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Aging and reprogramming: a two-way street

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Aging is accompanied by the functional decline of cells, tissues, and organs, as well as a striking increase in a wide range of diseases. The reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) opens new avenues for the aging field and has important applications for therapeutic treatments of age-related diseases. Here we review emerging studies on how aging and age-related pathways influence iPSC generation and property. We discuss the exciting possibility that reverting to a pluripotent stem cell stage erases several deficits associated with aging and offers new strategies for rejuvenation. Finally, we argue that reprogramming provides a unique opportunity to model aging and perhaps exceptional longevity.

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Introduction

Aging is associated with a dramatic increase in a wide range of age-related diseases, including cancer, cardiovascular dysfunction, metabolic disorders, and neurodegeneration. Even in the absence of identifiable disease, the physiology of organs and tissues declines throughout life. Within a tissue, both differentiated cells and adult stem cells are susceptible to intrinsic and extrinsic changes during aging. For example, old cells accumulate genomic damage and aggregated proteins, and display telomere erosion and mitochondrial dysfunction [1–6]. In addition to these intrinsic damages, cellular aging is also influenced by exposure to extrinsic factors, including inflammatory cytokines [7–9]. Despite the seemingly irreversible nature of age-associated wear and tear, aging is not just a one-way street toward decline. Several examples highlight the plasticity of the aging process. Genetic pathways (e.g. insulin-FoxO, TOR, AMPK, and Sirtuin) as well as environmental interventions (e.g. dietary restriction and rapamycin treatment) can delay aging, even if initiated late in life [10–14]. Furthermore, interventions such as parabiosis – joining the circulatory

system of an old animal to that of a young one – can reverse some aspects of aging in somatic stem and differentiated cells of the old individual, in tissues as diverse as muscle and brain [15,16].

Reprogramming of somatic cells into cells with embryonic stem cell (ESC) properties termed induced pluripotent stem cells (iPSCs) is a major scientific breakthrough for many areas of biology and medicine [17]. For the aging field, such a discovery has several fundamental implications. First, many age-related pathologies such as neurodegenerative diseases could benefit from regenerative therapies. In this way, patient-derived iPSCs hold great promise for this type of clinical application as they bypass issues regarding immune rejection and ethical concerns related to human embryonic stem cell (hESC) derivation. Second, reprogramming can be considered to mimic the ‘resetting’ that occurs during meiosis and fertilization, which allows the formation of a new individual from two older individuals. Thus, reprogramming may hold the key to ‘immortality’ and provide pivotal insights into possible rejuvenation strategies [18]. Finally, reprogramming allows the generation of patient-specific iPSCs with genetic predisposition to premature aging or exceptional longevity, thereby facilitating drug screening and understanding of the fundamental mechanisms underlying aging and longevity.

Many important questions stem from the interface of aging and reprogramming. How does age of the donor influence the generation and quality of iPSCs? Can genes involved in aging impact the reprogramming process? Are defects associated with cellular aging erased by reprogramming? Is it possible to model aging or longevity using iPSCs? This review will highlight emerging answers to these questions as well as remaining gaps in knowledge. A better understanding of the influence of age on reprogramming may help improve the current low reprogramming efficiency of somatic cells. Elucidation of the possible erasure of age-related defects in somatic cells by reprogramming may provide new tools to prevent or even reverse aging *in vivo*.

Do aged cells reprogram as well as young cells?

The first studies that tested how age of the donor affects reprogramming were conducted in mice (Table 1). Compared to humans, mice have the advantage of identical genetic background and controlled environment, thereby allowing one to focus on age as the main parameter. These initial studies suggest that cells from older mice tend to reprogram less efficiently than cells from young

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Table 1

Summary of the current literature on the impact of age on reprogramming. *Bona fide* iPSCs are defined as iPSCs expressing early and late markers of pluripotency, *in vitro* and/or *in vivo* differentiation potential into the three germ layers

Species	Cell type	Protocol	Age groups	Reprogramming Efficiency	Bona fide iPSCs	Re-differentiation potential	Reference
Mouse	Dermal fibroblasts from ear punches (C57BL/6 mice)	OSKM	2 vs >24 months	2.2-fold higher in younger	Not assessed	Not assessed	Li <i>et al.</i> , 2009 [19**]
Mouse	Dermal fibroblasts (B6CBAF1 mice)	OSKM	Juvenile vs 12 months	5-fold higher in younger	Yes	Haematopoietic cells and osteoblasts No age comparison performed	Kim <i>et al.</i> , 2010 [21**]
Mouse	Bone marrow cells (C57BL/6 mice)	OSKM	2 vs 23 months	5-fold higher in younger, and twice as fast	Yes	Myeloid cells No age comparison performed	Cheng <i>et al.</i> , 2011 [22*]
Mouse	Muscle-derived fibroblasts (C57BL/6-background <i>mdx</i> mice)	OSKM	1.5 vs 6 vs 14 months	6-fold higher in 1.5 and 6 months compared to 14 months	Yes	Skeletal muscle lineages No age comparison performed	Wang <i>et al.</i> , 2011 [20]
Human	Fibroblasts from various tissues of donors of both sexes and with different disease states	OSKM	8–64 years	No correlation with age	Yes	Definite endoderm. No age comparison performed	Somers <i>et al.</i> , 2010 [24]
Human	Dermal fibroblasts	OSKM + NANOG + LIN28	70 years	Not assessed	Yes	Fibroblast No age comparison performed	Suhr <i>et al.</i> , 2010 [28]
Human	Dermal fibroblasts of donors of both sexes and with various disease states	OSK and OSKM	29–82 years	Not assessed	Yes	Motor Neurons Efficiency not correlated to age	Boulting <i>et al.</i> , 2011 [25]
Human	Dermal fibroblasts	OSKM	84 years	Not assessed	Yes	Neuronal lineages No age comparison performed	Prigione <i>et al.</i> , 2011 [30*]
Human	Senescent and proliferative dermal fibroblasts	OSKM + NANOG + LIN28	74–101 years	Not assessed	Yes	Fibroblast No age comparison performed	Lapasset <i>et al.</i> , 2011 [26**]
Human	Keratinocytes	OSKM	56–78 years	Not assessed	Yes	Insulin-producing cells	Ohmine <i>et al.</i> , 2012 [27]
Human	Dermal fibroblasts	OSKM	106–109 years	Not assessed	Yes	Neural cells No age comparison performed	Yagi <i>et al.</i> , 2012 [29*]

