Bursts of Reprogramming: A Path to Extend Lifespan?

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In a thought-provoking study, Ocampo et al. show that the cyclic expression of stem cell reprogramming factors in vivo increases the lifespan of a mouse model of premature aging and provides health benefits to chronologically old, normal mice.

Repaiiring of somatic cells to induced pluripotent stem cells (iPSCs) not only holds great promise to model and treat many diseases but can also provide insights into aging and rejuvenation mechanisms (Studer et al., 2015). Indeed, reprogramming of aged somatic cells into iPSCs reverts some hallmarks of aging—including telomere length, mitochondrial fitness, abnormal nuclear morphologies, loss of heterochromatin markers, and gene expression—to a youthful embryonic state (Studer et al., 2015). In this issue of Cell, Ocampo et al. (2016) apply stem cell reprogramming factors in vivo to improve the health of an organism and to prolong its lifespan. They first find that transient in vitro expression of the four Yamanaka reprogramming factors—Oct4, Sox2, Klf4, and c-Myc (OSKM)—reverts age-associated features in mouse and human cells, without the loss of cellular identity. Remarkably, the authors show that at the organism level, short-term induction of the four reprogramming factors improves the physical state of a mouse model of premature aging and extends its lifespan. Importantly, they also provide evidence that transient expression of the four factors has beneficial effects in physiologically aged, normal mice.

An intriguing yet unexplored question in the aging and reprogramming field has been whether it is possible to uncouple the rejuvenation and the dedifferentiation process associated with iPSC reprogramming. Although previous studies have shown that transient expression of the four factors can induce a partially dedifferentiated state (without a complete loss of cellular identity) (Kurian et al., 2013), whether this partial state is sufficient to revert age-associated features remained unclear. The authors now show that short-term expression of the four Yamanaka factors is sufficient to revert some age-associated features in mouse and human cells without dedifferentiation. Using fibroblasts from a transgenic mouse model of premature aging (a model for Hutchinson Gilford Progeria Syndrome) that also carries an inducible OSKM polycistronic cassette, they show that short-term induction of OSKM (2 to 4 days) reduces markers of DNA damage (γ-H2AX), senescence, and mitochondrial impairment (reactive oxygen species production), without the loss of cellular identity. Moreover, expression of reprogramming factors restores the levels of two epigenetic marks of heterochromatin that have previously been associated with aging and progeria, namely trimethylated lysine 9 on histone H3 (H3K9me3) and H4K20me3, but the reprogramming model used, whose cells carry a genetic marker, thus constitutes a key target for future rejuvenation strategies. It would be interesting to determine if transient pulses of OSKM are sufficient to erase other hallmarks of aging—including metabolism defects, increased inflammation, and increased protein aggregates—and determine their hierarchy compared to epigenetic events in this process.

Interestingly, 8 days after OSKM induction, the progeria-associated features slowly come back, suggesting that OSKM expression provides a transient reversion of premature aging phenotypes. However, the re-establishment of age-associated features may be specific to the progeria model used, whose cells carry a genetic alteration. Additional experiments will be required to examine how well these findings recapitulate in cells from normal old mice.

What drives the reversion of these age-associated features? Ocampo et al. (2016) provide evidence that epigenetic changes may be at the core of this reversion. In time-course experiments, the epigenetic changes precede the reversion of DNA damage and senescence markers, and chemical inhibition of H3K9me3 methyltransferases abrogates the rejuvenation effect. However, many questions remain; for example, it is unclear which precise histone mark is required. The authors show restored levels of H3K9me3 and H4K20me3, but the reprogramming factors induce a complete remodeling of the chromatin landscape (Takahashi and Yamanaka, 2016). Alterations of the other histone marks (e.g., H3K4me3 and H3K27me3, which also change with age) required, and to what extent? Interestingly, H3K9me3 marks mainly constitutive heterochromatin and is often found coating retrotransposon elements, thereby preventing their spurious reactivation. Expression of retrotransposon elements has been associated with cellular senescence and organismal aging, and suppression of these elements can reverse the senescence phenotype, at least in vitro (Pal and Tyler, 2016). These findings suggest a critical role of epigenetic reprogramming in the rejuvenation process, perhaps by improving genomic stability. These results raise the exciting possibility that epigenetic changes supersede/precede other aging hallmarks in the physiological aging process, as well, and may thus constitute a key target for future rejuvenation strategies. It would be interesting to determine if transient pulses of OSKM are sufficient to erase other hallmarks of aging—including metabolism defects, increased inflammation, and increased protein aggregates—and determine their hierarchy compared to epigenetic events in this process.
Arguably, the most provocative question of this study is whether cellular reprogramming can also reset aging hallmarks at the organismal level (in vivo). Previous studies have been hampered by the fact that continuous expression of the four factors in vivo has resulted in extensive cancer development and high rate of mortality (Abad et al., 2013). The authors circumvent these issues by optimizing a protocol for in vivo cyclic induction of the reprogramming factors. When applied to a mouse model of premature aging that carries a mutation in the Lmna gene and produces a truncated Lamin A (LAKI mice), the mice exhibit improvements in features associated with their disease state, including physical appearance, histological changes in organs, as well as cardiovascular functions. Importantly, they also exhibit increased median and maximal lifespan (though the number of animals is relatively low \([n < 30]\)). A central question is whether these findings can be extended to physiological aging. Interestingly, the authors show that cyclic expression of the four factors improves the regenerative capacity of pancreas and muscle following injury and increases lifespan in a premature aging mouse model, potentially via induced epigenetic changes. Only the organs/tissues that exhibit a rejuvenation/reverted phenotype in wild-type (WT) mice are indicated. “Blood” includes changes in immune system, NT, not tested. *, shown to also improve in young mice.

How, mechanistically, does cyclic induction of reprogramming factors improve health span and lifespan of an organism? A key question is whether the extension of lifespan is solely due to the cellular rejuvenation provided by reprogramming or whether it is triggered by another mechanism. As observed in vitro, short-term induction of OSKM expression can be initiated to still provide a beneficial effect. Ultimately, it will be interesting to compare and contrast transient reprogramming with other interventions known to delay or reverse aging (Figure 1), including elimination of senescent cells (genetically or via senolytics) (Trabucco and Zhang, 2016), parabiosis (the fusion of young and old animals by blood circulation [Conboy et al., 2013]), rapamycin (Arriola Apelo and Lamming, 2016), and a fasting-mimicking diet (Brandhorst et al., 2015).
A Breakdown in Cooperativity Leads to Cardiac Identity Crisis

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Using induced pluripotent stem cells, Ang et al. elucidate how a mutation in the transcription factor GATA4 causes congenital heart disease. They find that, although the recruitment of GATA4 to cardiac super-enhancers is retained, it no longer functions in partnership with another key transcription factor, leading to misexpression of non-cardiomyocyte genes.

Congenital heart disease (CHD) is the most common cardiac malformation in newborns, occurring in nearly 1% of the population worldwide (Hoffman, 1995). An additional 5% of the population has mild cardiac abnormalities that eventually develop into cardiac problems as they age, including ventricular dysfunction and sudden death (Fahed et al., 2013, Brickner et al., 2000). The etiologies of CHD have a strong genetic component, which can include disruption of developmentally regulated cardiac transcription factors such as TBX5, NKX2-5, and GATA4 (Schott et al., 1998, Garg et al.,