Long Live Partial Reprogramming

Rosa M. Marión, Maria A. Blasco

In vivo amelioration of age-associated hallmarks by partial reprogramming.
Ocampo et al
Cell. 2016;167:1719–1733.

Aging can be defined as the progressive loss of physiological integrity that leads to tissue dysfunction and increased risk for developing age-associated human pathologies, such as cancer, diabetes mellitus, cardiovascular disorders, and neurodegenerative diseases.[1] Therapeutic strategies to delay or prevent aging are aimed to decrease the deleterious effects of aging and to improve the quality of life in aged individuals and eventually to extend the human life span. In addition, deep molecular understanding of the mechanisms of aging may lead to new treatments for age-related diseases. In line with this, a recent article in *Cell*[2] shows that cyclic induction of Yamanaka reprogramming factors in vitro, what the authors called "partial reprogramming," reduces age-associated features in mouse and human cells. Strikingly, partial reprogramming induced in vivo in a mouse model of premature aging ameliorates some aging-associated hallmarks and extends their prematurely shortened life span. Importantly, these beneficial effects are not accompanied by dedifferentiation or loss of cellular identity. Finally, the authors show that partial in vivo reprogramming increases the regenerative capacity of physiologically aged wild-type mice.

In the past few years, many research efforts to treat age-related pathologies and the effects of physiological aging have been focused on the use of cellular reprogramming strategies.[3] In vitro reprogramming of differentiated cells into iPS cells by the expression of the so-called Yamanaka factors (SOX2, OCT4, c-MYC, and KLF4) has the striking capacity to reverse some of the molecular hallmarks associated with aging in cells from healthy normal individuals and in cells from patients with progeria syndromes.[4–6] Thus, it has been shown that cellular reprogramming induces telomerase-mediated telomere elongation and opening of telomeric chromatin (decreased H3K9m3 and H4K20m3 marks, increased telomeric transcription),[6] decreases DNA damage in the resulting reprogrammed cells throughout a p53-dependent mechanism,[7] increases mitochondrial function,[8] reduces the presence of senescence markers, and resets gene expression profiles.[5] Full cellular reprogramming, however, also leads to full dedifferentiation, pluripotency, and teratoma tumor formation in vivo.[9] Remarkably, Ocampo et al[2] report that the amelioration of some age-associated phenotypes can be achieved by the induction of partial reprogramming, characterized by the expression of the reprogramming factors without the loss of cellular identity or acquisition of pluripotency, thus without induction of tumors at least during the life span of the progeria model, which is extended by 30% after cyclic induction of the reprogramming factors. Partial reprogramming is achieved by cyclic expression of the Yamanaka factors. The authors first show that in vitro partial reprogramming of fibroblasts from a transgenic mouse model of premature aging (LAKI [knock-in Lamin A G609G] mice, carrying a mutation in the gene *Lmna* that leads to the accumulation of a truncated form of lamin A, called progerin) relieves age-associated molecular and cellular phenotypes. Thus, short-term induction of the reprogramming factors in these cells (2 days of doxycycline administration followed by 5 days of doxycycline withdrawal) reduced the levels of markers of stress response (p53 tumor suppressor pathway), senescence (metalloproteinase MMP13 and interleukin-6), DNA damage (53BP1 and histone γ-H2AX proteins), and mitochondrial dysfunction (reactive oxygen species). Finally, it restored the levels of 2 heterochromatin markers H3K9m3 and H4K20m3 that are downregulated and upregulated, respectively, during aging.[10] Interestingly, the authors find that this restoration not only precedes the effects of age-associated phenotypes, but it is required for these effects to take place because partial reprogramming induced in the presence of a methyltransferase inhibitor did not ameliorate the aging phenotypes in the LAKI cells (Figure).

Importantly, partial reprogramming did not result in loss of fibroblast marker THY or in the expression of pluripotency marker NANOG, indicating that the reverse in aging phenotypes were obtained without dedifferentiation. This improvement of aging hallmarks is not restricted to cells from the premature aging model because they recapitulate the results in late-passage wild-type (WT) mouse and human cells, showing that the beneficial effects of partial reprogramming in vitro also apply to physiologically aged WT cells. Thus, this work shows for the first time that in vitro partial reprogramming without loss of cellular identity or acquisition of pluripotency can revert cellular age-associated phenotypes.

Would partial reprogramming in vivo reduce aging hallmarks in the organism? We previously reported that in vivo reprogramming leads to telomere elongation, a clear sign of cellular rejuvenation.[11] The use of in vivo reprogramming in regenerative medicine would avoid the need of cell transplantation.