mice [19**,20,21**,22*]. For example, dermal fibroblasts from old mice (>2-years) exhibit a two-fold reduction in their ability to generate colonies that stain positive for a stem cell marker, alkaline phosphatase (AP), compared to fibroblasts from young adult mice (2 months-old) upon expression of the four Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc) [19**]. Even more strikingly, dermal fibroblasts from middle-aged mice (1 year-old) were shown to result in a five-fold lower frequency of AP+ colonies compared to juvenile mice (exact age not stated) upon expression of the four Yamanaka factors [21**]. Furthermore, bone marrow cells from old mice (23 months-old) generated at least five-fold fewer AP+ colonies than cells from young adults (2 months-old), and the reprogramming process was reported to take twice as long [22*]. Collectively, these studies suggest an age-dependent decline in reprogramming efficiency in mice. However, several important points remain to be tested before such a

general conclusion can be reached. First, most studies only compared two age groups – old versus young, with the young being 2 months or less. It is not yet clear whether the observed impact of age on reprogramming is truly a difference of aging or a difference between post-natal development and mature adults. Furthermore, many of the studies based their reprogramming efficiency analysis on the number of clones that are positive for alkaline phosphatase, an early marker of pluripotency. It will be important to assess quantitatively other characteristics of iPSCs to provide a *bona fide* measure of reprogramming efficiency. Moreover, some of the studies used only one mouse per age group. Including a larger number of mice per age group to identify potential variations in reprogramming efficiency will be necessary.

While there is an apparent decline in reprogramming efficiency in aged populations of cells, it also appears

that some *bona fide* iPSC lines could be derived from cells from old donors, as measured by the qualitative expression of pluripotency markers and by *in vitro* and *in vivo* differentiation potential. Thus, aging may be a barrier for the initiation of the reprogramming process, but once the process is initiated, it appears to proceed in a relatively complete manner, at least to the iPSC state. An important remaining question is whether iPSCs derived from old donors have the full capacity to differentiate into functional cell types or whether they keep a 'memory' of the initial age of the donor. Another interesting point is that reprogramming potency seems to be impaired to a greater extent in bone marrow cells than in dermal fibroblasts [19^{••},22[•]], suggesting that different tissues may have varying sensitivity to age-dependent changes related to reprogramming efficiency.

Generating iPSCs from old human cells

Human studies are inherently difficult to interpret because differences in genetic background could supersede the impact of age on iPSC quality. Indeed, genetic background strongly influences reprogramming efficiency in mice [23]. Furthermore, the conditions under which primary fibroblasts are generated from human biopsies and the number of passages these fibroblasts undergo before reprogramming may also significantly impact reprogramming efficiency. Initial studies in humans suggest that, contrary to what has been observed in mice, aging does not drastically impair the ability of human cells to reprogram into *bona fide* iPSCs (Table 1). Somers and colleagues generated >100 iPSC lines from fibroblasts of 12 individuals with an age-range of 8–64 years using the four Yamanaka factors. The reprogramming efficiency ranged from 0.1 to 1.5%, but this variation was not significantly correlated with donor's age. *Bona fide* iPSC clones could be derived from all subjects regardless of age, as measured by expression of pluripotency markers and by teratoma formation assays. Furthermore, all the iPSC lines tested could give rise to definite endoderm cells, suggesting that age has no major impact on the re-differentiation potential of iPSCs, at least into endoderm [24[•]]. However, it is important to note that in this study, fibroblasts were obtained from different tissues, the donors had varying forms of lung diseases, and were of both sexes, all parameters that might have obscured a potential impact of age on reprogramming.

Several other studies have also succeeded in deriving *bona fide* iPSC lines from older patients [25,26^{••},27,28,29[•],30[•]] (Table 1). Suhr and colleagues derived *bona fide* iPSCs from primary fibroblasts of a 70-years-old patient using a six-factors cocktail containing OCT4, SOX2, KLF4, c-MYC, NANOG and LIN28, and succeeded in re-differentiating them into fibroblasts. However, no specific age comparisons were made [28]. Boulting and colleagues compared 16 iPSC lines from seven individuals of varying age (29–82 years old), sex, and health

