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iPSC technology to study human aging and aging-related disorders

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A global aging population, normally accompanied by a high incidence of aging-associated diseases, has prompted a renewed interest in basic research on human aging. Although encouraging progress has been achieved using animal models, the underlying fundamental mechanisms of aging remain largely unknown. Here, we review the human induced pluripotent stem cell (hiPSC)-based models of aging and aging-related diseases. These models seek to advance our knowledge of aging molecular mechanisms and help to develop strategies for treating aging-associated human diseases.

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Introduction

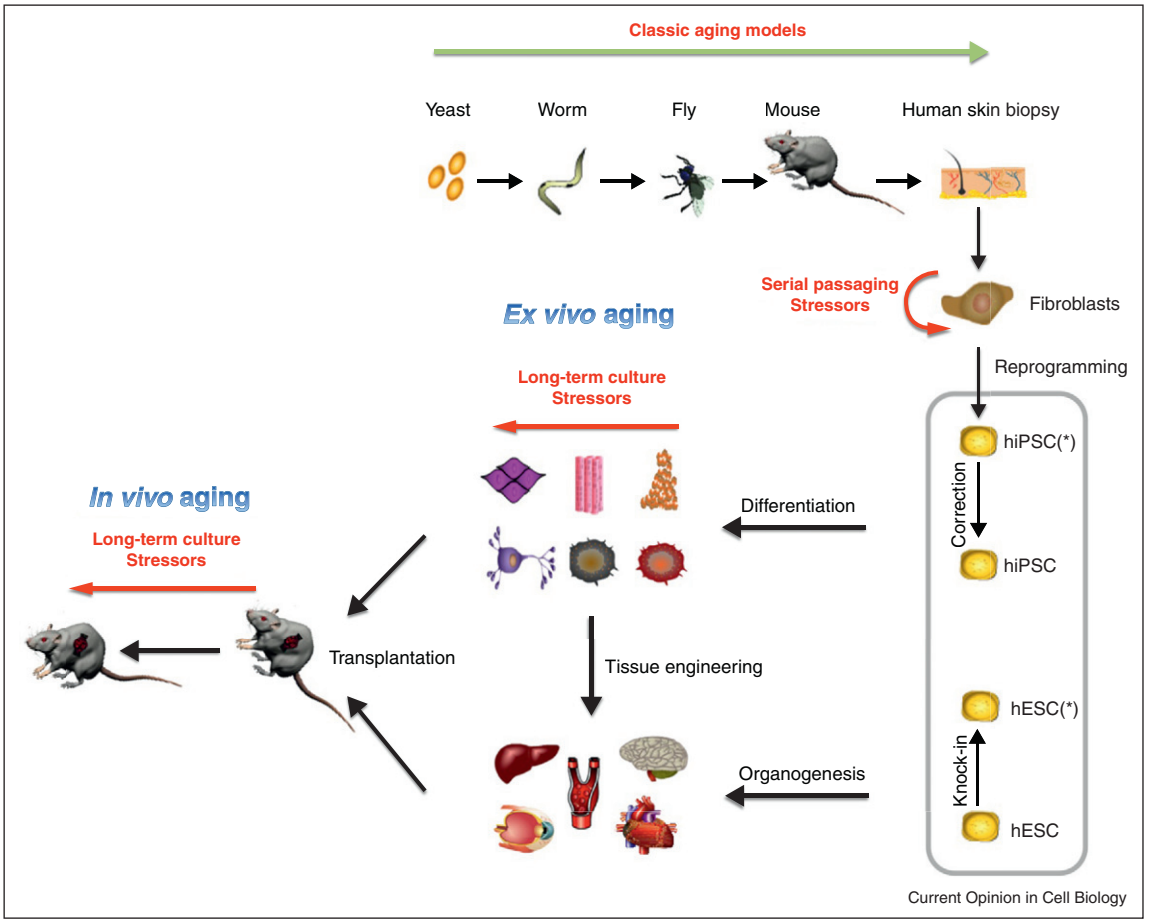
Owing to increased lifespan and subdued fertility, the world population aged 60 and over is anticipated to increase to 21.8% of the total population by 2050 [1]. Many individuals in an aging population will be inflicted with aging-associated diseases, such as various neurodegenerative disorders [2]. This phenomenon is of public concern and has thus spurred research in this area. It is believed that healthy aging could be accomplished if mechanisms underlying human aging were to be elucidated. Modern biological theories of human aging are classified into programmed theories and error theories. The programmed theories demonstrate that aging is regulated by some intrinsic mechanisms — by altered switch genes, changed hormones or even a dysfunctional immune system. On the contrary, the error theories emphasize cumulative environment-caused damage, such as reactive oxygen species, cross-linked macromolecules, DNA damage, and broken energy machines [3]. However, neither of these theories alone can explain all the phenomena and mechanisms at the root of aging. In fact, to date, the fundamental mechanisms of human physiological aging remain largely unknown.

