

# Restoring Stem Cell Function in Aged Tissues by Direct Reprogramming?

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Adult stem cells are responsible for the cellular turnover of many organs, and an impairment in their function leads to aging and disease. In efforts to reverse the process of tissue stem cell aging, we speculate on the promise and challenges of *in vivo* direct reprogramming strategies.

Aging correlates with a decline in the function of the adult stem cells (i.e., somatic stem cells) present in many organs of an adult mammal. A decrease in the stem cell pool and/or a restriction in the somatic stem cell differentiation potential affects tissue homeostasis and regeneration. Here, we discuss the possibility of replacing or rejuvenating dysfunctional adult stem cells from an aged tissue or organ using a direct reprogramming approach *in situ*.

## Limitations of Generating Differentiated Somatic Cells for Anti-Aging Purposes

The ability to induce pluripotency in somatic cells has heralded a new era in the field of regenerative medicine, because induced pluripotent stem cells (iPSCs) specific to individual patients could provide an unlimited source of specialized cell types for replacing diseased or aged tissues. However, several technical problems must be resolved before safely translating the iPSC technology to the clinic. For therapeutic purposes, the iPSCs must be differentiated into the required cell types. Currently available differentiation protocols aim to recapitulate *in vitro* the embryonic events that occur in the early embryo *in vivo*. However, the underlying developmental mechanisms are not well understood, making their faithful modeling *in vitro* a difficult undertaking and preventing the derivation of sufficient quantities of transplantable cells. In addition to transplantation and proper engraftment, the risk for teratoma formation

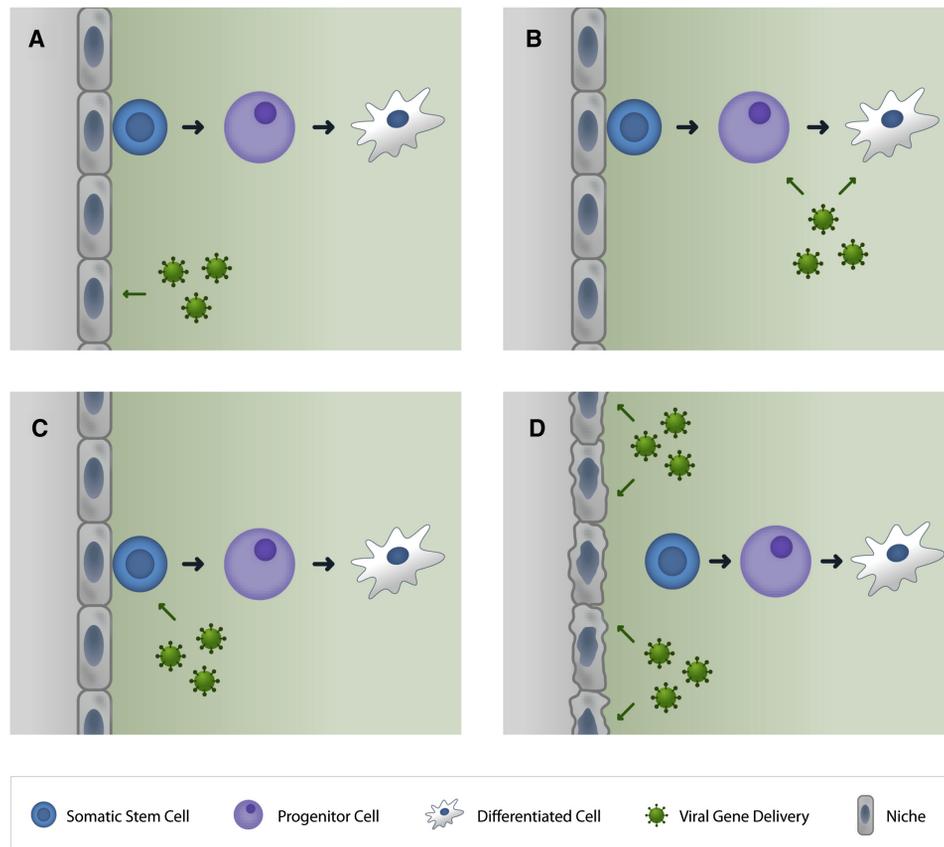
remains a major issue with somatic cells differentiated from iPSCs (Cohen and Melton, 2011).