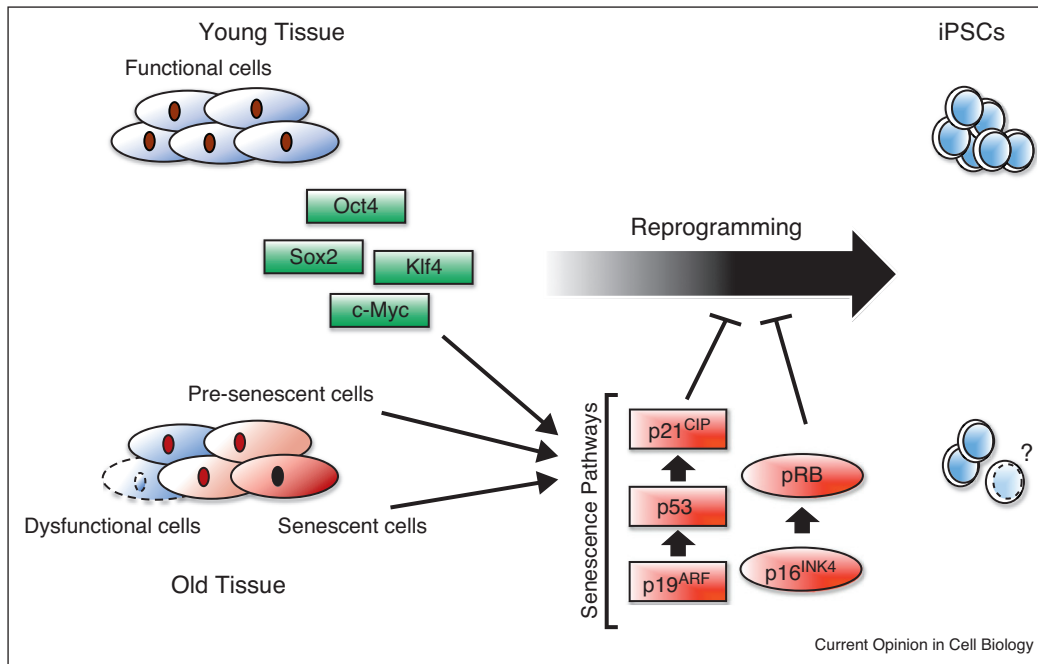
status in terms of differentiation potential. There was no correlation between donor age and the re-differentiation efficiency of the iPSC lines. These lines expressed pluripotency markers, formed the three germ layers *in vitro* and *in vivo* and differentiated into functional motor neurons at comparable levels [25]. Ohmine and colleagues derived iPSC lines from human keratinocytes of four individuals with an age-range of 56–78 year-old, using the four Yamanaka factors [27]. Although reprogramming efficiency was very low in this study (~0.0001%), *bona fide* iPSC lines could be derived, and these lines expressed a range of pluripotency markers and exhibit the ability to differentiate into the three germ layers. Furthermore, these iPSC lines could give rise to insulin-producing cells regardless of age [27]. Finally, two independent studies succeeded in reprogramming centenarian human fibroblasts into iPSCs using either the conventional four factors [29[•]] or a six-factor cocktail [26^{••}]. iPSC lines from centenarian fibroblasts expressed pluripotency markers and gave rise to the three embryonic lineages, showing *bona fide* iPSCs could be generated from exceptionally old donors [26^{••},29[•]]. Thus, one common conclusion from studies in humans is that iPSCs can be generated from old patients and these cells appear to be able to re-differentiate in different tissues. Thus, it is conceivable that patient-derived iPSCs could be used for degenerative diseases, even when they originate from older patients. However, understanding the exact impact of age on human reprogramming will require more quantitative assays to accurately determine reprogramming efficiency and re-differentiation capabilities as a function of donor's age. The investigation of larger numbers of subjects with controlled genetic and environmental backgrounds will also be needed to appropriately isolate the age parameter. Moreover, as aging is accompanied with accumulation of genomic mutations that may compromise the cells' natural defenses against tumor development [31] (discussed below), further investigation is required to evaluate whether iPSCs derived from old donors are more prone to develop cancer than iPSCs derived from young donors.

Cellular senescence, aging, and reprogramming

Cellular senescence, which is associated with aging, may be one of the mechanisms by which aging impairs reprogramming efficiency, at least in mice [19^{••},32^{••},33^{••},34–38]. Senescent cells are characterized by a virtually irreversible cell cycle arrest, p16^{INK4} induction, and heterochromatin foci [39,40]. Because cell cycle progression is believed to be a key parameter for the reprogramming process [41,42], the cell cycle arrest due to cellular senescence may represent a major barrier to reprogramming. The number of senescent cells increases in the body during aging [40,43,44] and in patients with genetic diseases that recapitulate some aspects of aging such as Hutchinson–Gilford Progeria syndrome (HGPS) [45,46]

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Figure 1



Cellular mechanisms by which age may impact reprogramming of somatic cells into iPSCs. Studies in mice indicate that aging hampers reprogramming efficiency [19**,20,21**,22*]. This decrease may partly be due to the fact that old tissues contain a more heterogeneous pool of cells, including normal, pre-senescent, senescent and dysfunctional cells that may not reprogram. Senescence pathways including the p19^{ARF}/p53/p21^{CIP1} and the p16^{INK4}/pRB pathways have been shown to constitute barriers to the reprogramming process [19,32**,33**,35–38]. The reprogramming process *per se* induces a stress similar to senescence [33**] that may push pre-senescent cells into a fully senescent state, which may further hamper reprogramming. Although initial studies suggest that iPSCs derived from old donors are as potent as their young counterparts in terms of pluripotency and re-differentiation potential, further studies are required to fully understand the impact of age on the iPSCs, including their tumorigenic potential.

