AGEING

Retinal rejuvenation

> changes in the pattern of DNA methylation are needed for the beneficial effects of OSK expression after injury

Ageing impairs CNS function and the ability to regenerate after injury. Ageing is proposed to be associated with the accumulation of epigenetic 'noise', but whether such epigenetic changes partly underlie the effects of ageing on the CNS is unknown. Now, Lu et al. show that reprogramming retinal ganglion cells (RGCs) in mice in a way that renders their DNA methylation pattern more 'youthful' can enable these cells to regenerate after injury and can restore visual function in aged mice.

Previous research indicated that ectopic expression of four transcription factors — OCT4, SOX2, KLF4 and MYC (known as the Yamanaka factors) — can reprogramme somatic cells into pluripotent stem cells, and might prevent premature ageing in a mouse model of progeria. Here, Lu et al. tested the effects of virus-mediated ectopic expression of OCT4, SOX2 and KLF4 (collectively called OSK) in RGCs, as a model CNS cell type that shows loss of function and regenerative ability with ageing.

Credit: Robert Trevis-Smith/Getty

The authors excluded *Myc*, given its oncogenic profile.

Strikingly, viral induction of OSK expression in RGCs of young (4-week-old) or aged (12-month-old) mice 2 weeks before optic-nerve crush increased RGC survival (without increasing proliferation), and promoted axonal regeneration, as measured using a tracer. The effects were specific to the OSK combination, as expressing any one or two of the three transcription factors did not have the same effect.

The Yamanaka factors are known to reverse DNA methylation signatures associated with ageing in vitro. Here, Lu et al. showed that, 4 days after optic-nerve crush, RGCs exhibited changes in DNA methylation pattern that mimic those seen with ageing, whereas OSK expression in injured RGCs prevented these changes without altering global DNA methylation levels. DNA is methylated by ten-eleven translocation (TET) enzymes. Lu et al. showed that OSK expression upregulated TET1 and TET2 levels, and that knockdown of *Tet1* or *Tet2* blocked the pro-survival, pro-regenerative effects of OSK expression after optic-nerve crush. These data suggest that changes in the pattern of DNA methylation are needed for the beneficial effects of OSK expression after injury.

In cultured human neurons, OSK expression prevented axonal loss and protected against accelerated ageing-related changes in DNA methylation after treatment with the chemotherapeutic drug vincristine, which causes axonal injury. Moreover, knockdown of *TET2* in these cells similarly blocked the beneficial effects of OSK expression in this model, indicating that the effects of OSK expression may be conserved in mice and humans.

In glaucoma, increased intraocular pressure leads to degeneration of RGCs and their axons, resulting in vision loss. Lu et al. increased intraocular pressure in mice by injecting microbeads into the eye, leading to RGC axonal loss and a loss of visual acuity (as measured in an optomotor response task) after 4 weeks. The authors then induced OSK expression in RGCs for another 4 weeks. This treatment restored RGC axon density to levels observed in non-glaucomatous eyes, reversed the loss of visual acuity and normalized RGC electrical activity measured using pattern electroretinogram. Thus, OSK expression can restore vision in this model of glaucoma.

Next, the authors tested the effects of OSK expression in ageing. Inducing OSK expression for 4 weeks in the RGCs of 11-month-old mice restored the visual acuity of these animals to a level comparable to that in 5-month-old mice. RNA sequencing of RGCs from 5-month-old and 12-month-old mice revealed that the transcription of 464 genes is altered with ageing, and that about 90% of these changes are reversed by OSK expression. The authors used machine learning to characterize a DNA methylation signature of ageing, and showed that OSK expression countered this signature in 12-month-old mice. The ageing signature was enriched with CpG sites associated with genes that recruit TET enzymes, and knockdown of *Tet1* or *Tet2* blocked the restorative effects of OSK expression on RGC function and vision of aged mice.

Together, these results indicate that OSK expression induces reprogramming of the DNA methylation pattern of aged or injured RGCs to a more 'youthful' state.

Natasha Bray

ORIGINAL ARTICLE Lu, Y. et al. Reprogramming to recover youthful epigenetic information and restore vision. *Nature* **588**, 124–129 (2020)