The reprogramming of somatic cells into iPSCs entails the use of a set of specific transcription factors that can establish a pluripotent program in a differentiated cell. This method of reprogramming suggests that other cell fates could be induced if the right transcription factor cocktail is used. Recent studies have indeed shown that specific transcription factor combinations can be used to convert fibroblasts directly into neurons, cardiomyocytes, and hepatocytes, bypassing a pluripotent intermediate and essentially removing the risk for teratoma formation. The cell types generated by such direct lineage reprogramming typically exhibit an immature phenotype resembling that of fetal or neonatal cells. Nevertheless, these cells might acquire a fully functional and mature profile after engraftment into the host and subsequent exposure to the appropriate environmental cues (Cohen and Melton, 2011). However, terminally differentiated cells, such as postmitotic neurons, do not proliferate and cannot be expanded prior to transplantation. Therefore, the generation of a sufficient number of cells for ensuring a successful transplantation remains a challenge.

A strategy that might circumvent this obstacle is the direct reprogramming of somatic cells into self-renewing somatic stem cells. The low reprogramming efficiency associated with this approach would not limit the clinical applicability of this strategy because the generated

cells would be able to proliferate. In addition, the somatic stem cells could be transplanted into the host niche, where endogenous stimuli could promote acquisition of a fully mature phenotype. Moreover, transplanted somatic stem cells could self-renew and/or differentiate into cells that would respond to inflammatory signals and migrate toward damaged tissues, thus facilitating proper engraftment for regenerative purposes. Recently, we and others reported the direct conversion of mouse fibroblasts into self-renewing induced neural stem cells (iNSCs) that could differentiate into neurons, astrocytes, and oligodendrocytes, as reviewed in Zhou and Tripathi (2012).

A successful direct reprogramming strategy requires the use of culture conditions that are optimal for the desired cell type. However, such conditions have not yet been established for all of the specialized cell types of the adult organism. To overcome this problem, one could perform direct lineage conversion *in vivo*, where the right environment already exists to promote the survival of the induced cells. As a proof of principle, exocrine pancreatic cells have been directly reprogrammed *in vivo* into  $\beta$  cells (Zhou et al., 2008). Reprogramming *in situ* circumvents the need to determine culture conditions for the maintenance of the induced cells *in vitro*, provides the right niche for achieving a fully mature and functional phenotype, and eliminates the transplantation step. However, as in the *in vitro* scenario, *in vivo* conversion into somatic cells is limited by the



**Figure 1. Potential Strategies for Restoring Stem Cell Function in Aged Organs and Tissues**

(A) Stem cells contain a genetic modification that is transmitted to their progenitor and daughter cells. In this scenario, somatic cells located close to the niche and not derived from the mutated adult stem cell would be reprogrammed by factors (e.g., expressed via viruses) into de novo stem cells.

(B) Stem cells contain an epigenetic modification that is transmitted to the progenitors. In this case, progenitor or differentiated cells would be dedifferentiated into stem cells, and the detrimental epigenetic marks would be erased.

(C) The stem cell transcriptional network is missing specific transcription factors that would be delivered specifically to the stem cells.

(D) Malfunction of the stem cells is due to an aged niche, which would be the target for the in vivo reprogramming.

inefficiency of the reprogramming process and by the nonproliferative nature of many terminally differentiated cells. Thus, the number of somatic cells directly converted in vivo might not suffice to rescue the tissue deficiency. This problem could again, in principle, be overcome by the direct conversion in vivo of differentiated cells into proliferative tissue-specific somatic stem cells. Therefore, direct reprogramming in vivo into somatic stem cells might potentially be able to circumvent many of the obstacles that stand in the way of being able to use direct lineage reprogramming in a therapeutic setting.