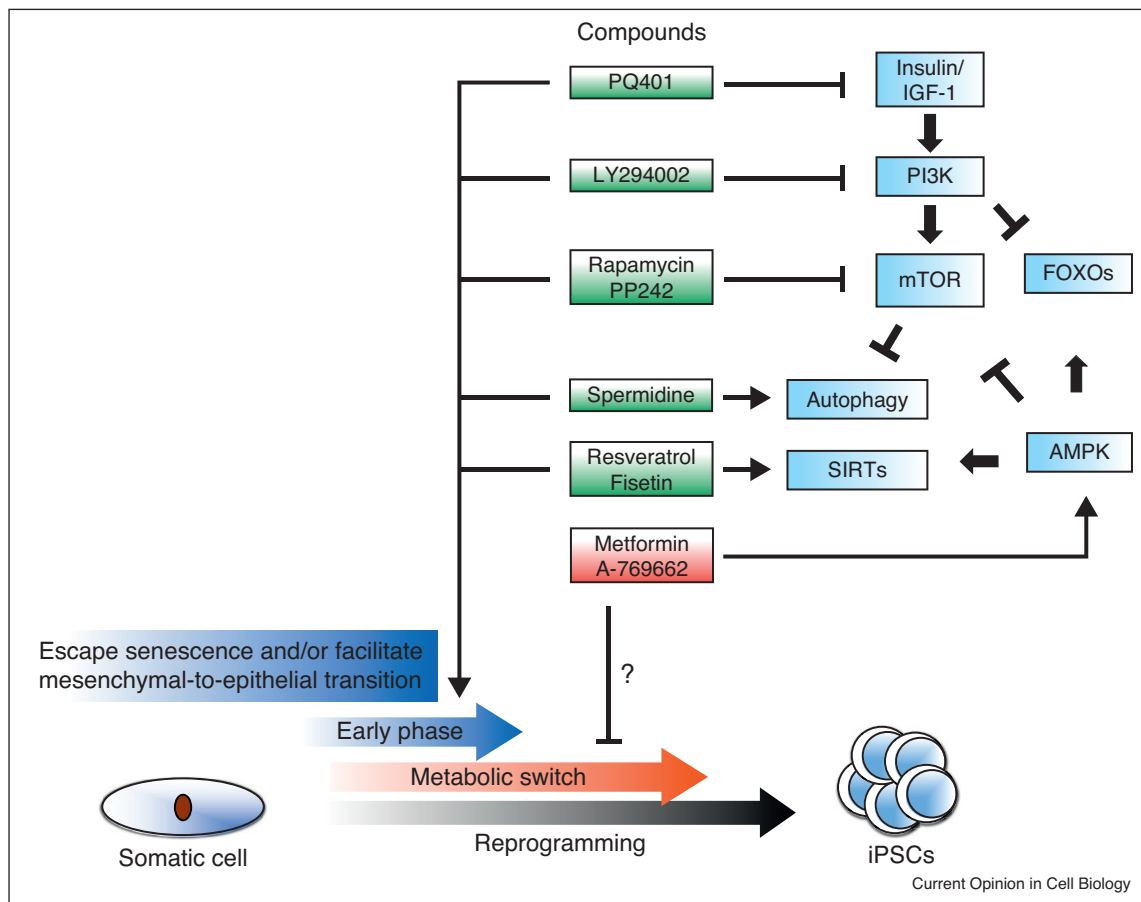
and Dyskeratosis congenita (DC) [47]. Consequently, cell populations obtained from old donors may contain greater number of senescent, pre-senescent and dysfunctional cells (Figure 1), which would decrease the reprogramming efficiency. Moreover, the reprogramming process itself appears to trigger a stress response similar to senescence, called reprogramming-induced senescence (RIS) [33**]. Cells from old donors may have intrinsic senescence pathways already activated (pre-senescent cells) and may therefore be more sensitive to RIS and more difficult to reprogram (Figure 1). Indeed, the age-dependent decline in reprogramming efficiency of mouse fibroblasts correlated with increased expression of the Ink4/Arf locus, which contains the two anti-proliferative genes *p16^{INK4}* and *p19^{ARF}* [19**]. Interestingly, the age-dependent decline in reprogramming efficiency could be counteracted by silencing Ink4/Arf expression using shRNAs [19**]. Consistently, knockdown of genes that are important for cellular senescence, such as *p53* and *p21^{CIP1}*, increase the efficiency of mouse and human reprogramming by both accelerating the reprogramming process as well as increasing the yield of iPSC colonies [19**,36–38]. These data suggest that cellular senescence

is at least partly responsible for the decrease in reprogramming associated with aging. However, senescence does not appear to be an insurmountable barrier to reprogramming because human senescent cells have been successfully reprogrammed to iPSCs using a six-factor reprogramming cocktail [26**].

Aging pathways, metabolism, and reprogramming

An intriguing question is whether interfering with aging pathways involved in energy metabolism ameliorate the ability of old cells (or even young ones) to generate functional iPSCs. One of the best example of a pathway involved in aging and metabolism is the insulin/IGF-1 pathway [13]. Deficiency in the insulin/IGF-1 receptor extends lifespan from worms to mammals [13] and is associated with extreme longevity in humans [48]. While it is not yet known if genetic deficiency in the insulin/IGF-1 pathway affects reprogramming, a recent study showed that several chemical inhibitors of this pathway improved reprogramming. Pharmacological inhibitor of the insulin/IGF-1 signaling pathway, such as the inhibitor of IGF1 receptor (PQ401) and a PI3K inhibitor

Figure 2



Compounds that modulate well-known aging and metabolism pathways affect somatic cell reprogramming [49**,62*]. Green boxes depict compounds that enhance reprogramming, and red boxes depict compounds that inhibit the process. The pro-reprogramming compounds have been shown to act in the early phase of reprogramming, potentially by preventing hyper activation of insulin/IGF-1 and mTOR pathways that may in turn induce cellular senescence and/or facilitate mesenchymal-to-epithelial transition [49**]. It is not clear yet whether treatment with metformin hampers reprogramming or enhances it [49**,62*].

(LY294002), increase reprogramming efficiency by 2–4 fold of mouse embryonic fibroblasts (MEFs) transduced by retroviral vectors expressing OCT4, SOX2, KLF4 and c-MYC [49**] (Figure 2).