Practical tools for studying aging encompass many model organisms. For instance, the insulin/insulin-like growth factor signaling pathway, a major molecular aging pathway, was first found in *Caenorhabditis elegans* and subsequently found conserved in all other model animals and humans [4]. As an ideal system to translate knowledge from lower organism models into mammalian species, a number of mouse aging models have been generated including those caused by mitochondrial oxidative stress [5], deficient DNA repair ability [6], overexpressed tumor suppressor genes [7] or abnormal genes involved in human premature aging syndromes [8]. However, mice have been separated from humans for 84–120 million years with distinct evolutionary pressures [9]. One of the many consequences is, for instance, the extension of telomere length (40–60 kb) in mice compared to humans (5–15 kb). Another difference is *p16* pathway, which appears to be uniquely employed in human aging [10]. Moreover, mice do not spontaneously develop neurodegenerative disorders, the major causes of disability and mortality among elderly people [11]. These pitfalls call for advanced human models. Here, we will focus on the evolution of human aging models (Figure 1), and will summarize results related to recently established hiPSC-based disease models for aging and various aging-related neurodegenerative disorders (Figure 2; Tables 1 and 2).

Classic cell models for human aging

In 1961 primary human cells were found to undergo population doubling only 50–100 times before encountering an inevitable proliferation arrest in culture. This phenomenon is termed replicative senescence, and represents the first popular human cellular aging model (Figure 1). The feasibility of replicative senescence is based on the fact that the number of times human fibroblasts can be passaged in culture is inversely proportional to the age of the donor [12]. Following this, normal human somatic cells were discovered to also undergo senescence upon exposure to aging-associated stresses such as DNA damaging and oxidative stress agents [13], or upon overactivation of oncogenes (*e.g.* Ras, Raf, and E2F2) [14]. Currently, to evaluate the progress of cell aging, several molecular hallmarks have been used, including

Figure 1



Evolution of aging models. Research on aging utilizes different model organisms including budding yeast, nematode worms, fruit flies, mice, and human beings. Classic human aging research models employ successive passaging resulting in replicative senescence and stress stimulations that can induce cell senescence in an accelerated way. Somatic reprogramming followed by directed differentiation, in combination with targeted gene editing technologies, is providing an unprecedented avenue to obtain various human cell and tissue types *in vitro* with which studying human aging and aging-related diseases becomes feasible. Moreover, cell and organ derivatives from patient-specific induced pluripotent stem cells (iPSCs) can be transplanted into animal models and the integrated human living materials could provide an opportunity to study human tissue and organ aging or disorders in an *in vivo* context. * indicates the cells bearing pathogenic mutation(s).

proliferative markers Ki67 and PCNA, senescence-associated β-galactosidase (SA-βgal) [15], senescence-associated heterochromatin foci (SAHF) [16], p16 [17••], and IL-8 [18••]. Another popular approach to study human aging is the investigation of dermal fibroblasts isolated from patients with premature aging syndromes (Figure 1), which share many similar features and mechanisms with physiological aging [19]. These syndromes include Hutchinson–Gilford progeria syndrome (HGPS), and Werner syndrome [20]. However, the fact that specific living tissue samples, such as neurons and vessel wall cells, are inaccessible severely hampers advancements in this field.