#### Reprogramming In Vivo into Somatic Stem Cells Could Compensate for Tissue Aging

In theory, if tissue stem cells could be generated from somatic cells in vivo using

the right combination of factors, then the in situ reprogrammed adult stem cells could be used to ameliorate the effects of an age-related decline in the stem cell pool and/or a restriction in the stem cell differentiation potential. Depending on the mechanisms underlying the age-related stem cell dysfunction, we have divided the different strategies that could be explored into genetic abnormalities, epigenetic modifications, dysregulated gene expression, and niche defects.

In the case of intrinsic genetic modifications in the adult stem cells (Figure 1A), such as chromosomal translocations or mutations, de novo somatic stem cells would need to be generated. Because daughter cells could also have inherited the genetic alterations that are responsible for the dysfunction, the progeny of stem cells that need to be replaced could not be the cells subjected to the direct

conversion. Therefore, somatic cells located close to the stem cell niche that are not derived from the damaged tissue stem cells would be the target cell of choice. Some cell types can present advantages for the reprogramming process that should be taken into consideration. For instance, epithelial cells (e.g., keratinocytes) can be reprogrammed into iPSCs more rapidly than cells from a mesenchymal origin because they do not need to undergo a mesenchymal-to-epithelial transition step (Stadtfeld and Hochedlinger, 2010). However, the potential impact of these parameters in the direct conversion into somatic stem cells needs to be investigated.

In another scenario (Figure 1B), adult stem cell dysfunction could be caused by epigenetic modifications (Pollina and Brunet, 2011). In this case, progenitor or daughter cells differentiated from the

aged adult stem cell would be suitable for conversion, because the reprogramming process would most likely reset the epigenome, correcting the detrimental modifications. Progenitor cells lack the unlimited self-renewal activity of somatic stem cells and are committed to differentiation. However, somatic stem cells and the progenitors derived from them have very similar transcriptional networks. For instance, neural stem cells and neural progenitor stem cells both express Sox2 (Zhou and Tripathi, 2012). During the reprogramming of NSCs into iPSCs, Sox2 does not need to be exogenously introduced, because it is already expressed by the NSCs, reducing the number of reprogramming factors needed (Stadtfield and Hochedlinger, 2010). Thus, we would suggest that the conversion of progenitor or early-committed cells into somatic stem cells might require fewer transcription factors than conversion of more differentiated cells because it could take advantage of genes that are already being endogenously expressed. Finally, whereas the epigenetic memory in somatic stem cells might be substantial when reprogrammed from cells that originated from a different germ layer (Han et al., 2012), using progenitor or early-committed cells from the same lineage as the initial target cell would minimize its potential impact.

Another scenario would involve alterations in the expression levels of specific genes leading to an adult stem cell malfunction (Figure 1C). For example, deletion of the transcription factors *FoxO1*, *FoxO3*, and *FoxO4* reduces the number of NSCs in the mouse adult brain, impairing neurogenesis. Similar effects are seen with deletion of the transcription factor *Tlx* and the enzyme *Telomerase*. In these situations, it is possible that forced expression of single genes could improve the performance of the aged adult stem cells. Consistent with this idea, the overexpression of *Telomerase* could restore NSC self-renewal and neurogenesis in aged telomerase-deficient mice (Pollina and Brunet, 2011). The overexpression of *Tlx* in NSCs also delayed the age-associated NSC decline, but, in addition, it led to the formation of glioma-like lesions (Pollina and Brunet, 2011). As in this case, many genes involved in stem cell maintenance have also been related to tumor formation. Considering that, to

date, in vivo transplantation of somatic stem cells generated by direct reprogramming has not been associated with any tumor formation, it appears that induction of a de novo cell phenotype may present a lower risk for tumorigenesis than the overexpression of single genes in aged somatic stem cells (e.g., *Tlx*).

In the generation of de novo somatic stem cells, the old (i.e., aged) and young stem cells will cohabitate in the same niche. If the aged stem cells exhibit a decline in self-renewal activity, we would expect a dilution of the old stem cell pool due to the higher proliferative ability of the de novo-induced tissue stem cells. In addition, the new stem cells would be able to compensate for the restricted differentiation potential of the aged stem cells. However, the aged stem cells exhibiting genetic alterations cannot be eliminated from the stem cell niche and would continue to accumulate mutations, presenting a tumorigenic risk.