Another conserved protein that regulates aging is the protein kinase mTOR. Genetic or pharmacological inhibition of the mTOR pathway promotes longevity from yeast to mammals [12,50]. mTOR regulates longevity partly by modulating autophagy [51]. Two inhibitors of the mTOR pathway (rapamycin and PP242) and an inducer of autophagy (spermidine) also improved the reprogramming efficiency of MEFs 4–5 fold (Figure 2). The iPSC lines generated in the presence of the two mTOR inhibitors expressed pluripotency markers, were karyotypically normal, and could give rise to germline chimeras [49**]. It may seem counter-intuitive that inhibition of two signaling pathways (insulin/IGF-1 and

mTOR) that promote cell proliferation actually enhances reprogramming, as proliferation is highly correlated to reprogramming potency [41,42]. However, hyper-activation of mTOR can also induce senescence [52,53]. Inhibitors of insulin/IGF-1 and mTOR may thus act to fine-tune the activity of these pathways to avoid cellular senescence [54]. Accordingly, inhibition of both pathways was shown to improve the initial phase of reprogramming [49**], a phase in which senescence acts as a major barrier to reprogramming. In addition, these inhibitors may act by facilitating a mesenchymal-to-epithelial transition (MET) [49] (Figure 2), as cells of mesenchymal origin such as fibroblast are thought to undergo MET before they can initiate the reprogramming process into pluripotent state [55,56]. Whether these inhibitors may help the reprogramming of old cells even more than that of MEFs is not known. It will be interesting to further explore the molecular and cellular mechanisms by which

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modulation of these pathways influences reprogramming, in particular, whether and how autophagy is involved in iPSC generation and function.

The AMP-dependent protein kinase (AMPK) and the NAD⁺-dependent histone deacetylases of the Sirtuin family have been implicated in promoting longevity and metabolic health, in particular in response to some dietary restriction regimens [57–61]. An initial study showed that while activation of Sirtuins by two compounds (Resveratrol and Fisetin) enhanced reprogramming 6-fold, activation of AMPK by metformin had no significant effect on iPSC generation [49**] (Figure 2). However, somewhat surprisingly, a second study reported that activation of AMPK with two chemical activators (metformin and A-769662) in fact reduced the generation of AP⁺ cells from MEFs and adult human fibroblasts by 60–90% [62*] (Figure 2), although it is not clear whether these AP⁺ cells were indeed *bona fide* iPSCs. The authors suggest that AMPK activation may prevent the metabolic switch from oxidative to glycolytic metabolism [62*], a transition that is essential for the initiation for somatic cell reprogramming [63–65].

Taken together, these studies suggest a functional correlation between regulation of cell reprogramming and pathways involved in aging and metabolism. This connection could be harnessed for better reprogramming efficiency and iPSC quality. These preliminary results also raise the possibility that depending on the metabolic state of the organism (for example, caloric restriction or obesity), donor cells may not be more or less conducive to reprogramming.

Epigenetic regulators of aging

Aging is accompanied with alterations in chromatin states triggered by changes in DNA methylation, post-translational modifications of histones, and histone protein levels. In many vertebrate species, old cells exhibit an age-dependent global loss of DNA methylation [66,67], an epigenetic mark generally associated with gene repression [68]. Moreover, histone marks associated with active chromatin (e.g. H4K16ac) tend to increase with age, at least in yeast [69]. H3K4me3 levels, which is also generally associated with active chromatin, has been shown to change at specific genes with age in human brain [70]. Conversely, histone marks associated with repressed chromatin (e.g. H3K27me3, H3K9me3) decrease with age in species ranging from worms to humans [71–74]. Finally, aging is associated with a decrease in the abundance of the core histone proteins H2A, H3 and H4 [69,75–79]. Thus, chromatin states appear to become less repressed with age, which might either increase the expression of aging genes and promote general increased transcription/translation, thereby possibly leading to excessive protein misfolding or increase accessibility of DNA to damage.

Consistent with a causative role for age-dependent changes in chromatin states, chromatin modifiers can influence longevity in several species. Decreasing H4K16Ac via the Sir2 deacetylase promotes longevity in yeast [69]. Deficiency in members of the COMPASS complex, which is responsible for generating H3K4me3, extends lifespan in *C. elegans* [80,81]. Conversely, over-expression of RBR-2, an H3K4me3 demethylase, promotes lifespan extension in *C. elegans* [81], and RBR-2 mutants in both *C. elegans* and *Drosophila* are short-lived [81,82]. Depletion of UTX, an H3K27me3 demethylase, also extends lifespan in *C. elegans* [71,80]. These results suggest that manipulating chromatin states can influence longevity, at least in invertebrates.