Induced pluripotent stem cell (iPSC)-based models for human aging

An important breakthrough in human aging models is the reprogramming of human somatic cells into hiPSCs by overexpression of OCT4, SOX2, KLF4, and c-MYC [21••]. Since then, powerful tools for establishing iPSC models for aging-related diseases have also emerged [22]. Owing to the self-renewal ability and pluripotency of hiPSCs, and established hiPSC directed differentiation protocols toward multiple lineages [23], various aged or diseased cell types can be massively cultured in a dish to re-establish patient-specific tissues and even organs for mechanism studies and drug discovery and testing (Figure 1).

Modeling human premature aging syndromes with iPSCs

Studying progeroid syndromes could lead to a greater understanding of normal human aging. Genetic background and disease characteristics are thoroughly studied for some progeroid syndromes such as HGPS. HGPS

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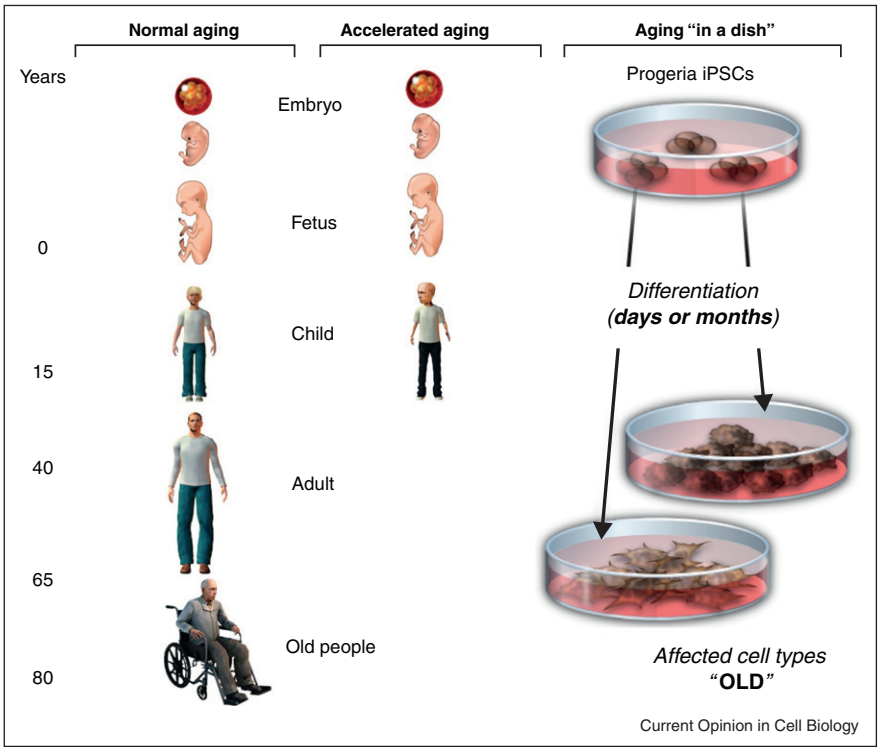
Figure 2



iPSC-based human aging models. Normal human aging expands several decades, and is affected and complicated by genetic and environmental factors. The length of this process hampers the study of the molecular and cellular mechanisms underlying aging. The use of iPSCs and their derivatives from patients with accelerated aging (like those with Hutchinson–Gilford progeria syndrome) may partially recapitulate the aging process *in vitro* and thus be an alternative model to study human aging in a dish.

