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Putting aging on ICE

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Abstract

A recent report by Yang *et al.* in *Cell* demonstrates that faithful DNA double strand breaks and repair cycles phenocopy many aspects of aging in mice. Whether this progeroid phenotype is caused by a loss of epigenetic information remains to be conclusively determined.

Aging is a complex phenotype characterized by an array of biological changes that lead to an increase in an organism's frailty and in the likelihood of its death. Conserved hallmarks of aging across taxa have been described¹, including genomic instability, epigenetic alterations, senescent cell accumulation and exhaustion of stem cells. However, there is still much debate about which hallmarks are primary drivers, secondary mediators, or simply downstream consequences of the aging process.

A recent paper by Yang and colleagues² attempts to test a unifying theory of aging, dubbed the "Information Theory of Aging". Central to this theory is the notion that cellular identity is determined by a precise epigenomic landscape. As a by-product of cellular metabolism and exposure to external insults, DNA-damage inevitably accumulates with time. Repair of DNA-damage is coordinated by chromatin modifiers, which also play a key role in maintaining cellular epigenomes and, thus, cell identity. Based on this theory, the repeated relocalization of chromatin modifiers to DNA-damage sites over a lifetime leads to progressive loss of epigenomic identity, ultimately manifesting in what we know as aging at the organismal level. Crucially, the theory distinguishes itself from prior DNA-based theories of aging in that it posits that the loss of epigenomic, rather than genomic, information is the primary driver of aging².

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Declaration of Interests

The authors have no conflict of interest.

In order to test this theory, the authors use a mouse model expected to scramble epigenetic information while keeping genetic information intact, dubbed ICE for Inducible Changes to the Epigenome². In this model, mice carry 2 transgenes: (i) a ubiquitously expressed Cre recombinase localizing to the nucleus upon exposure to tamoxifen [TAM], and (ii) a TAM-stabilized and nuclear-localized I-*PpoI* endonuclease fusion protein transgene at the *Rosa26* locus, which can only be expressed once an upstream floxed stop cassette is excised by Cre. Using this system, transgenic mouse cells exposed to TAM should inducibly express nuclear I-*PpoI*, which can cut 20 unique canonical sites in the mouse genome. To note, one of these sites is located within the 28S rDNA sequence, of which mice carry hundreds of genomic copies³. Since using I-*PpoI* to induce Double-Stranded DNA Breaks [DSBs] should create “sticky” 4-basepair overhangs, these DSBs are expected to have low mutagenic potential.

The authors first characterized the ICE system using mouse embryonic fibroblasts [MEFs]. After induction, ICE cells showed strong signs of dsDNA damage compared to controls, while displaying limited mutational load post-treatment, compatible with the notion that the ICE system increases the rate of mutation-less DSBs. ICE MEFs showed increased estimated age based on a DNA-methylation [DNAm] epigenetic clock, increased expression of senescence-associated genes, and increased frequency of markers of cell senescence. The group tested the ICE system *in vivo*, by inducing I-*PpoI* expression in young adult mice, and performed phenotypic characterization 1 and 10 months after induction. Consistent with the known progeroid impact of DNA-damage⁴, ICE mice exhibited visible signs of accelerated organismal aging (Figure 1). Specifically, young adult ICE mice phenocopied organismal aging phenotypes usually observed only by 24-30 months of age, such as lower body weight, reduced fat mass, decreased respiratory exchange rate, decreased activity, increased frailty, kyphosis, decreased bone density, and hair greying. System-specific age-mimicking phenotypes were also observed at the level of kidney architecture, impaired learning and increased glial cell activation in the brain, and decreased muscle function. ICE mice also exhibited accelerated DNAm epigenetic aging in multiple tissues. Thus, relatively faithful DNA damage in ICE mice seems to recapitulate many aspects of aging. To provide molecular insights into the epigenetic impact of the ICE system, the authors profiled chromatin states in ICE MEFs using mass spectrometry of histone modifications and ChIP-seq of select histone marks. Consistent with the disruptive effect of DNA damage signalling on chromatin⁵, the authors observed many chromatin remodelling events in ICE MEFs. For instance, there was an enrichment for neuronal-related genes among genes with decreased levels of the repressive histone mark H3K27me3 and indeed, ICE MEFs were more easily reprogrammed into neurons, which the authors hypothesize is the result of cell identity erosion in ICE cells.