The opinions expressed in this Commentary are not necessarily those of the editors or of the American Heart Association.

Commentaries serve as a forum in which experts highlight and discuss articles (published here and elsewhere) that the editors of *Circulation Research* feel are of particular significance to cardiovascular medicine.

From the Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Research Centre (CNIO), Madrid, Spain.

Commentaries are edited by Aruni Bhatnagar & Ali J. Marian.

Correspondence to Maria A. Blasco, PhD, Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Research Centre (CNIO), 3 Melchor Fernandez Almagro St, Madrid E-28029, Spain. E-mail mblasco@cnio.es

(*Circ Res*. 2017;120:1381-1383.
DOI: 10.1161/CIRCRESAHA.117.310594.)

Circulation Research **is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.117.310594

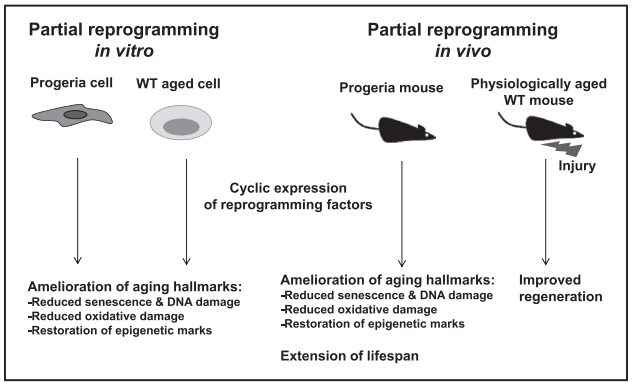

Figure. Beneficial effects of partial reprogramming in vitro and in vivo.

However, acquisition of pluripotency by in vivo reprogramming induces the formation of teratomas and cancer.[9] Importantly, Ocampo et al prove that in vivo partial reprogramming in the progeria LAKI mouse model reduces several age-associated organismal phenotypes but does not lead to teratoma formation, cancer development, or increased mortality, even after 35 cycles of doxycycline administration, thus avoiding the deleterious effects of full reprogramming to pluripotency in vivo. In particular, they report that partial reprogramming of LAKI mice reduces spine curvature, increases the dermal and epidermal thickness of the skin, and rescues defects in several organs such as the intestinal track, spleen, kidneys, and stomach. Interestingly, cardiovascular alterations present in LAKI mice, which mimics those observed during physiological aging and in progeria patients, such as degeneration of vascular smooth muscle cells or development of bradycardia, are partially rescued by the induction of in vivo partial reprogramming. Importantly, although cell proliferation rates were restored in several organs after partial reprogramming, they did not presented dysplasia or acquisition of pluripotency (NANOG-positive cells). Partial in vivo reprogramming reduced the presence of markers of stress response and senescence and restored normal level of the heterochromatin marks altered during aging. Interestingly, the exhaustion of cell population associated to aging was partially restored, at least in skeletal muscle and hair follicle. Most remarkably, the authors show that partial in vivo reprogramming leads to a dramatic extension of the life span in the premature aging LAKI mouse model, probably because of an increased regenerative capacity and tissue homeostasis derived from the improvement of the aging hallmarks in the organism. One important issue that should be addressed is whether the beneficial effects of partial reprogramming require continuous cyclic expression of the reprogramming factors. The authors observe that the age-associated phenotypes progressively return after in vitro partial reprogramming of LAKI cells, suggesting that, at least in these cells and set up, the amelioration of the aging hallmarks is not stable and requires continuous cycles of expression. Further experiments should be performed to determine the stability of the effects of in vivo partial reprogramming.

A key question raised by these results is whether in vivo partial reprogramming would have a beneficial effect and extend the life span in physiologically aged WT individuals. Although the authors do not address the last question, they demonstrate that partial reprogramming in vivo increases the regenerative capacity of WT aged mice after injuries. Thus, after the induction of ablation of β cells in the pancreas of physiologically aged WT mice, partially in vivo reprogrammed old mice showed an increase in the expansion of the β cell population and their regenerative capacity, as well as an improved glucose tolerance. Also, after the induction of muscular injury, they revealed an expansion of muscle cells and an improved muscle regenerative capacity. Because both the loss of the proliferative capacity of β cells in response to injury and the appearance of sarcopenia (loss of skeletal muscle mass) are tissue dysfunctions associated with age, these results suggest that partial in vivo reprogramming could also ameliorate the hallmarks of aging in physiologically aged WT individuals.