patients show growth retardation after one year of age, followed by the appearance of wrinkled and sclerotic skin, decreased joint mobility, cardiovascular problems, and die at a median age of 13. HGPS is usually caused by a single nucleotide substitution of *LMNA*, which encodes lamins A and C [24]. The prevalent *LMNA* (G608G) mutation activates a cryptic splicing site in prelamin A, leading to a truncated mutant of lamin A known as progerin. Progerin accumulation results in abnormal nuclear envelopes, mis-regulation of heterochromatin and nuclear lamina proteins, attrited telomeres, and genomic instability [25]. The pathogenic progerin is typically present in vascular smooth muscle cells (SMCs), mesenchymal stem cells (MSCs), and dermal fibroblasts of HGPS patients. In addition, progressive accumulation of progerin also occurs in cultured senescent cells and cells of elderly individuals [26]. Notably, accumulation of progerin in MSCs has been suggested to contribute to accelerated as well as physiological aging progress [27].

Recently, three independent groups have established accelerated human aging models with hiPSC technology [28••,29••,30•] (Figure 2; Table 1). In agreement with the report that pluripotent stem cells (PSCs) do not express A-type lamins [31], HGPS–iPSCs show absence of progerin and nuclear envelope abnormalities. More importantly, the nuclear envelope-associated chromatin aberrances were also reset as a result of induced pluripotency. Five hundred and eighty-six autosome genes were found to be methylated differently between HGPS and healthy fibroblasts, while in iPSCs, only thirty-three genes were methylated differently [28••]. However, progerin expression and aging-associated phenotypes were restored in several differentiated mesodermal cell types, such as MSCs [29••], vascular SMCs [28••,29••], and fibroblasts [29••,30•,32••]. Moreover, the progerin-expressed mesodermal cell types are much more vulnerable to apoptotic stress [29••,30•]. In addition, progerin knockdown or targeted genetic correction of mutated *LMNA* in HGPS–iPSCs can effectively reverse disease phenotypes of their mesodermal derivatives, demonstrating that these observed aging-associated phenotypes are progerin-dependent [32••]. Since these HGPS–iPSC models present most of the expected pathologic features, they could be employed as invaluable platforms to study the molecular mechanisms of human premature aging disorders [33]. Moreover, studies based on these iPSC models may result in the discovery of novel mechanistic clues for physiological human aging. For instance, DNAPKcs, a DNA repair and telomere capping-related

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Table 1

PSC-based human aging models

Diseases	Genetic mutation	Primary cells	Years old	Reprogramming approaches	Differentiation	Relevant phenotype	Animal models	Refs
HGPS	LMNA (G608G)	Fibroblasts	8, 14	Retrovirus; OSKC	SMCs	Progerin expression, misshapen nuclei and lost H3K9me3 are restored	No	[28••]
	LMNA (G608G)	Fibroblasts	8, 14	Retrovirus; OSKC	MSCs, SMCs, Fibroblasts	DNA damage, nuclear abnormalities, and apoptosis induced by stresses and hypoxia are increased	Yes	[29••]
HGPS, aWS	LMNA (G608G, E578V)	Fibroblasts	3, 13	Retrovirus; OSKC Lentivirus; OSKCNL	Fibroblasts	Nuclear abnormalities, senescence and susceptibility to apoptosis are increased	No	[30•]
	LMNA (G608G, E578V)	Fibroblasts	3, 13	Retrovirus; OSKC	SMCs, Fibroblasts	Progerin and misshapen nuclei are restored but not in corrected cells	No	[32••]
DC	DKC1 (del-L37)	Fibroblasts	7	Retrovirus; OSKC	No	TR and DKC1 are upregulated during reprogramming	No	[41••]
	DKC 1 (L54V, ΔL37) TCAB 1 (H376Y, G435R)	Fibroblasts	7–45	Retrovirus; Lentivirus; OSKC	No	Lengthening of telomeres is abrogated, and extended culture leads to progressive telomere shortening and eventual loss of self-renewal	No	[42••]
	TERT (P704S and R979W)	Fibroblasts	>15	Lentivirus; OSKC	No	Reduction in telomerase levels blunts the natural telomere elongation, and extended culture leads to progressive telomere shortening and eventual loss of self-renewal	No	[42••]
Centenarian	–	Fibroblasts	92–101	Lentivirus; OSKCNL	Fibroblasts	Rejuvenated physiology	No	[72••]

