptome of the human brain. *Nature* **478**, 483–489.
125 Miller JA, Ding S-L, Sunkin SM, Smith KA, Ng L, Szafer A, Ebbert A, Riley ZL, Royall JJ, Aiona K *et al.* (2014) Transcriptional landscape of the prenatal human brain. *Nature* **508**, 199–206.
126 Keil JM, Qalieh A and Kwan KY (2018) Brain transcriptome databases: a user's guide. *J Neurosci* **38**, 2399–2412.
127 Stein JL, de la Torre-Ubieta L, Tian Y, Parikshak NN, Hernández IA, Marchetto MC, Baker DK, Lu D, Hinman CR, Lowe JK *et al.* (2014) A quantitative framework to evaluate modeling of cortical development by neural stem cells. *Neuron* **83**, 69–86.
128 Nayler SP, Powell JE, Vanichkina DP, Korn O, Wells CA, Kanjhan R, Sun J, Taft RJ, Lavin MF and Wolvetang EJ (2017) Human iPSC-derived cerebellar neurons from a patient with ataxia-telangiectasia reveal disrupted gene regulatory networks. *Front Cell Neurosci* **11**, 321.
129 Camp JG, Badsha F, Florio M, Kanton S, Gerber T, Wilsch-Bräuninger M, Lewitus E, Sykes A, Hevers W, Lancaster M *et al.* (2015) Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. *Proc Natl Acad Sci USA* **112**, 15672–15677.
130 Velasco S, Kedaigle AJ, Simmons SK, Nash A, Rocha M, Quadrato G, Paulsen B, Nguyen L, Adiconis X, Regev A *et al.* (2019) Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature* **570**, 523–527.
131 Nowakowski TJ, Bhaduri A, Pollen AA, Alvarado B, Mostajo-Radji MA, Di Lullo E, Haeussler M, Sandoval-Espinosa C, Liu SJ, Velmeshev D *et al.* (2017) Spatiotemporal gene expression trajectories reveal developmental hierarchies of the human cortex. *Science* **358**, 1318–1323.
132 Tasic B, Yao Z, Graybuck LT, Smith KA, Nguyen TN, Bertagnolli D, Goldy J, Garren E, Economo MN, Viswanathan S *et al.* (2018) Shared and distinct transcriptomic cell types across neocortical areas. *Nature* **563**, 72–78.
133 Fan X, Dong J, Zhong S, Wei Y, Wu Q, Yan L, Yong J, Sun L, Wang X, Zhao Y *et al.* (2018) Spatial transcriptomic survey of human embryonic cerebral cortex by single-cell RNA-seq analysis. *Cell Res* **28**, 730–745.
134 Horvath S (2013) DNA methylation age of human tissues and cell types. *Genome Biol* **14**, R115.
135 Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y *et al.* (2018) An epigenetic biomarker of aging for lifespan and healthspan. *Aging* **10**, 573–591.
136 Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD *et al.* (2019) DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging* **11**, 303–327.
137 Huh CJ, Zhang B, Victor MB, Dahiya S, Batista LF, Horvath S and Yoo AS (2016) Maintenance of age in human neurons generated by microRNA-based neuronal conversion of fibroblasts. *eLife* **5**, e18648. https://doi.org/10.7554/eLife.18648
138 Lo Sardo V, Ferguson W, Erikson GA, Topol EJ, Baldwin KK and Torkamani A (2017) Influence of donor age on induced pluripotent stem cells. *Nat Biotechnol* **35**, 69–74.
139 He Y and Ecker JR (2015) Non-CG methylation in the human genome. *Annu Rev Genomics Hum Genet* **16**, 55–77.
140 Stroud H, Su SC, Hrvatin S, Greben AW, Renthal W, Boxer LD, Nagy MA, Hochbaum DR, Kinde B, Gabel HW *et al.* (2017) Early-life gene expression in neurons modulates lasting epigenetic states. *Cell* **171**, 1151–1164.e16.
141 Iwamoto K, Bundo M, Ueda J, Oldham MC, Ukai W, Hashimoto E, Saito T, Geschwind DH and Kato T (2011) Neurons show distinctive DNA methylation profile and higher interindividual variations compared with non-neurons. *Genome Res* **21**, 688–696.
142 Guo JU, Su Y, Shin JH, Shin J, Li H, Xie B, Zhong C, Hu S, Le T, Fan G *et al.* (2014) Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. *Nat Neurosci* **17**, 215–222.