Finally, the authors attempted to rescue the ICE phenotypes using partial reprogramming with OSK factors, which they previously showed can rescue aged phenotypes in mouse retinal ganglion cells [RGCs]⁶. After OSK treatment, select phenotypes induced in ICE cells and tissues appeared rescued (*e.g.* marker gene expression, H3K9me3 levels, DNAm age acceleration). In addition, OSK expression rescued RGC gene expression in aged ICE mouse retina tissue, consistent with transcriptional rejuvenation.

This study makes a convincing case that the ICE model leads to accelerated organismal aging, suggesting that high mutational load after DSBs is not a necessary intermediate for progeroid-effects of genomic instability^{1,4}. However, the notion that progeroid phenotypes are only directly mediated by observed changes to the epigenome (the so-called “Information Theory of Aging”) is not directly tested in this study. Importantly, the type of system used in this study (inducible nuclear localization of a restriction enzyme like *I-PpoI* or *SacI*) robustly induces the DNA-damage response and P53 signalling⁷⁻⁹, which can lead to cell senescence. Importantly, senescent cells secrete a panoply of pro-inflammatory factors, leading to a deleterious state of chronic sterile inflammation which can drive aspects of aging¹. This study identified increased senescence markers in ICE cells², suggesting that the secretory impact of senescent cells in the ICE model may need to be accounted for. Interestingly, transient nuclear localization of *I-PpoI* in mouse epidermal stem cells was recently found to promote selective elimination of damaged stem cells through differentiation¹⁰. Although stem cell niches were not directly queried in this study, loss of adult stem cell populations by ICE-activation mediated attrition could explain a number of age-related phenotypes in ICE mice, and can be addressed in future studies with single cell technologies. In summary, additional evidence will be required to prove or disprove the “Information Theory of Aging”. However, this study provided the field with a new model, whereby a short organism-wide burst of DSBs in adulthood can have a long-term detrimental impact on mice that resembles aging.

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Figure created with [BioRender.com](https://www.biorender.com). The authors thank Dr. Minho Kim for feedback on the manuscript. B.A.B. is supported by NIGMS R35 GM142395, NIA R01 AG076433, Simons Foundation award SF811217 and Pew Biomedical Scholar award #00034120.

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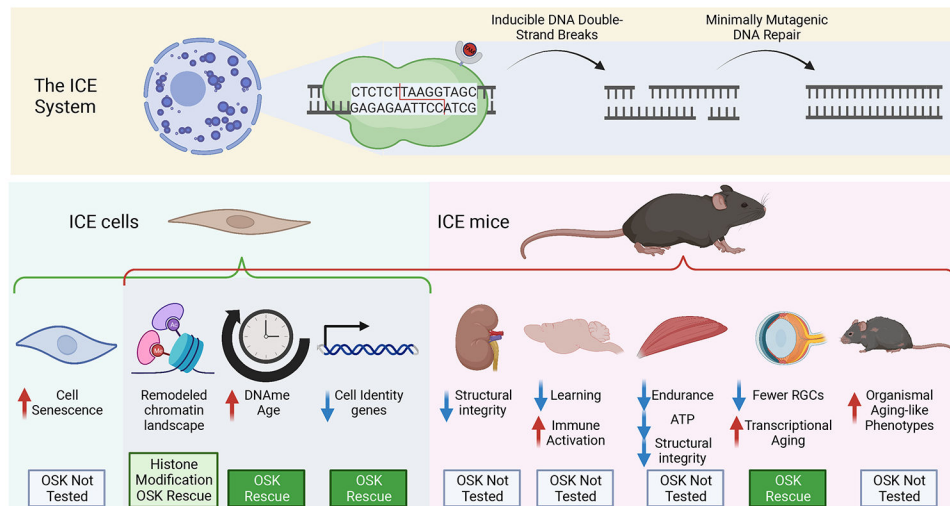


Figure 1: The ICE mouse model recapitulates many aspects of aging.

The ICE system creates inducible double strand breaks at 20 unique sites in the mouse nuclear genome, that are repaired with minimal mutagenesis. ICE cells show age-related signatures *in vitro* and ICE mice phenocopy many aspects of aging *in vivo*. Aspects of ICE-induced phenotypes can be rescued with OSK (Oct4-Sox2-Klf4) transient overexpression. Created with [BioRender.com](https://www.biorender.com)