HGPS, Hutchinson–Gilford progeria syndrome; aWS, atypical Werner syndrome; DC, dyskeratosis congenital; O, *Oct4*; S, *Sox2*; K, *Klf4*; C, *c-myc*; N, *Nanog*; L, *Lin28*; MSCs, mesenchymal stem cells; SMCs, smooth muscle cells.

protein kinase, is identified as a binding partner of progerin and is downregulated in HGPS fibroblasts, HGPS–iPSC-derived SMCs, as well as fibroblasts isolated from physiologically aged individuals [28••], thus providing a potential explanation of how progerin cooperates with dysfunctional telomeres or a defective DNA repair system to contribute to normal cellular aging [34].

Modeling human telomere dysfunction diseases with iPSCs

Telomeres are involved in the processes of both physiological aging and HGPS, supporting the idea that telomeres are a vital factor in aging. In fact, telomere length is regarded as a reliable marker for the age of human somatic cells [35••]. Telomeres are repeated sequences, which could be replenished by telomerase containing the telomerase reverse transcriptase (TERT) and telomerase RNA (TR). However, TERT exists only in pluripotent cells or cancer cells [36]. As a result, telomeres become gradually shorter in both mouse and human somatic cells with age [35,37] as well as cells with telomerase defects. Reprogramming of somatic cells into pluripotency provides a good platform to study telomere biology and aging mechanisms, because during reprogramming, telomerase activity is upregulated in both mouse and human cells [38•], although loss of telomerase results in compromised reprogramming efficiency [39••].

Dyskeratosis congenital (DC) is caused by mutations in the dyskerin gene (*DKC1*) resulting in shortened telomeres and accelerated cellular senescence [40]. An interesting question of whether or not DC fibroblasts could be reprogrammed and what the fate of telomeres in the resulting iPSCs would be has recently been addressed. DC-specific hiPSCs have recently been generated by the Daley and Artandi groups [41••,42••] (Table 1). Both studies demonstrate that telomerase impeccability is not necessarily required for the derivation of DC-hiPSCs. Using *DKC1* del-L37 mutant fibroblasts, the Daley group proved that telomeres became longer in DC-hiPSCs relative to DC fibroblasts through upregulation of *TR* and *DKC1*, and further *TR* upregulation was observed to be a common feature of pluripotent states during reprogramming of *DKC1*(del37L/A386T), *TR*($TR^{+/-}$) mutant