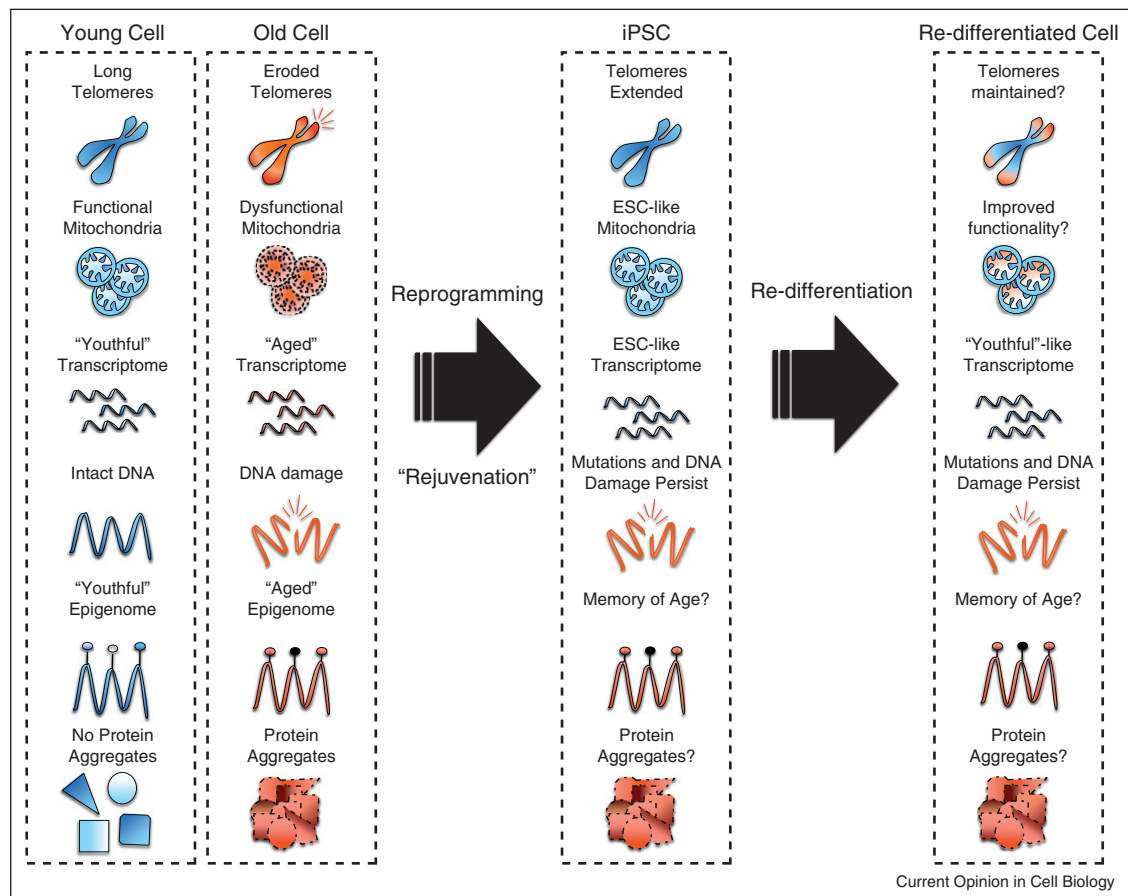
To the authors knowledge there are no studies to date that have examined if the epigenetic changes of old cells impact reprogramming efficiency or if age-dependent epigenetic alterations are themselves affected by reprogramming. However, emerging evidence points to possible antagonistic interactions between chromatin states associated with aging and those important for reprogramming. Several members of the H3K4me3 regulating COMPASS complex, whose deficiency extends lifespan in worms [80,81], are upregulated during the reprogramming process. Accordingly, knockdown of one member of the COMPASS complex (Wdr5) in MEFs dramatically decreases the number of AP⁺ and SSEA1⁺ clones [83**]. Conversely, inhibition of histone deacetylases by Valproic acid or Butyrate, which leads to increased levels of histone acetylation and chromatin opening, improves overall reprogramming efficiency and can even substitute for some of the reprogramming factors [84,85]. These results suggest that chromatin changes that are favorable for cellular reprogramming are detrimental for organismal longevity (and vice versa). Future investigation in this area is required to identify the epigenetic changes of old cells and how they are impacted by reprogramming. It would be particularly interesting to test if the antagonistic roles of chromatin states on aging and cellular reprogramming can be uncoupled.

Does reprogramming erase signs of age?

Reprogramming has the remarkable ability to reverse some cellular and molecular characteristics associated with aging, including cellular senescence, telomere erosion, mitochondrial dysfunction, and global changes in gene expression, suggesting that many of the age-associated characteristics that were once thought to be permanent are, in fact, reversible (Figure 3).

Senescent cells obtained by serial passaging of dermal fibroblasts of a 74-year-old donor could be reprogrammed using a six-factor cocktail. iPSC lines derived from senescent fibroblasts express pluripotency markers and can give rise to the three germ layers, showing that they are *bona fide* iPSCs. Interestingly, when iPSCs from

Figure 3



Aspects of aging that can be 'rejuvenated' or not by reprogramming.

senescent cells were re-differentiated into fibroblasts, these fibroblasts proliferated at a similar rate as young proliferative fibroblasts, and became senescent after a greater number of passage doublings compared to the donor cells [26^{••}]. Thus, cellular senescence, which is often considered an irreversible cell cycle arrest, can be reverted by potent reprogramming protocols.

Telomere erosion is a characteristic of aged and senescent cells [3,86,87]. Reprogramming triggers an increase in telomere length in cells from old mice and humans [26^{••},27,28,29[•],88,89] (Figure 3). However, whether the resulting iPSC lines can maintain their long telomere length over long-term passages is still subject to debate. It was shown that iPSC lines generated from a hES-derived cell line using a three-factor protocol (Oct4, Sox2 and Klf4) had shorter telomeres than their parental hESC on average. Interestingly, the iPSC lines initially exhibited long telomeres similar to the parental cells, but upon repeated passaging the iPSC lines lost telomere length. The telomere shortening correlated with reduced levels of telomerase activity in these iPSC lines,

suggesting that telomere maintenance cannot be fully restored by reprogramming. However, the authors also observed heterogeneity in telomere dynamics between the derived iPSC lines, where some iPSC lines were able to maintain and even continue extending their telomeres [89]. Similarly, it has been shown that upon re-differentiation, some iPSC lines can maintain their telomeres over extended time period, suggesting that a subpopulation of these cells might be transformed [28,89] (Figure 3). By contrast, iPSC lines that were generated from senescent and centenarian human fibroblasts using a six-factor protocol exhibit maintenance of telomere length over 110 passage doublings [26^{••}]. Given the variability of telomere length within iPSC lines derived from the same donor, it will be important to test additional iPSC lines to determine whether efficient reprogramming protocols can trigger re-elongation of eroded telomeres to iPSCs and progeny, without inducing cancer.