143 Lister R, Mukamel EA, Nery JR, Urich M, Puddifoot CA, Johnson ND, Lucero J, Huang Y, Dwork AJ, Schultz MD *et al.* (2013) Global epigenomic reconfiguration during mammalian brain development. *Science* **341**, 1237905.
144 Marchetto MCN, Winner B and Gage FH (2010) Pluripotent stem cells in neurodegenerative and neurodevelopmental diseases. *Hum Mol Genet* **19**, R71–R76.
145 Wu Y-Y, Chiu F-L, Yeh C-S and Kuo H-C (2019) Opportunities and challenges for the use of induced pluripotent stem cells in modelling neurodegenerative disease. *Open Biol* **9**, 180177.
146 Xie YZ and Zhang RX (2015) Neurodegenerative diseases in a dish: the promise of iPSC technology in disease modeling and therapeutic discovery. *Neurol Sci* **36**, 21–27.
147 Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL,

Boscolo FS *et al.* (2012) Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature* **482**, 216–220.
148 Young JE, Boulanger-Weill J, Williams DA, Woodruff G, Buen F, Revilla AC, Herrera C, Israel MA, Yuan SH, Edland SD *et al.* (2015) Elucidating molecular phenotypes caused by the SORL1 Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. *Cell Stem Cell* **16**, 373–385.
149 Mertens J, Stüber K, Poppe D, Doerr J, Ladewig J, Brüstle O and Koch P (2013) Embryonic stem cell-based modeling of Tau pathology in human neurons. *Am J Pathol* **182**, 1769–1779.
150 Mertens J, Stüber K, Wunderlich P, Ladewig J, Kesavan JC, Vandenberghe R, Vandenbulcke M, van Damme P, Walter J, Brüstle O *et al.* (2013) APP processing in human pluripotent stem cell-derived neurons is resistant to NSAID-based γ-secretase modulation. *Stem Cell Rep* **1**, 491–498.
151 Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Tu EY, Mandal PK, Vera E, Shim J, Kriks S *et al.* (2013) Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell* **13**, 691–705.
152 Frobel J, Hemeda H, Lenz M, Abagnale G, Joussen S, Denecke B, Šarić T, Zenke M and Wagner W (2014) Epigenetic rejuvenation of mesenchymal stromal cells derived from induced pluripotent stem cells. *Stem Cell Rep* **3**, 414–422.
153 Lapasset L, Milhavet O, Prieur A, Besnard E, Babled A, Aït-Hamou N, Leschik J, Pellestor F, Ramirez J-M, De Vos J *et al.* (2011) Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev* **25**, 2248–2253.
154 Prigione A, Fauler B, Lurz R, Lehrach H and Adjaye J (2010) The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells. *Stem Cells* **28**, 721–733.
155 Yang Y, Jiao J, Gao R, Le R, Kou X, Zhao Y, Wang H, Gao S and Wang Y (2015) Enhanced rejuvenation in induced pluripotent stem cell-derived neurons compared with directly converted neurons from an aged mouse. *Stem Cells Dev* **24**, 2767–2777.
156 Choi J, Lee S, Mallard W, Clement K, Tagliazucchi GM, Lim H, Choi IY, Ferrari F, Tsankov AM, Pop R *et al.* (2015) A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. *Nat Biotechnol* **33**, 1173–1181.
157 Tang Y, Liu M-L, Zang T and Zhang C-L (2017) Direct reprogramming rather than iPSC-based reprogramming maintains aging hallmarks in human motor neurons. *Front Mol Neurosci* **10**, 359.
158 Studer L, Vera E and Cornacchia D (2015) Programming and reprogramming cellular age in the era of induced pluripotency. *Cell Stem Cell* **16**, 591–600.
159 Vera E, Bosco N and Studer L (2016) Generating late-onset human iPSC-based disease models by inducing neuronal age-related phenotypes through telomerase manipulation. *Cell Rep* **17**, 1184–1192.
160 Zhu L, Sun C, Ren J, Wang G, Ma R, Sun L, Yang D, Gao S, Ning K, Wang Z *et al.* (2019) Stress-induced precocious aging in PD-patient iPSC-derived NSCs may underlie the pathophysiology of Parkinson's disease. *Cell Death Dis* **10**, 105.