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Table 2

PSC-based human aging-related neurodegenerative disease models

Disease	Genetic mutation	Primary cells	Years old	Reprogramming method	Gene correction	Neural differentiation	Relevant phenotype	Refs
PD	Sporadic, G20446[a]	Fibroblasts	57	Retrovirus; OSKC	No	No	No	[47•]
	Sporadic, G20442, G20443, G20445, G20446, G08395[a]	Fibroblasts	53–85	Lentivirus; excisable, OSKC, OSK	No	Yes	No	[48]
	Sporadic, G20442, G20443, G20445, G20446, G08396[a]	Fibroblasts	53–85	Lentivirus; excisable, OSKC, OSK	No	Yes	No	[49]
	AG20442, AG20443, AG20446[a]	Fibroblasts	53–85	Lentivirus; excisable, OSK	No	Yes	Reduced motor asymmetry in PD–iPSC transplanted Parkinsonian rats	[50]
	LRRK2 (G2019S)	Fibroblasts	60	Retrovirus; OSK	No	Yes	Elevated alpha-synuclein expression, increased sensitivity to cellular stressors	[51•]
	PINK1 (C1366T, T509G)	Fibroblasts	53–71	Retrovirus; OSKC	No	Yes	Less recruitment of Parkin to the mitochondria	[52]
	SNCA (A53T, E46K)	Fibroblasts, hESCs	–	Lentivirus; excisable, OSKC	Yes	Yes	No	[53••]
	SNCA triplication	Fibroblasts	48	Retrovirus; OSKC	No	Yes	Accumulation of alpha-synuclein, inherent overexpression of oxidative stress markers, and increased sensitivity to peroxide-induced oxidative stress	[56]
	SNCA triplication	Fibroblasts	55	Retrovirus; OSKC	No	Yes	SNCA expression doubled	[54•]
	Parkin (ΔExon 3 or and 5)	Fibroblasts	–	Lentivirus; OSKC	No	Yes	Increased spontaneous DA release, decreased DA uptake and elevated ROS	[58]
	Sporadic, LRRK2 (G2019S)	Fibroblasts	51–66, 44–68	Retrovirus; OSK	No	Yes	Fewer and shorter neurites and a significant increase of apoptotic cells	[59]
AD	Sporadic	Fibroblasts	–	Lentivirus; OSK	No	Yes	Functional β-secretases and γ-secretases expressed	[66]
	PS1 (A246E), PS2 (N141I)	Fibroblasts	56, 81	Retrovirus; OSKNL	No	Yes	Increased amyloid β 42 secretion, sharply responsible to γ-secretase inhibitors and modulators	[55•]
	Sporadic, APP duplication	Fibroblasts	78, 46–53	Lentivirus; OSKC	No	Yes	Increased amyloid β, Pi-tau and aGSK-3β in sAD2 and APP^{Dp}	[65••]
	Trisomy of chromosome 21	Fibroblasts	–	Lentivirus; OSKC	No	Yes	Increased amyloid β peptide production and aggregates, phosphorylation and redistribution of tau	[67]
ALS	SOD1 (L144F)	Fibroblasts	82	Retrovirus; OSKL	No	Yes	No	[38•]
	TDP-43 (M337V)	Fibroblasts	56	Retrovirus; OSKC	No	Yes	Higher levels of soluble and detergent-resistant TDP-43, decreased survival, and increase vulnerability to PI3K pathway inhibition	[71]

PD, Parkinson's disease; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; O, *Oct4*; S, *Sox2*; K, *Klf4*; C, *c-myc*; N, *Nanog*; L, *lin28*.
[a] From Coriell Institute for Medical Research.

fibroblasts. By contrast, the Artandi group found that the same biochemical defects in original fibroblasts with *TCAB1*(H376Y/G435R), *TERT*(P704S/R979W) or *DKC1* (L54V/del37L) mutations were still present in DC-hiPSCs, including diminished telomeres and reduced telomerase activity. Moreover, the telomeres of *DKC1*–DC-hiPSCs are progressively shortened during extended culture, ultimately leading to loss of self-renewal. The authors claim that these processes accurately recapitulate the features of DC development. Although inconsistency has been observed between these two studies, both reports highlight the role of shorter telomeres in DC pathogenesis. A recent

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study by Wang *et al.* revealed the molecular mechanism of telomere heterogeneity of iPSCs [43], which may provide clues to the above contradiction. Reprogramming of telomerase and telomeres was found to be gradual, being influenced by telomerase gene activation, passage number and other telomerase-independent mechanisms in mouse iPSCs. Even some wild-type mouse iPSCs failed to lengthen telomeres, particularly at early passages. Moreover, for $TR^{-/-}$ iPSCs, telomeres were gradually shortened with increased chromosome fusion during later passages. This indicates that telomerase deficiency could not block reprogramming onset but is essential for telomere maintenance and chromosomal stability of iPSCs. Such conclusions are in line with the situations encountered with human *DKC1*–DC iPSCs.

hiPSC-based models for aging-related degenerative diseases

Aging is perhaps the biggest risk factor for many human diseases. During aging, there are a number of cellular alterations, such as accumulated mis-folded proteins, that may contribute to aging-related diseases. For example, mis-folded proteins are found in many neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). Incidence of these diseases increases with aging. iPSC technology has shown huge potential to study these degenerative diseases.