Mitochondrial properties also appear to be restored to an ESC-like state by the reprogramming process [26^{••},27,30[•],90] (Figure 3). iPSCs from senescent and aged cells

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acquire mitochondrial properties similar to those of ESCs, in terms of metabolism, mitochondrial membrane potential, numbers, distribution and morphology [26^{••}]. Similarly, a reversion of mitochondrial morphology and functionality to ESC-like state in iPSC lines derived from an old human subject was observed [30[•],90]. Transcriptome analysis of iPSC lines derived from elderly patients further confirmed a rejuvenation of mitochondria pathways [26^{••},27,30[•]]. Taken together, these studies suggest that mitochondrial properties in aged iPSCs are restored to a similar status to that of young iPSCs and ESCs. Whether mitochondrial properties remain in a rejuvenated state upon re-differentiation is still unclear [90–92]. It will also be interesting to test whether the age-dependent accumulation of damaged macromolecules, such as proteins and lipids [93] (Figure 3), and the age-dependent changes in metabolism [94,95], are rejuvenated by reprogramming.

Interestingly, gene expression profiles of iPSC lines derived from old mouse and human subjects have also been shown to be reset to an embryonic-like state [19^{••},20,26^{••},27,30[•]] (Figure 3). Transcriptome analysis from several groups reveals significant downregulation of senescence/apoptosis-related genes in iPSC lines from older individuals. These genes include p16^{INK4A} and p15^{INK4B} in the p16^{INK4A}/RB pathway and p21^{CIP1} in p19^{ARF}/p53 pathway, as well as proapoptotic genes such as FAS, CASP8, CASP7, BAD and TP53AIP1 [19^{••},27]. Comparison of the gene expression profiles of proliferative and senescent fibroblasts derived from a 74-year-old patient, the corresponding six-factor cocktail-induced iPSC lines, and publicly available gene expression datasets of hESC and iPSC lines derived by four-factors revealed that the aged fibroblasts have an ‘aging signature’ regardless of their proliferation status, and that a six-factor protocol resets this aging signature more efficiently than a four-factor protocol. Furthermore, the transcriptome of a fibroblast re-differentiated from the proliferative and senescent fibroblasts of the 74-year-old patient and a centenarian clustered better with fibroblasts derived from hESCs than the parental cells, indicating a ‘rejuvenation’ of the transcriptome [26^{••}]. Collectively, these studies suggest that the transcriptome of old cells is ‘rejuvenated’ by the reprogramming process. However, the gene expression profiles of the iPSCs are not identical to hESCs, and although the re-differentiated fibroblasts clustered better with young fibroblasts than with their parental cells, they are still distinguishable from the young fibroblasts. Thus, these differences in the transcriptome between re-differentiated ‘rejuvenated’ fibroblasts and young fibroblasts may be reminiscence of old age.

One tantalizing aspect of reprogramming-induced rejuvenation that has not been investigated thoroughly is the epigenetic status of iPSCs derived from old donors

(Figure 3). Epigenetic changes to the chromatin are now widely accepted as part of organismal aging, in particular alterations in DNA methylation status [96–101]. Recent evidence suggests that iPSCs from mouse and human retain an epigenetic memory, namely DNA methylation signatures, of their tissue of origin, and that this impacts their differentiation potential [21^{••},102–105]. Interestingly, this epigenetic memory does not necessarily manifest itself at the pluripotency stage – iPSCs derived from different tissues were shown to exhibit proper morphology, express pluripotency markers, and were able to differentiate into the three embryonic layers *in vitro* and *in vivo*. Instead, this epigenetic memory may manifest later, during re-differentiation into specific cell types that require the specific loci that have residual epigenetic marks [21^{••},102,105]. As of yet, no studies have directly characterized old cells in regard to their epigenetic state before and after reprogramming. It would be interesting to investigate whether iPSCs derived from aged cells retain a memory of their age, in the form of DNA methylation or histone modifications, and whether this memory affects their differentiation potential. Interestingly, a recent study shows that reprogramming of cancer cells into a pluripotent state makes the tumor cells less aggressive *in vivo*. The authors observed that the reprogramming process led to a major re-structuring of the epigenome, resetting the epigenetic status of oncogenes from an active into a bivalent or inactive state [106]. This suggests that the reprogramming process *per se* is able to affect the epigenome of aberrant cells.

Aspects of aging that cannot be reset by reprogramming

Many aspects of aging are resettable by reprogramming, which opens avenues for novel therapeutic interventions of age-related symptoms and diseases. However, the accumulation of nuclear and mitochondrial DNA damage that is associated with aging [31] is likely to be an aspect of aging that cannot be reversed by reprogramming (Figure 3). This accumulated genomic damage may represent a safety issue when mutations occur in important cellular maintenance genes such as p53, rendering the cells more prone to tumor development. Indeed, several studies have shown that p53 deficiency in MEFs give rise to iPSCs with increased chromosomal instability, persistent damaged DNA, and malignant tumor-forming potential [32^{••},107]. Accordingly, several studies reported genetic aberrations in iPSC lines derived from old individuals [25,30[•]]. However, genomic abnormalities have also been shown to occur during the reprogramming process, while maintaining the iPSCs in culture and upon re-differentiation [108–111]. Therefore, further investigation is required to clarify whether these genetic aberrations are truly an age-dependent risk or whether they are due to the reprogramming process or the protocols used (or a combination of all three). In addition, the iPSC lines

that were derived from old donors and that carried karyotypic abnormalities were as susceptible to drug-induced apoptosis as their young counterparts. The authors interpreted this result as an indication that iPSCs from aged donors are not predisposed to cancer [30^{*}]. It will be interesting to compare mice derived from iPSCs originating from young and old donors to determine if tumorigenesis is positively correlated to donor's age.

