Review

The aging signature: a hallmark of induced pluripotent stem cells?

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Summary

The discovery that somatic cells can be induced into a pluripotent state by the expression of reprogramming factors has enormous potential for therapeutics and human disease modeling. With regard to aging and rejuvenation, the reprogramming process resets an aged, somatic cell to a more youthful state, elongating telomeres, rearranging the mitochondrial network, reducing oxidative stress, restoring pluripotency, and making numerous other alterations. The extent to which induced pluripotent stem cell (iPSCs) mimic embryonic stem cells is controversial, however, as iPSCs have been shown to harbor an epigenetic memory characteristic of their tissue of origin which may impact their differentiation potential. Furthermore, there are contentious data regarding the extent to which telomeres are elongated, telomerase activity is reconstituted, and mitochondria are reorganized in iPSCs. Although several groups have reported that reprogramming efficiency declines with age and is inhibited by genes upregulated with age, others have successfully generated iPSCs from senescent and centenarian cells. Mixed findings have also been published regarding whether somatic cells generated from iPSCs are subject to premature senescence. Defects such as these would hinder the clinical application of iPSCs, and as such, more comprehensive testing of iPSCs and their potential aging signature should be conducted.

Key words: aging; differentiation; epigenetic; induced pluripotent stem; reprogramming; telomeres.

Introduction

It was discovered early on that somatic cells could be reset to a pluripotent state through somatic cell nuclear transfer (Gurdon, 1962; Tada et al., 1997; Hochedlinger & Jaenisch, 2002; Wilmut et al., 2007) and cell fusion (Tada et al., 2001). A landmark experiment in the cell reprogramming field was performed by Takahashi and Yamanaka, demonstrating that adult somatic cells could be restored to pluripotency through the exogenous expression of four transcription factors: Oct4, Sox2, Klf4, and c-Myc. These induced pluripotent stem cells (iPSCs) expressed markers exclusive to embryonic stem cells (ESCs), mimed their morphology and growth properties, and could differentiate into all three germ layers (Takahashi & Yamanaka, 2006).

Since their initial discovery, multiple methods of reprogramming have been generated. Adult somatic cells have been successfully induced into pluripotency using viral vectors (Zhou & Freed, 2009), nonintegrating episomes (Yu et al., 2009), and minicircle vectors (Jia et al., 2010). Pluripotency can also be induced by the use of reprogramming proteins, either by direct addition of purified protein (Zhou et al., 2009) or with extracts from cells stably expressing reprogramming factors (Kim et al., 2009). More recently, effective reprogramming was achieved using synthetic mRNA (Warren et al., 2010), a technique our group has used to derive iPSCs from disease and healthy donors. More comprehensive listings of successfully employed methods have been reviewed elsewhere (Gonzalez et al., 2011).

With regard to aging and age-related disease, iPSCs represent enormous therapeutic potential. Reprogramming adult, somatic cells allows for the generation of patient-specific models that have already been used to generate a wealth of information regarding disease pathogenesis, drug testing, and drug discovery (Bellin et al., 2012). It was previously proposed that the ability to reprogram a cell to a youthful state without affecting the differentiation program may be an effective strategy for rejuvenating an aged organism (Rando & Chang, 2012). In order for such a method to be viable, reprogramming would have to reset the aging clock, clearing the damage that accrues with age and restoring a cell to a youthful state. This would require multiple types of restoration, as somatic cells accumulate nuclear and mitochondrial mutations as well as damaged macromolecules with age. Furthermore, aging cells are characterized by distinct changes in the epigenome, telomere shortening, increased oxidative stress, and numerous other alterations (Kirkwood, 2005; Haigis & Yankner, 2010; Johnson et al., 2012). Such restoration is not impossible, however, as evidenced by the fertilization process, where an aged sperm and egg fuse to form a zygote devoid of aging damage or any evidence of the age of the parental cells (Rando & Chang, 2012).

There are currently conflicting data regarding the ability of reprogramming to fully rejuvenate an aged somatic cell and the extent to which iPSCs mimic ESCs. Moreover, contentious data exist suggesting that cells derived from iPSCs may be subject to premature senescence. This review highlights recent data relevant to these controversies and discusses the conclusions that can be currently drawn.

Does reprogramming reset the aging clock?