The beneficial effects of in vitro reprogramming to pluripotency on age-associated phenotypes were already known.[12] On the contrary, work from the same group had previously described that partial dedifferentiation of cells can be achieved by expression of the Yamanaka factors, without total loss of cell identity,[13] but the effect of this partial reprogramming of hallmarks of aging was not known. Thus, the central question of whether reprogramming-induced dedifferentiation was required to revert these phenotypes was still unanswered. The authors now shed light on this issue as they clearly show that the beneficial effects of reprogramming can be dissociated from dedifferentiation, because partial reprogramming alleviates signs of aging without loss of cellular identity or acquisition of pluripotency. This question is of key importance in the case of in vivo reprogramming because in vivo reprogramming to pluripotency induces the appearance of dysplasia and teratomas.[9] The establishment of a protocol to induce partial reprogramming in vivo while avoiding cell dedifferentiation and thus its deleterious effects is one of the major accomplishes of this work.

The results presented in this work suggest that partial in vivo reprogramming could eventually be of use as a therapeutic approach in the treatment of diseases that cause premature aging. However, an essential question remains unsolved: can partial in vivo reprogramming extend the life span of normal physiologically aged individuals? The authors do not address this question, so additional experiments to investigate the effects of partial reprogramming in the life span of WT old mice will be required. Interestingly, they do show an important beneficial effect of in vivo partial reprogramming in the regenerative capacity of physiologically aged mice. Altogether, these results suggest that partial in vivo reprogramming could be used to manipulate the health status and ultimately the aging process of an individual.

What is the molecular mechanism behind the reversion of the age-associated phenotypes? The question remains unsolved. The authors show that the epigenetic changes are early events during the process of partial reprogramming in vitro. Importantly, use of chaetocin, an inhibitor of histone lysine methyltransferases, abolished the rejuvenation effect of reprogramming in vitro, suggesting that epigenetic changes act as drivers of reprogramming, although it was not tested whether this results can be recapitulate during partial reprogramming in vivo. These results open the interesting question of whether physiological aging is also ignited by epigenetic changes. This would allow the manipulation of the aging process by modulators of epigenetic marks. However, more insights into

the molecular mechanism of partial reprogramming are not provided, and further experiments should be performed to characterize the effect of epigenetic remodeling in the aging phenotypes and the specific marks involved in the process.

The authors describe partial restoration of the number of adult stem cells in several tissues and structures, such as skeletal muscle and hair follicle. This suggests that the reversion of the regenerative capacity and the rescue of the age-associated phenotypes could be explained by recovery of the adult stem cells exhaustion associated with aging. A key and yet unsolved question is the nature of the reprogrammed cells: are all the cells in a specific tissue reprogrammed and rejuvenated or are there a specific type of cells, that is, adult stem cells, more prone to reprogramming? Partial reprogramming of adult stem cells in the tissues, and the associated number recovery, could impact in the reversion of hallmarks of aging in somatic cells and the increased regenerative capacity and tissue homeostasis. Further studies should be performed to define the nature of the in vivo reprogrammed cells.

The authors did not show the effects on other well-established hallmarks of aging, such as telomere length, which has been shown to limit human and mouse longevity.[14] As mentioned above, we have recently described that in vivo reprogramming induces the activation of telomerase and the elongation of telomeres in the reprogrammed cells.[11] Thus, increased expression of telomerase contributes to the rejuvenation of reprogrammed cells. Telomere shortening is a hallmark of aging and is associated with premature appearance of diseases associated with aging.[15] Because telomere length influences longevity, it could be speculated that induction of partial reprogramming in vivo could activate the expression of telomerase and, subsequently, the elongation of telomeres, contributing to erase the aging phenotypes. This important question is not addressed in this work, so further experiments should be performed to answer it.

The key question raised by this work is whether partial in vivo reprogramming could be applied as a valid approach for age-associated diseases and antiaging therapies for humans. However, numerous challenges must be overcome before its use in medicine. An important technical issue to address is the delivery method. Gene therapy approaches are promising as new safer vectors are being developed. Also, chemical reprogramming emerges as a possible system to induce reprogramming. Because of the importance of epigenetic changes in the process, a possible scenario would allow to modulate reprogramming by the use of chemicals that modify specific epigenetic marks, although a deeper knowledge of the epigenetic events necessary for partial reprogramming is required. In any case, safety issues should be carefully monitored, and the expression levels of the reprogramming factors should be fine-tuned to avoid dedifferentiation and the development of cancer and teratomas. Also, it should be determined whether whole-organism reprogramming is required to obtain a beneficial effect or whether reprogramming could be directed to specific organs. Organ-specific partial reprogramming could potentially revert the effects of aging or tissue damage and regenerate the desired organ. In the particular case of cardiac regenerative medicine, this work demonstrates that partial reprogramming rescues cardiovascular alterations present in patients with premature aging syndromes and during normal aging, suggesting that this organ could be a target for the use of partial reprogramming in regenerative medicine. It would be of great importance to determine whether direct partial reprogramming of the adult heart after a myocardial infarction could also increase the regenerative capacity of the tissue and thus induce heart repair.