PD is one of the most common aging-associated neurodegenerative disorders, characterized by accumulation of Lewy body inclusions and preferential loss of dopamine (DA) neurons in the substantia nigra pars compacta [44]. Notably, mutations in α-synuclein (*SNCA*) and leucine-rich repeat kinase 2 (*LRRK2*) genes frequently cause autosomal dominant PD, while loss-of-function mutations in PTEN-induced putative kinase 1 (*PINK1*) or Parkin (*PRKN*) are implicated in autosomal recessive PD [45]. Direct studies of such neurological disorders in humans are impractical, since vital neuron isolation is difficult. One alternative has been the use of neuroblastoma cell lines [46]. This and other models, however, are far from mimicking a physiological setting, and limited toward the elucidation of cellular and molecular mechanisms as well as clinical applications. iPSC technology makes it a reality for PD specific neuronal cells to be studied *in vitro*. In recent years, about a dozen PD–iPSC models have been established [47•,48–50,51•,52,53••,54•,55•,56–59] (Table 2). Specific aspects of PD-associated phenotypes have been successfully recapitulated. Consistent with α-synuclein aggregates and DA neuron loss in PD patients, *SNCA* triplication iPSC-derived DA neurons display a twofold increase of α-synuclein [54•], increased expression of oxidative stress markers, and higher sensitivity to oxidative injuries [56]. A similar situation was observed for *LRRK2* G2019S iPSC-derived DA neurons [51•], which is consistent with the notion that *LRRK2* and *SCNA* may share a common pathogenic pathway in PD [60]. As for recessive PD, in *PRKN* (Exon 3 and/or 5 lost) mutant iPSC-derived DA neurons, Parkin dysfunction reduces DA uptake and enhances DA release. These alterations may result from the increased expression of oxidative stress and monoamine oxidase, which are important roadblocks of precise neurotransmission [58]. In *PINK1* (C1366T, T509G) mutant iPSC-derived DA neurons, recruitment of Parkin to mitochondria is impaired, and neurotransmission is consequently abnormal [52]. These findings indicate that these recessive PD mutations may result in DA neuron dysfunction through a defective Pink1–Parkin pathway.

AD is a prevalent age-dependent neurodegenerative disorder [61]. Extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau proteins are two definitive traits for diagnosed AD. It is unknown, however, whether and how these tangles and plaques contribute to disease progression [62]. Mutations of APP, presenilin 1 (PS1), and presenilin 2 (PS2) are identified in early-onset (<60 years) familial AD (FAD) [63] which accounts for less than 5% of AD cases, while the vast majority (>95%) are attributed to late-onset sporadic AD (SAD). In the past two years, hiPSC models have been successfully established for both SAD and FAD caused by PS1 mutation (A246E [55•]/L166P [64]) or PS2 mutation (N141I) [55•] or APP gene duplication (APP^{Dp}) [65••,66] (Table 2). All of these iPSCs are able to differentiate into neuronal or glial cells, recapitulating features of AD to different extents. The neurons derived from FAD–iPSCs with PS1 or PS2 mutations showed increased Aβ42 secretion, which was sharply reduced by treatment with γ-secretase inhibitors [55•,64,66]. Using iPSC-derived AD neurons, Israel *et al.* reported some new AD-related phenotypes including the presence of aberrant early endosomes and elevated GSK3β activity. Of importance, they reasoned that it was primary products of APP processing rather than the previously conceived end product Aβ that drove tau phosphorylation and aggregation [65••]. Additionally, other diseases frequently accompany AD. Recently, cortical neurons derived from iPSCs from a Down syndrome patient showed enhanced hyperphosphorylated tau protein and secretion of Aβ [67]. Comprehensive study of these diseased neurons with different genetic aberrances will result in a better understanding of the disease mechanisms.