Epigenetic memory

Epigenetic modifications such as histone acetylation and DNA methylation play a paramount role in regulating gene expression and exhibit unique changes during aging and age-related disease (Fraga et al., 2007; Johnson et al., 2012). Modifications to epigenetic machinery can directly impact longevity (Lin et al., 2005) and health (Klein et al., 2011) as well as prevent differentiation of stem cells into somatic tissues...
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tissue of origin that favor differentiation into lineages related to the donor cell.
compared to ESCs, retaining residual methylation signatures characteristic of their
cells. iPS cells have been reported to feature incomplete epigenetic reprogramming
regarding the epigenetic memory of induced pluripotent stem cell (iPSC)s and
found between the cell type of origin and the propensity of an iPSC
varied in their differentiation propensity. Moreover, no correlation was
More recently, genetically matched human iPSC clones from dermal
fibroblasts and bone marrow stromal cells of the same donor were
generated and differentiated into osteogenic and chondrogenic lin-
eges. The authors found that, although the iPSCs exhibited an
epigeneric memory characteristic of the donor tissue used, the clones
varied in their differentiation propensity. Moreover, no correlation was
found between the cell type of origin and the propensity of an iPSC
clonetodifferentiate into bone and cartilage (Nasu et al., 2013). Further
work is required to determine whether this epigenetic memory affects

It was recently shown that low-passage iPSCs can feature incomplete
epigeneric reprogramming compared to ESCs, retaining residual DNA
methylation signatures that are characteristic of their tissue of origin and
favor differentiation into lineages related to the donor cell (Fig. 1). iPSCs
developed from mouse neonatal progenitors, for example, contained meth-
lyomic signatures at loci important for hematopoietic differentiation,
resulting in a decreased propensity for differentiating into hematopoietic
cell types. Treatment with chromatin-modifying compounds reduced
DNA methylation at these loci and increased the blood-forming potential of
the low-passage iPSCs, suggesting that the effects of these epigenetic
marks can be attenuated via pharmaceutical intervention (Kim et al.,
2010). Conflicting data exist regarding the retention of these methyl-
ation signatures with passage number. Some iPSC clones derived from human neonatal keratinocytes and umbilical cord blood cells were
documented to maintain tissue-specific methylation memory at high
passage numbers (Kim et al., 2011), while iPSCs derived from mouse myogenic cells, fibroblasts, and hematopoietic cells reportedly lost their
epigeneric memory with continued passage in culture (Polo et al., 2010).
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Telomere length
Unlike stem cells, somatic cells have a limited division capacity and
senescence in vitro. The inability to further replicate is termed replicative
senescence and can be induced by a plethora of factors, such as short
and uncapped telomeres, oxidative stress, and mitochondrial DNA
damage (Fig. 2) (Chen et al., 2007). The enzyme telomerase, which
maintains telomere length and long-term self-renewal potential of stem
cells, is strongly expressed in ESCs and is inactive in most somatic cells.
As such, telomere length gradually decreases with every cell division in a
typical somatic cell, eventually resulting in replicative senescence (Harley
et al., 1990). The lifespan of normal human fibroblasts can be extended
in vitro by exogenous introduction of plasmids expressing the catalytic
subunit of telomerase hTERT, resulting in an increased telomerase
activity (Bodnar et al., 1998). Donor cells that are difficult to reprogram
can be more efficiently induced into the pluripotent state by adding
hTERT and SV40 large T antigen to the reprogramming factors originally
used by Yamanaka’s laboratory (Park et al., 2008). Furthermore,
telomerase-deficient mice exhibit a sharp reduction in reprogramming
efficiency that can be restored by the reintroduction of telomerase
(Marion et al., 2009), highlighting the imperative role telomerase plays
in iPSC reprogramming.

Through the use of factor-based reprogramming, Yu et al. reported
that human iPSCs display levels of telomerase activity characteristic of
ESCs (Yu et al., 2007). In a recent report using mouse iPSCs, it was
found that, at passage eight, iPSCs have shorter telomeres than ESCs,
yet longer telomeres than the embryonic fibroblasts they were derived
from. It was only after subsequent cell divisions and further passaging
that telomeres were restored to the lengths found in ESCs (Marion et al.,
2009). In six of seven iPSC cell lines derived from human skin fibroblasts,
telomeres were substantially elongated compared to their parental cells
and had telomere lengths comparable to ESCs at passage five (Suhr
et al., 2009) (Table S1). At passage 25, several iPSC clones derived from
human neonatal foreskin fibroblasts had significantly longer telomeres
than their parental donors (Yehezkel et al., 2011). iPSCs reprogrammed
from senescent and centenarian human cells exhibited telomere lengths
that were identical to or greater than the lengths observed in ESCs and
did not shorten with increased passage number (Lapasset et al., 2011)
(Table S1).