161 Chung CY, Khurana V, Auluck PK, Tardiff DF, Mazzulli JR, Soldner F, Baru V, Lou Y, Freyzon Y, Cho S *et al.* (2013) Identification and rescue of α-synuclein toxicity in Parkinson patient-derived neurons. *Science* **342**, 983–987.
162 Li L, Roh JH, Chang EH, Lee Y, Lee S, Kim M, Koh W, Chang JW, Kim HJ, Nakanishi M *et al.* (2018) iPSC modeling of Presenilin1 mutation in Alzheimer's disease with cerebellar ataxia. *Exp Neurobiol* **27**, 350–364.
163 Moreno CL, Della Guardia L, Shnyder V, Ortiz-Virumbrales M, Kruglikov I, Zhang B, Schadt EE, Tanzi RE, Noggle S, Buettner C *et al.* (2018) iPSC-derived familial Alzheimer's PSEN2 N141I cholinergic neurons exhibit mutation-dependent molecular pathology corrected by insulin signaling. *Mol Neurodegener* **13**, 33.
164 Jovičić A, Mertens J, Boeynaems S, Bogaert E, Chai N, Yamada SB, Paul JW, Sun S, Herdy JR, Bieri G *et al.* (2015) Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat Neurosci* **18**, 1226–1229.
165 Chen H, Qian K, Du Z, Cao J, Petersen A, Liu H, Blackbourn LW, Huang C-L, Errigo A, Yin Y *et al.* (2014) Modeling ALS with iPSCs reveals that mutant SOD1 misregulates neurofilament balance in motor neurons. *Cell Stem Cell* **14**, 796–809.
166 Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R *et al.* (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* **321**, 1218–1221.
167 The HD iPSC Consortium (2012) Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. *Cell Stem Cell* **11**, 264–278.
168 Arbab M, Baars S and Geijsen N (2014) Modeling motor neuron disease: the matter of time. *Trends Neurosci* **37**, 642–652.
169 Kiskinis E, Sandoe J, Williams LA, Boulting GL, Moccia R, Wainger BJ, Han S, Peng T, Thams S, Mikkilineni S *et al.* (2014) Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant SOD1. *Cell Stem Cell* **14**, 781–795.

170 Gunhanlar N, Shpak G, van der Kroeg M, Gouty-Colomer LA, Munshi ST, Lendemeijer B, Ghazvini M, Dupont C, Hoogendijk WJG, Gribnau J *et al.* (2018) A simplified protocol for differentiation of electrophysiologically mature neuronal networks from human induced pluripotent stem cells. *Mol Psychiatry* **23**, 1336–1344.
171 Schäuble S, Klement K, Marthandan S, Münch S, Heiland I, Schuster S, Hemmerich P and Diekmann S (2012) Quantitative model of cell cycle arrest and cellular senescence in primary human fibroblasts. *PLoS ONE* **7**, e42150.
172 Hoffman GE, Schrode N, Flaherty E and Brennand KJ (2019) New considerations for hiPSC-based models of neuropsychiatric disorders. *Mol Psychiatry* **24**, 49–66.
173 Ghaffari LT, Starr A, Nelson AT and Sattler R (2018) Representing diversity in the dish: using patient-derived in vitro models to recreate the heterogeneity of neurological disease. *Front Neurosci* **12**, 56.
174 Chang J-H, Tsai P-H, Wang K-Y, Wei Y-T, Chiou S-H and Mou C-Y (2018) Generation of functional dopaminergic neurons from reprogramming fibroblasts by nonviral-based mesoporous silica nanoparticles. *Sci Rep* **8**, 11.
175 Jin Y, Lee JS, Kim J, Min S, Wi S, Yu JH, Chang G-E, Cho A-N, Choi Y, Ahn D-H *et al.* (2018) Three-dimensional brain-like microenvironments facilitate the direct reprogramming of fibroblasts into therapeutic neurons. *Nat Biomed Eng* **2**, 522–539.
176 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.
177 Pfisterer U, Wood J, Nihlberg K, Hallgren O, Bjermer L, Westergren-Thorsson G, Lindvall O and Parmar M (2011) Efficient induction of functional neurons from adult human fibroblasts. *Cell Cycle* **10**, 3311–3316.
178 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.
179 Addis RC, Hsu F-C, Wright RL, Dichter MA, Coulter DA and Gearhart JD (2011) Efficient conversion of astrocytes to functional midbrain dopaminergic neurons using a single polycistronic vector. *PLoS ONE* **6**, e28719.