iPSCs from individuals with premature aging or exceptional longevity

Hutchinson–Gilford Progeria syndrome (HGPS) is a rare genetic disease in which children show several signs of premature aging, including increased risk of cardiovascular diseases and hair loss [45,46]. The disease is caused by a mutation in the lamin A (*LMNA*) gene, which leads to the production of a truncated and toxic version of the lamin A protein termed progerin. Cells from HGPS patients display abnormal nuclear morphology, loss of heterochromatin markers H3K9me3, HP1 α and HDAC1, and increased DNA damage [74,112]. Two recent studies showed that it is possible to derive iPSCs from fibroblasts of HGPS patients [113^{**},114^{**}]. HGPS-derived iPSC lines expressed pluripotency markers, formed the three germ layers *in vitro* and *in vivo*, and were able to re-differentiate into a variety of different cell-types. Upon reprogramming, HGPS-derived iPSC lines no longer expressed progerin (similar to ESCs). Interestingly, the nuclear defects and epigenetic alterations associated with the disease were reverted back to normal, confirming that the reprogramming process is able to restore several defects associated with premature aging syndromes as well as physiological aging. However, upon differentiation, HGPS-derived iPSC lines start to re-express the deleterious form of Lamin A, and as a consequence, these cells exhibit signs of premature senescence such as reduced telomere length even at early passages [113^{**},114^{**}]. The premature senescence of progeny of HGPS-derived iPSCs is particularly evident in mesenchymal stem cells (MSCs) and vascular smooth muscle cells (VSMCs). MSCs generated from HGPS-derived iPSCs are more sensitive to hypoxic conditions [114^{**}]. As MSCs are normally found in low O₂ niches *in vivo*, this observation raises the possibility that MSC exhaustion underlies HGPS pathology [114^{**}]. Furthermore, VSMCs generated from HGPS-derived iPSCs are more sensitive to a number of stress stimuli (e.g. electrical stimulation) that these cells normally endure *in vivo* [114^{**}], which could explain the specific vulnerability of VSMCs in HGPS patients. These studies are examples of how disease modeling of premature aging syndromes by iPSCs can give novel insights into the pathology of aging. It will be interesting to use relevant HPSG-derived cells (in particular MSCs and VSMCs) as models for chemical or genetic screens to identify ways to alleviate age-related characteristics.

On the other end of the age spectrum are individuals with exceptional longevity, such as centenarians and super-centenarians (110 years old or more). In these individuals, longevity is known to be largely due to genetic factors. It will be interesting to determine if iPSC lines derived from these individuals exhibit specific qualities compared to cells from individuals with normal lifespan, and whether some lineages are more affected than others. Centenarian-derived iPSC lines could also serve as cellular models to understand the molecular mechanisms of exceptional longevity.

Concluding remarks

The past few years have seen significant advances in understanding the relationship between aging and reprogramming. While it is now clear that cells from old patients can be reprogrammed, more studies will be needed to better understand how age impacts iPSC generation and quality. Larger numbers of individuals, additional age groups, and fewer confounds will be critical to isolate the age parameter from other components. As there is significant variation among iPSC lines derived from the same individual, it will also be important to characterize several iPSC lines from the same individual. Such systematic studies will be particularly essential for human iPSCs, given the heterogeneous genetic background and uncontrolled environment of humans, two factors that impact organismal longevity and presumably affect reprogramming.

It will also be important to investigate the combined impact of age and tissue of origin on iPSC generation and quality. There is emerging evidence that tissue from different niches may exhibit differing sensitivity to age-dependent decline in reprogramming properties [19^{**},20,22^{*}]. Such differences in how age impacts iPSC depending on the tissue of origin would be particularly important from a clinical standpoint, as some sources of cells for iPSC derivation could be detrimental for stem cell therapies.

Characterizing the epigenetic state of old cells before and after reprogramming and differentiation will be a key step in identifying the age-dependent epigenetic changes that are reversible and those that remain as a 'memory' of the aged state. Such a knowledge may open new avenues for mimicking rejuvenation at the molecular level and may also provide ways of improving the reprogramming efficiency and iPSC quality.

Could one derive iPSC lines from an older patient, generate specific committed progenitors, and use these cells to alleviate aging or age-related disease in the same patient? Several hurdles might need to be overcome to use aged patient-derived iPSCs for stem cell therapies. One potential issue may be that even though reprogramming erases most molecular signs of aging, iPSCs derived

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from old individuals might still be more prone to genomic instability and neoplastic risk due to unrepaired damage. Thus, more iPSC lines might need to be screened, and their genome sequenced to ensure that key tumor suppressor or oncogenic pathways are not affected. Another key hurdle will be whether the reimplantation of the cells in the old environment of the patient will turn the clock forward in an accelerated manner and 're-age' the cells. This is a particularly important problem, given the systemic impact of an aged environment on stem cell function in several tissues, including muscle and brain [8,9].

Despite hurdles for the clinical use of iPSC cells, the process of reprogramming has opened many new avenues for the field of aging. iPSC lines could be used to model the genetic basis of aging and longevity, thereby allowing the screening and identification of novel factors that improve premature aging or affect exceptional longevity. In the likely event that not all signs of aging are reverted by reprogramming, iPSCs from old vs young individuals would also provide a model for aging in the tissue culture dish. Finally and importantly, the rejuvenation of many age-related characteristics by reprogramming also provides an opportunity for the field to understand the basis of 'immortality'.

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