A study conducted by Vaziri et al. compared telomerase activity and
telomere length in iPSCs to hESCs. The authors reported that five well-
studied human iPSC cell lines had significantly shorter telomeres than
three commonly used ES cell lines as well as reduced levels of telomerase
activity. Six iPSC cell clones were then generated from the ESC-derived cell
line EN13 and, after further culturing, telomeres in five of the six lines
shortened to lengths comparable to those observed in the widely used
iPSC cell lines. One clone, however, expressed high levels of telomerase
and displayed a progressive increase in telomere length for over 60 days,
procuring a length equivalent to that of the parental ES cell line (Vaziri
et al., 2010). Mathew et al. also reported the variability in telomere
length and telomerase expression in human iPSC clones. Only clones
with the highest level of telomerase expression were observed to have
telomere lengths comparable to ESCs (Mathew et al., 2010). In iPSCs
derived from patients with dyskeratosis congenita, a disease character-
ized by shortened telomeres and defects in telomerase function,
reprogramming was capable of restoring telomere length in some iPSCs
(Agarwal et al., 2010), but not others (Batista et al., 2011) (see Table S1
for a detailed overview of telomere length data). For those iPSCs where

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telomere length was elongated, telomere length was observed to increase with further passaging (Agarwal et al., 2010). When telomere chromatin is transcribed, long noncoding RNA transcripts referred to as telomeric-repeat-containing RNA (TERRAs) are generated. TERRA levels are positively correlated with telomere length and may act as regulators of telomerase expression (Schoeftner & Blasco, 2008). In mouse iPSCs, TERRAs were observed to be increased compared to differentiated cells, yet lower compared to ESCs at passage eight (Marion et al., 2009). At passage 25, human iPSCs had higher TERRA levels than their parental source, yet these levels were found to vary from clone to clone (Yehezkel et al., 2011).

These results indicate that, although telomerase activity is clearly reconstituted during the reprogramming process, there is a significant variability in telomere length among various iPS cell lines. This length is sensitive to passage number and can even vary among cell lines derived from the same tissue or parental cell type. This is not unique, however, as it was recently shown that substantial variability in telomere length and telomerase expression exists for ESCs as well. For both iPSCs and ESCs, telomere length was found to be highly correlated with proliferation efficiency and developmental pluripotency (Huang et al., 2011). As such, caution should be made when drawing comparisons for telomere length, ensuring that passage number is controlled for and that appropriate controls (e.g., numerous ES cell lines and donor tissue) are used.

Mitochondrial alterations and oxidative stress

Aging in somatic cells is accompanied by mitochondrial dysfunction and oxidative stress (Fig. 2) (Passos et al., 2007; Moiseeva et al., 2009). Compared to somatic cells, ESCs have less mitochondrial mass, decreased ATP levels, reduced reactive oxygen species (ROS), and more active repair mechanisms that mitigate mitochondrial DNA damage (Cho et al., 2006; Saretzki et al., 2008). Mitochondria in ESCs have also been reported to be sparser, have underdeveloped or condensed cristae, and rely more on anaerobic glycolysis for energy (Prigione et al., 2010; Suhr et al., 2010).

It was recently shown that the reprogramming process reorganizes mitochondria, causing them to adopt the immature morphology, distribution, and DNA content characteristic of the pluripotent state. Like ESCs, these human iPSCs also displayed reduced levels of oxidative stress, decreased amounts of intracellular ATP, and increased production of lactate. Expression of nuclear factors involved in mitochondrial biogenesis was also comparable (Prigione et al., 2010). Age does not appear to limit the ability of reprogramming to mediate these changes, as iPSCs with chromosomal anomalies from an 84-year-old woman exhibited restructured mitochondria and decreased levels of oxidative stress and DNA damage. These changes were similar to those observed for iPSCs generated from young donors (Prigione et al., 2011) (see Table S1 for details). In senescent and centenarian human fibroblasts, the reprogramming process reset the expression of genes associated with mitochondrial metabolism and oxidative stress. Mitochondrial morphology and distribution were also both comparable to ESCs (Lapasset et al., 2011). In iPSC clones derived from human dermal fibroblasts, mitochondrial biogenesis and ROS stress defense mechanisms were analogous to those observed in ESCs (Armstrong et al., 2010). Resetting of metabolic signature and restructuring of mitochondria have also been observed by others (Suhr et al., 2010). Varum et al. obtained more mixed results (Fig. 2), finding that human iPSCs exhibit a metabolic signature that, while not completely identical to ESCs, clusters more closely with ESCs than with differentiated cells. It was also observed that the mitochondrial morphology in iPSCs is more similar to ESCs than somatic cells, yet still distinct (Varum et al., 2011). Although different

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reports have been published regarding the extent of rejuvenation, these
clearly demonstrate that the reprogramming process resets
mitochondria and related metabolic and stress mechanisms to a more
youthful state (Fig. 2). Further studies are required to illuminate the
extent of this rejuvenation, however, and why varied results have been
obtained.