180 Li S, Shi Y, Yao X, Wang X, Shen L, Rao Z, Yuan J, Liu Y, Zhou Z, Zhang Z *et al.* (2019) Conversion of astrocytes and fibroblasts into functional noradrenergic neurons. *Cell Rep* **28**, 682–697.e7.
181 Li H, Jiang H, Yin X, Bard JE, Zhang B and Feng J (2019) Attenuation of PRRX2 and HEY2 enables efficient conversion of adult human skin fibroblasts to neurons. *Biochem Biophys Res Commun* **516**, 765–769.
182 Wan X, Xu L, Li B, Sun Q, Ji Q, Huang D, Zhao L, Xiao Y, Ji Q, Ji Q *et al.* (2018) Chemical conversion of human lung fibroblasts into neuronal cells. *Int J Mol Med* **41**, 1463–1468.
183 Lee SW, Oh YM, Lu Y-L, Kim WK and Yoo AS (2018) MicroRNAs overcome cell fate barrier by reducing EZH2-controlled REST stability during neuronal conversion of human adult fibroblasts. *Dev Cell* **46**, 73–84.e7.
184 Guo Z, Zhang L, Wu Z, Chen Y, Wang F and 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.
185 Heinrich C, Blum R, Gascón S, Masserdotti G, Tripathi P, Sánchez R, Tiedt S, Schroeder T, Götz M and Berninger B (2010) Directing astroglia from the cerebral cortex into subtype specific functional neurons. *PLoS Biol* **8**, e1000373.
186 Torper O, Pfisterer U, Wolf DA, Pereira M, Lau S, Jakobsson J, Bjorklund A, Grealish S and Parmar M (2013) Generation of induced neurons via direct conversion in vivo. *Proc Natl Acad Sci USA* **110**, 7038–7043.
187 Hu X, Qin S, Huang X, Yuan Y, Tan Z, Gu Y, Cheng X, Wang D, Lian X-F, He C *et al.* (2019) Stem cell reports article region-restrict astrocytes exhibit heterogeneous susceptibility to neuronal reprogramming. *Stem Cell Rep* **12**, 290–304.
188 Cheng L, Gao L, Guan W, Mao J, Hu W, Qiu B, Zhao J, Yu Y and Pei G (2015) Direct conversion of astrocytes into neuronal cells by drug cocktail. *Cell Res* **25**, 1269–1272.
189 Robinson M, Fraser I, McKee E, Scheck K, Chang L and Willerth SM (2018) Transdifferentiating astrocytes into neurons using ASCL1 functionalized with a novel intracellular protein delivery technology. *Front Bioeng Biotechnol* **6**, 173.
190 Zhang L, Yin J-C, Yeh H, Ma N-X, Lee G, Chen XA, Wang Y, Lin L, Chen L, Jin P *et al.* (2015) Small molecules efficiently reprogram human astroglial cells into functional neurons. *Cell Stem Cell* **17**, 735–747.
191 Gao L, Guan W, Wang M, Wang H, Yu J, Liu Q, Qiu B, Yu Y, Ping Y, Bian X *et al.* (2017) Direct generation of human neuronal cells from adult astrocytes by small molecules. *Stem Cell Rep* **8**, 538–547.
192 Hao L, Xu Z, Sun H, Luo W, Yan Y, Wang J, Guo J, Liu Y and Chen S (2017) Direct induction of functional neuronal cells from fibroblast-like cells derived from adult human retina. *Stem Cell Res* **23**, 61–72.

193 Liang X-G, Tan C, Wang C-K, Tao R-R, Huang Y-J, Ma K-F, Fukunaga K, Huang M-Z and Han F (2018) Myt1l induced direct reprogramming of pericytes into cholinergic neurons. *CNS Neurosci Ther* **24**, 801–809.

194 Omrani MR, Yaqubi M and Mohammadnia A (2018) Transcription factors in regulatory and protein subnetworks during generation of neural stem cells and neurons from direct reprogramming of non-fibroblastic cell sources. *Neuroscience* **380**, 63–77.

195 Marro S, Pang ZP, Yang N, Tsai M-C, Qu K, Chang HY, Südhof TC and Wernig M (2011) Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. *Cell Stem Cell* **9**, 374–382.