Although there is clearly much work to be done and many challenges lie ahead, this work open new roads to explore future therapies for age-related diseases, damaged organs, and eve physiological aging.

Sources of Funding

Work in the laboratory of M.A.B. is funded by the Spanish Ministry of Economy and Competiveness (PLAN RETOS) and by the Fundación Botín.

Disclosures

None.

References

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153:1194–1217. doi: 10.1016/j.cell.2013.05.039.
2. Ocampo A, Reddy P, Martinez-Redondo P, et al. In vivo amelioration of age-associated hallmarks by partial reprogramming. *Cell*. 2016;167:1719–1733.e12. doi: 10.1016/j.cell.2016.11.052.
3. Ocampo A, Reddy P, Izpisua Belmonte JC. Anti-aging strategies based on cellular reprogramming. *Trends Mol Med*. 2016;22:725–738. doi: 10.1016/j.molmed.2016.06.005.
4. Liu GH, Barkho BZ, Ruiz S, et al. Recapitulation of premature ageing with iPSCs from Hutchinson-Gilford progeria syndrome. *Nature*. 2011;472:221–225. doi: 10.1038/nature09879.
5. Lapasset L, Milhavet O, Prieur A, Besnard E, Babled A, Aït-Hamou N, Leschik J, Pellestor F, Ramirez JM, De Vos J, Lehmann S, Lemaitre JM. Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev*. 2011;25:2248–2253. doi: 10.1101/gad.173922.111.
6. Marion RM, Strati K, Li H, Tejera A, Schoeftner S, Ortega S, Serrano M, Blasco MA. Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell*. 2009;4:141–154. doi: 10.1016/j.stem.2008.12.010.
7. Marión RM, Strati K, Li H, Murga M, Blanco R, Ortega S, Fernandez-Capetillo O, Serrano M, Blasco MA. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature*. 2009;460:1149–1153. doi: 10.1038/nature08287.
8. Suhr ST, Chang EA, Tjong J, Alcasid N, Perkins GA, Goissis MD, Ellisman MH, Perez GI, Cibelli JB. Mitochondrial rejuvenation after induced pluripotency. *PLoS One*. 2010;5:e14095. doi: 10.1371/journal.pone.0014095.
9. Abad M, Mosteiro L, Pantoja C, Canamero M, Rayon T, Ors I, Grana O, Megias D, Dominguez O, Martinez D, Manzanares M, Ortega S, Serrano M. Reprogramming in vivo produces teratomas and iPS cells with totipotency features. *Nature*. 2013;502:340–345.
10. Pal S, Tyler JK. Epigenetics and aging. *Sci Adv*. 2016;2:e1600584. doi: 10.1126/sciadv.1600584.
11. Marión RM, López de Silanes I, Mosteiro L, Gamache B, Abad M, Guerra C, Megías D, Serrano M, Blasco MA. Common telomere changes during in vivo reprogramming and early stages of tumorigenesis. *Stem Cell Reports*. 2017;8:460–475. doi: 10.1016/j.stemcr.2017.01.001.
12. Studer L, Vera E, Cornacchia D. Programming and reprogramming cellular age in the era of induced pluripotency. *Cell Stem Cell*. 2015;16:591–600. doi: 10.1016/j.stem.2015.05.004.
13. Kurian L, Sancho-Martinez I, Nivet E, et al. Conversion of human fibroblasts to angioblast-like progenitor cells. *Nat Methods*. 2013;10:77–83. doi: 10.1038/nmeth.2255.
14. Blasco MA. Telomere length, stem cells and aging. *Nat Chem Biol*. 2007;3:640–649. doi: 10.1038/nchembio.2007.38.
15. Bar C, Blasco MA. Telomeres and telomerase as therapeutic targets to prevent and treat age-related diseases. *F1000Res*. 2016;5. doi: 10.12688/f1000research.7020.1.

Long Live Partial Reprogramming

Rosa M. Marión and Maria A. Blasco

Circ Res. 2017;120:1381-1383
doi: 10.1161/CIRCRESAHA.117.310594
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571