Effect of donor age on reprogramming efficiency
It is currently possible to generate iPSCs from human centenarian cells
(Lapasset et al., 2011; Yagi et al., 2012) that can differentiate into all
three germ layers (Yagi et al., 2012). The reprogramming process causes
senescent and centenarian cells to acquire a more youthful signature
after reprogramming, resetting telomere lengths and gene expression
profiles to those observed in ESCs (Lapasset et al., 2011). Although
these data demonstrate that old age does not abrogate the ability to
reprogram somatic cells, there are conflicting data on whether donor
age impacts reprogramming efficiency.

Using human tissue, Boulting and colleagues (Boulting et al., 2011)
characterized 16 iPSCs from seven individuals of different ages. The
authors found that, although the lines had variable expression of early
pluripotency markers, all 16 lines passed stringent tests for differen-
tiation capacity (Boulting et al., 2011). In mice, dermal skin fibroblasts
procured from old mice (121 weeks old) had shorter telomeres than
those obtained from young mice (22 weeks old). Despite the difference
in telomere length, iPSCs could be derived from both old and young
donors and the resultant iPSCs had equally elongated telomeres
(Marion et al., 2009). iPSCs generated from bone marrow of old mice
(23 months old) were more difficult to produce than those generated
from the bone marrow of young mice (2 months old), requiring twice
as much time to reprogram (Cheng et al., 2011). In iPSCs sourced from
14-month-, 6-month-, and 6-week-old mice, reprogramming efficiency
declined with age. Unlike those from younger mice, iPSCs from 14-
month-old mice exhibited unstable pluripotency, regressing after
expansion in culture and exhibiting faint staining of the pluripotency
marker alkaline phosphatase. A gradual loss of expression in the ESC
marker Nanog was also observed in elderly mice. This was ameliorable,
however, as inhibition of BMP and TGF-β signaling helped to stimulate
self-renewal as well as to stabilize Nanog expression (Wang et al.,
2011).

A few age-related genes have been identified that play inhibitory
roles in the reprogramming process. Using mouse and human fibro-
blasts, Li et al. reported that reprogramming efficiency declined with
age. Knockdown of the Ink4/Arf locus, which encodes for tumor
suppressors and is upregulated with age, was sufficient to rescue the
age-dependent defect in reprogramming efficiency (Li et al., 2009).
Similarly, expression of lamin A, a protein that maintains nuclear
structure integrity, increases with age in somatic cells and is expressed
at lower levels in undifferentiated cells. Using human fibroblasts, an inverse
correlation was observed between reprogramming efficiency and the
expression level of lamin A. Like the Ink4/Arf locus, knockdown of lamin
A using short hairpin RNA accelerated the rate of iPSC induction.
Furthermore, overexpression of lamin A severely hindered reprogram-
m (Zuo et al., 2012). Interestingly, mutations in lamin A/C cause
defects in the nuclear envelope and underlie Werner syndrome and
Hutchinson Gilford progeria, two diseases of accelerated aging.
Recently, iPSCs were generated from patients suffering from these
disorders. Compared to their donor fibroblasts, these iPSCs had normal
nuclear membrane morphology, suggesting that the reprogramming
process could rejuvenate nuclear defects (Ho et al., 2011).

Although additional age comparisons are necessary, these results
suggest that mammalian aging may decrease reprogramming efficiency
(for an overview of donor age of the generated iPSC lines, see Table S1,
and for factors used for reprogramming, see Table S2). Old age does not
prevent successful reprogramming, however, as these studies demon-
strate that somatic cells of any age—even those that are senescent—can
be coaxed into a more youthful, pluripotent state. Moreover, the loss in
efficiency can be mitigated via inhibition of specific signaling pathways
and genes. Hence, old age is unlikely to nullify the rejuvenative potential
of iPSCs.

Do cells derived from iPSCs age prematurely?
Recent data have emerged suggesting that cells derived from iPSCs may
exhibit signs of premature senescence (please see Fig. 2 for an overview
of premature senescence in iPSCs). As with epigenetic memory and
telomere length, these data are also controversial.