In the scenarios described above, the decline in tissue homeostasis was caused by intrinsic modifications in the stem cells. But adult stem cells could also be affected by extrinsic factors (Figure 1D). The environment or stem cell niche plays an essential role in regulating the self-renewal and differentiation potential of somatic stem cells. For instance, a reduction in the number of spermatogonial stem cells (SSCs) with age is associated with reduced fertility of male mice, suggesting a decline in SSC self-renewal ability. However, SSCs isolated from young mouse testes could be serially transplanted multiple times into young recipients and could self-renew for a longer time than the life span of a normal mouse (Ryu et al., 2006). In this case, the proliferation decay is not intrinsic to the SSCs but rather is induced by the aged environment. This phenomenon has been also observed in other adult stem cells (e.g., hematopoietic stem cells). Therefore, the tissue stem cell niche can be considered to be one of the main players in preventing, reverting, and/or delaying stem cell aging. At this point, we would like to speculate on the possibility of reprogramming or rejuvenating the aged niche through the overexpression of a minimum set of genes. Unfortunately, very little is known about the transcriptional networks governing the different cell types that form

a stem cell niche. Glial cell-line-derived neurotrophic factor (GDNF) is a ligand secreted by Sertoli cells that ensures the self-renewal of SSCs in mouse testes. Interestingly, aged testes show reduced GDNF levels (Ryu et al., 2006), establishing a connection between an alteration in the SSC niche and a decline in SSC proliferation ability. Although mice that express GDNF ubiquitously are infertile due to an inability of the SSCs to differentiate (Meng et al., 2000), it would be interesting to assess the effect of GDNF overexpression solely by Sertoli cells on aged SSC. Nevertheless, it seems unlikely that only one gene could restore the function of an aged stem cell niche. In addition, the aging of the stem cell niche is also affected by systemic factors, as is evident from parabiosis experiments in which circulating factors from the blood of young mice can modify the stem cell niche of old mice and can revert some aging effects (studies that have been recently reviewed in Wagers, 2012). In those cases in which the main cause for the dysfunctional niche relies on the alteration of systemic factors, in situ transcription-mediated reprogramming of the aged adult stem cell niche would not be the strategy of choice. Overall, further knowledge in niche regulation is needed to ascertain better strategies for rejuvenating the stem cell environment and, indirectly, restoring the somatic stem cell function—a promising approach, yet one fraught with many challenges.

#### Methods for Specifically Delivering the Reprogramming Factors

For any transcription-factor-mediated reprogramming approach, a robust technique for efficiently delivering reprogramming factors to a specific cell type of choice would be required but is currently still lacking. The recent demonstration that lentiviral vectors are able to recognize and infect, in vivo, cells presenting with specific cell-surface antigens is promising (Anliker et al., 2010). However, lentiviral vectors integrate into the host genome, precluding their use in medical applications. Unfortunately, the efficiency of the conversion process is significantly compromised when using integration-free methods, as demonstrated in iPSC reprogramming experiments (Stadtfield and Hochedlinger, 2010). Therefore, further efforts are needed to overcome

the technical challenges associated with the current reprogramming techniques. Progress in the area of nanotechnology may help develop systems that allow efficient directed delivery of reprogramming factors to regions within tissue and organs.

### Future Perspectives

From graying hair to a decline in neurogenesis, aging affects everyone. Though an *in vivo* transcription-factor-mediated approach cannot be considered as an elixir for immortality, it could serve as the basis for further discussion about tissue stem cell rejuvenation. Future efforts should be directed toward the characterization of the transcriptional networks of tissue stem cells and their niches and the development of delivery methods for specific cell types. In addition, identification of the molecular modifications that stimulate adult stem cell longevity in

response to environmental modifications (e.g., dietary restrictions) would provide crucial information for improving anti-aging reprogramming strategies. The challenges that lie ahead should be an impetus for uncovering the missing links in direct reprogramming and for restoring stem cell function in aged cells.

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