ALS is a fatal neurodegenerative disorder typically affecting people between the ages of 50 and 60, characterized by the degeneration of upper and lower motor neurons [68]. Sporadic ALS (SALS) and familial ALS (FALS) demonstrate similar pathological features, including the atrophy of dying motor neurons, intracytoplasmic abnormalities of neurofilaments and the formation of Bunina bodies. Mutations of the superoxide dismutase

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1 (*SOD1*) gene are the most well studied causative gene, making up for about 20% of FALS, while mutations of two other genes, *FUS/TLS* and *TDP-43*, make up for about 5–10% [69]. hiPSC-derived neurons provide a potential experimental system to study motor neuron degenerative disorders (Table 2). Initially, human embryonic stem cell (hESC)-derived motor neurons over-expressing three different mutations (G93A, A4V, and I113T) of *SOD1* were obtained, demonstrating characteristics of ALS-related degeneration such as expansive neural cell death and decreased neurite extension [70]. Further, the Dimos and Bilican groups generated ALS-specific iPSCs from two patients bearing *SOD1* (L144F) and *TDP-43* (M337V) mutations respectively [38•,71]. For the former, ALS iPSCs could be effectively differentiated into mature motor neurons and glia, although no obvious ALS-related phenotypes were identified. For the latter, *TDP-43* mutated motor neurons displayed elevated vulnerabilities toward PI3K pathway blocking, consistent with motor neuron degeneration in ALS. Taken together, ALS, despite its aging-dependent penetrance, can be modeled in a dish in an accelerated way.

Conclusions and perspectives

Human aging is a progressive process resulting in gradual defects of the genome, epigenome, and molecular and organelle hemostasis in different cells and tissues. Reprogramming toward pluripotency enables resetting of the cellular clock and removal of most, if not all, of the aging-associated cellular hallmarks [72••,73,74]. So far, almost all types of aged or diseased iPSCs can be generated, even in certain cases where reprogramming was previously thought to be impossible [72••,75]. These iPSCs, therefore, hold the potential to recapitulate phenotypes of various aging-related diseases (Figures 1 and 2). Instead of the several decades needed for human physiological aging, a period of only days or months is needed before cell aging and disease phenotypes are displayed in culture conditions, probably due to a complex interplay between endogenous genetic defects and suboptimal culture systems.

To obtain appropriate culture conditions that can induce aging-related phenotypes, extended culture time is usually a required condition. However, optimization of 'pro-aging medium' could be a catalyst for enabling successful recapitulation of aging-associated features. In some cases, supplementation in culture with aging-associated stresses, such as oxidative stress inducers, DNA damaging agents, or proteasome inhibitors, could be a better way. Apart from the proven feasibility of modeling aging-related diseases in a dish, other approaches, including developing hiPSC-derived organs *in vitro* [76,77••,78,79] or animals integrated with hiPSC derivatives [80••,81], could be superior strategies for obtaining closer physiological settings for disease modeling. Therefore, by utilizing hiPSC disease models, we can not only gain insight into the molecular mechanisms of human aging, but also create an unprecedented platform for developing novel drugs to realize healthy aging and prevent or cure various aging-related diseases. Even more attractive is the potential to combine gene-targeting technologies [32••,53••,82] with patient-derived iPSCs and their derivatives to obtain corrected, safe and advanced transplantation materials for treatment of aging-related degenerative disorders in the future.

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Papers of particular interest, published within the period of review, have been highlighted as:

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