Suhr et al. reprogrammed human skin fibroblasts into iPSCs and then
produced differentiated cell lines derived from three iPSC-teratoma
explants. Although one line exhibited elongated telomeres, the other
two displayed telomere lengths comparable to the input fibroblasts (Suhr
et al., 2009). The same group examined the mitochondria of iPSCs
generated from human fibroblasts as well as fibroblasts re-derived from
iPSCs. The authors observed that the quality and function of mitochon-
drial complement of the re-derived fibroblasts was dramatically
improved compared to the input fibroblasts (Suhr et al., 2010). Upon
differentiation, the mitochondrial network and metabolic signature of
both human ESCs and iPSCs changed to match features observed in
fibroblasts. Expression of the antioxidant GPX1 was higher in fibroblasts
differentiated from iPSCs, however, suggesting that iPS-derived somatic
cells may differ with regard to their handling of ROS (Prigione et al.,
2010). Feng et al. successfully differentiated human iPSCs into multiple
cell types, although the efficiency was markedly lower than it was for
ESCs. Moreover, the authors observed that, unlike cells derived from
ESCs, somatic cells derived from iPSCs exhibited early senescence and
possessed dramatic defects in expansion capability (Feng et al., 2010)
(for an overview of all iPSC lines tested, see Table S1). This fate is not
inexorable, however, as others have generated somatic cells from iPSCs
that do not exhibit premature senescence (Gokoh et al., 2011).

Although it is too early to conclusively determine, issues of premature
senescence may, like telomere length, vary considerably from line to line.
Subsequent research drawing detailed comparisons between cell types
derived from multiple ESC and iPSC lines will help resolve this
contention.

Conclusions
It is quite clear that the reprogramming reverses many aspects of aging.
Even iPSCs derived from senescent and centenarian cells exhibit a more
youthful signature, displaying elongated telomeres and gene expression
profiles comparable to ESCs (Lapasset et al., 2011). Metabolic signa-
tures, mitochondrial networks, handling of ROS, telomerase expression,
and other factors are all reset to a state characteristic of pluripotency
(Suhr et al., 2009; Prigione et al., 2010, 2011). These data are
disputed, however, as differential reports have been published
regarding the extent to which reprogramming rejuvenates aged, somatic
cells and whether iPSCs exhibit aging signatures (summarized in Table
S1).

Telomere length, for example, has been observed to be shortened
(Vaziri et al., 2010), similarly sized, or even elongated compared to ESCs
Considerable variation in telomere length as well as telomerase activity has also been reported for both iPSCs and ESCs, however, suggesting that telomere profiles may be unique to the stem cell line used (Huang et al., 2011). The same may be true for epigenetic memory, as Nasu et al. found no correlation between the differentiation potential of an iPSC clone and the type of progenitor cell used. Instead, the authors observed that differentiation propensity varied substantially from clone to clone (Nasu et al., 2013). Mitochondrial networks have been reported to be rearranged to a state indistinguishable from ESCs (Pirigone et al., 2010, 2011) or to a mixed phenotype in between that of ESCs and somatic cells (Varum et al., 2011). Some groups have reprogrammed cells from an elderly organism with no comment on decreased reprogramming efficiency (Marion et al., 2009), while others have observed reprogramming efficiency to decline with age (Li et al., 2009). Similar discrepancies are noted regarding premature senescence, which has been observed in somatic cells derived from some iPSCs (Feng et al., 2010), but not others (Gokoh et al., 2011).

That variations in differentiation propensity are observed in iPSCs derived from the same donor (Nasu et al., 2013) indicates that the reprogramming process may not reset cells to a younger state in an invariable manner. Furthermore, many of the studies cited used different reprogramming protocols as well as donor cells from different cell types or species (summarized in Table S2). Conflicting reports regarding the extent to which adult, somatic cells are rejuvenated may also be explained by the distinct protocols and materials used. Regardless, it is apparent that some iPSCs and their differentiated progeny may exhibit age-related defects. Whether these defects are cell line specific or a broader problem with iPSCs requires additional data to determine. Moreover, aging is a complex disease marked by innumerable changes, and thus far, only a small set of age-related alterations has been investigated in iPSCs. To properly assess whether iPSCs exhibit an aging signature and are less youthful than ESCs, additional research into other aspects of aging is required.

Acknowledgments

Thanks go to Mr. Pooyan Naghsh for graphical design and making tables. The work presented in this paper was made possible by funding from the German Federal Ministry of Education and Research (BMBF 1315883).

Author contributions

L. R., A. A., and A. A. J. involved in collection and/or assembly of data. L. R. involved in graphical design and making tables. A. A. J. involved in editing. A. S. provided the concept and conclusions as well as major editing of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Table S1 Aging signatures of different iPSC cell lines and derivatives.

Table S2 iPSC cell lines generated from different cell types or species.